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# UCC

**University College Cork, Ireland**  
Coláiste na hOllscoile Corcaigh

1 Research Article

2 **Effect of an egg ovalbumin-derived protein hydrolysate on blood pressure and cardiovascular risk in adults**  
3 **with a mildly elevated blood pressure: a randomized placebo-controlled crossover trial**

4

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6

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13

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15 Total word count (no of characters incl. spaces): 6,725 (44,134).

16 **Abbreviations:** **ACE**, angiotensin-converting enzyme; **AE**, adverse events; **BP**, blood pressure; **cfPWV**, carotid  
17 to femoral pulse wave velocity; **CVD**, cardiovascular disease; **NO**, nitric oxide; **PWA**, pulse wave analysis; **RCT**,  
18 randomized controlled trial.

19

20 **Abstract**

21 **Purpose** While animal and *in vitro* data demonstrate vasodilatory effects of egg-white derived peptides, human  
22 studies are lacking. We investigated for the first time the effects of an egg ovalbumin-derived protein hydrolysate  
23 on blood pressure (BP) and cardiovascular risk.

24 **Methods** A double-blind, placebo-controlled randomized crossover trial was implemented in 75 adults aged 50-  
25 70yrs with systolic BP (130– ≤150 mmHg). Participants were randomized to an egg ovalbumin derived-protein  
26 hydrolysate (3g/d) or placebo (3g/d). Participants completed two 6-week periods separated by a 3-week washout.

27 **Results** Data from 65 participants with a mean systolic BP (135.1±11 mmHg) were included. Mean office and  
28 central BP and arterial stiffness (assessed by carotid-femoral pulse wave velocity (cfPWV) or pulse wave analysis  
29 (PWA)) did not change over time and no significant differences were observed between the egg-protein  
30 hydrolysate and placebo groups ( $P > 0.05$ ). Similarly, no significant effects of this egg ovalbumin-derived protein  
31 hydrolysate on blood lipid and glucose concentrations ( $P > 0.05$ ) were observed.

32 **Conclusion** This is the first dietary intervention to investigate the effects of egg ovalbumin-derived protein  
33 hydrolysates on cardiovascular risk in humans. Despite promising findings from animal and *in vitro* studies, this  
34 RCT does not support the hypothesis that consumption of an egg ovalbumin-derived-protein hydrolysate for 6-  
35 weeks in adults with a high-normal BP results in a reduction in BP or the modification of cardiovascular risk.

36

37 **Keywords:** Bioactive peptides, blood pressure, cardiovascular risk, egg white, ovalbumin.

38

39 **Introduction**

40 Cardiovascular disease (CVD) is the leading global cause of mortality, accounting for ~31% of deaths [1].  
41 Hypertension is one of the major controllable risk factors associated with CVD [2]. Dietary factors play a  
42 significant role in the prevention of hypertension and the maintenance of normal blood pressure (BP) [3, 4];  
43 therefore, efforts are being placed on the development of foods with anti-hypertensive activity.

44 Many dietary proteins contain peptide sequences (between 2-50 amino acid residues) encrypted within  
45 their primary structure that are capable of modulating specific physiological functions once released by digestive  
46 enzymes during gastrointestinal transit or by fermentation or ripening during food processing [5]. There is  
47 increasing evidence that these bioactive peptides naturally present in dairy, cereals and fish may reduce vascular  
48 risk most notably by reducing BP [6 -8]. The formulation of these peptides into foods provides an opportunity to  
49 support physiological functions beyond that of nutrition.

50 Eggs are a highly nutritious food and a key dietary source of high biological protein. It has been observed  
51 that the consumption of an egg protein-derived hydrolysate compared to the original egg protein may demonstrate  
52 greater bioactivity. Miguel et al. [9, 10] reported that in spontaneous hypertensive rats, the long-term (20-weeks)  
53 administration of an egg-protein hydrolysate showed a clear impact on BP, whereas egg white protein  
54 demonstrated no extensive biological effects. Interestingly, animal trials using egg-white derived peptide  
55 hydrolysate fractions have demonstrated reductions in arterial pressure in spontaneously hypertensive rats [9, 11],  
56 with no effect in normotensive rats [12]. Plat et al. [13] have recently demonstrated a BP-lowering effect of an  
57 egg-white derived peptide from the protein lysozyme in a sample of mildly hypertensive adults; however, human  
58 studies investigating peptides isolated from the egg white protein, ovalbumin, on BP and cardiovascular risk are  
59 lacking.

60 This is the first randomised placebo controlled trial with a crossover design to investigate the efficacy of an egg  
61 ovalbumin-derived protein hydrolysate fraction on BP and cardiovascular risk in adults with a high-normal  
62 systolic BP.

63

64 **Materials and Methods**

65 **Study population**

66 Participants were recruited from the free-living community in the city of Cork, Republic of Ireland, via flyers and  
67 advertisements in local newspapers. From a total of 310 volunteers who were phone-screened, 208 attended a

68 screening visit; of these 75 adults (41 male; 34 female) met the inclusion criteria and were enrolled onto the study  
69 protocol. The participant flow from screening to final analysis is described in Fig 1.

70 Participants were included if they met the following criteria: aged 50-70 yrs; in good health; had a systolic  
71 BP: 130 – ≤150 mmHg (office BP, average of 3 readings taken after 5 minutes rest (Omron 705IT monitor,  
72 ProMed, Ireland)); had a BMI: 25.0 – 35.0 kg/m<sup>2</sup>. Main exclusion criteria included: smoking, hypertension,  
73 depressed or elevated BP measurements (systolic/diastolic: <95/55 mm Hg or >150/90 mm Hg), history of  
74 cardiovascular events, medical illness including diabetes mellitus (Types 1 & 2), egg allergy, chronic kidney  
75 disease and gastro-intestinal diseases, and medication use that may affect outcomes: anti-hypertensive  
76 medications and lipid lowering therapies.

77 The study protocol was approved by the Clinical Research Ethics Committee of the Cork Teaching  
78 Hospitals, University College Cork (UCC), Ireland (Ref ECM 4(u) 06/08/13) and was conducted in accordance  
79 with the principles of Good Clinical Practice and the Declaration of Helsinki. Detailed information about the  
80 study was provided to all volunteers, eligible volunteers provided their written informed consent at screening.  
81 This trial was registered at [clinicaltrials.gov](https://clinicaltrials.gov): Identifier NCT02223169/  
82

### 83 **Study design**

84 This study was a double-blind, randomized, placebo-controlled crossover trial with two 6-week periods separated  
85 by a 3-week washout (Online resource 2). Participants completed a 2-week run-in prior to commencing the trial.  
86 Enrolled volunteers were randomly assigned to their treatment using a block randomization scheme. Separate  
87 randomization schedules were generated for men and women. The randomization process and treatment  
88 allocation was conducted by a senior scientist not involved in the implementation or analysis of the trial. For the  
89 research team, a double-blinded protocol was maintained for the duration of the study and analysis.

90 At baseline, participants received 42 blinded sachets in a sealed envelope. Participants were randomized  
91 in a 1:1 ratio to receive either the active treatment (powdered egg ovalbumin-derived protein hydrolysate fraction,  
92 3g/d) or placebo-control (maltodextrin powder, 3g/d) for 6 weeks (period 1), after washout, participants received  
93 the alternative intervention for a further 6-weeks (period 2), followed by a final 3-week washout. The duration of  
94 the washout was in line with other peptide dietary intervention studies [14, 15].

95 The primary outcome was systolic BP (by office and central measurement); secondary outcomes  
96 included diastolic BP, changes in arterial stiffness (assessed by augmentation index by pulse wave analysis (PWA)  
97 and carotid-femoral, pulse wave velocity (cfPWV), fasting plasma lipids and glucose concentrations.

98

99 **Study treatments**

100 The egg ovalbumin-derived protein hydrolysate fraction (3g/d) and matching placebo (maltodextrin, 3g/d) were  
101 produced by BioActor, The Netherlands, in a plant (Bouwhuis Enthoven) which complies with food-grade  
102 conditions. Both powders were manufactured to appear and taste similar. Participants consumed one sachet each  
103 morning as part of a meal. Participants were instructed to add their sachet to a fruit juice (~150 mL) and to mix  
104 prior to consumption using the beverage shaker provided. The energy content of the egg albumin-derived protein  
105 hydrolysate fraction and the maltodextrin sachets were 1584 kJ/100g and 1757 kJ/100g, respectively (for nutrient  
106 compositions, see Online resource 1). A full characterization of these egg-derived protein hydrolysates as well as  
107 an *in vitro* evaluation of their ACE-inhibitory activity was undertaken prior to study commencement by BioActor  
108 (Table 1). For potential mechanistic data underpinning the biological activity of this peptide digestate fraction,  
109 the manufacturers measured angiotensin converting enzyme inhibition (IACE) as well as anti-oxidant activity of  
110 the egg ovalbumin-derived protein hydrolysate fractions using an Oxygen Radical Absorbance Capacity (ORAC)  
111 assay, prior to trial commencement. The ACE inhibitory activity ( $\mu\text{M}$ ) and antioxidant capacity ( $\mu\text{moleq Trolox}$ )  
112 were directly comparable to data reported by Miguel et al. [16] and Davalos et al. [17]. The *in vitro* digestion and  
113 bioavailability of the egg-albumin derived protein hydrolysate fractions used in the current study are  
114 comprehensively described by Grootaert et al. [18]].

115 In terms of safety, a number of animal studies were published in which egg albumin-derived peptide  
116 hydrolysates were administered without adverse events [9, 11, 19-20]. In addition, a pilot safety study at a similar  
117 level of supplementation (3g) of this specific egg ovalbumin-derived protein hydrolysate digestate fraction was  
118 conducted in adults ( $n$  20); where, no adverse events (AE) associated with consumption were observed. In the  
119 current study, the occurrence of any potential AE was checked routinely by the research team. Any AE  
120 experienced during the study were documented in detail on a clinical report form and reported to the Ethics  
121 Committee for the project and the Principal Investigator who assessed the relation of the AE to the treatment.  
122 Participant compliance was assessed by counting the used and remaining sachets a participant returned.

123

124 **Study visits**

125 Study visits took place at the Human Nutrition Studies Unit at UCC between May 2014 and February 2016.  
126 Participants were requested to maintain their habitual diet, level of physical activity and body weight. Participants  
127 were asked to refrain from consuming eggs and egg dishes for the study duration. Participants were monitored to

128 encourage compliance with the protocol. At screening, data on BP, weight and height were collected. Participants  
129 attended the study unit in a fasted state between 08:00 and 10:00 am at the baseline and endpoint of each study  
130 period and post the final washout (5 sampling points). Measurement of office and central BP, arterial stiffness  
131 and anthropometric assessments were completed at each visit. Health and lifestyle information were also  
132 collected.

133

#### 134 *BP*

135 BP was measured in accordance with the European Society of Hypertension guidelines [21]. Office BP was  
136 measured on fasted study-participants, between 08:00 – 10:00 am, prior to blood sampling, on the same arm at  
137 each visit and in accordance with a standardised protocol. BP was measured after the participant was in a seated  
138 position in a quiet room for at least 5 minutes. Office BP was measured 3 times with using a validated  
139 oscillometric semiautomatic arm device (Omron 705IT monitor) with 2-3 minute intervals between readings and  
140 the mean reading was calculated.

141

#### 142 *Central BP and assessment of arterial stiffness index*

143 Central BP, augmentation index by PWA and carotid-femoral cfPWV, a known indicator of arterial stiffness and  
144 cardiovascular risk, was measured with the participant in the semi-supine position using a non-invasive  
145 oscillometric technique with the Vicorder<sup>®</sup> Tonometric device (Skidmore medical, UK) (for further details see  
146 [22]).

147

#### 148 *Anthropometry*

149 Height was measured using a wall-mounted stadiometer without shoes. Body weight was measured in participants  
150 without shoes and in light clothing using a SECA weighing scales (ProMed, Ireland). Waist circumference was  
151 measured midway between the lowest rib and the iliac crest using a SECA tape measure (ProMed, Ireland). Body  
152 composition (fat mass and lean mass) was assessed during one visit by dual-energy X-ray absorptiometry (DXA)  
153 scan on a Lunar DXA device (GE Medical Systems, UK).

154

#### 155 *Blood sampling*

156 Five fasting blood samples (30ml) were collected under standardised conditions. For the 24 hours prior to their  
157 visit, participants refrained from alcohol consumption and strenuous physical activity. Blood samples were

158 processed and immediately stored at  $-80^{\circ}\text{C}$  until analysis. Post-blood sampling participants received their  
159 breakfast.

160

### 161 **Biochemical analysis**

162 All fasting samples for each participant were analysed within the same batch. Plasma lipids (total-cholesterol,  
163 HDL-cholesterol and triglycerides) and glucose were measured using an automated bench top clinical chemistry  
164 analyser (RX Autoanalyser, Randox laboratories, NI) according to the manufacturer's instructions. The co-  
165 efficient of variation (CV) for inter-day variations was  $<10\%$ . LDL-cholesterol was estimated using Friedewald's  
166 formula [23].

167

### 168 **Sample size calculation**

169 Sample size was calculated based on systolic BP data derived from baseline values of 700 adults who had  
170 previously participated in RCTs at UCC. This study was powered to detect a reduction of 2 mmHg systolic BP  
171 at 80% power and with a significance level of 5%, and a within-subject standard deviation of 4.0 mmHg. In total  
172 65 participants were required to complete the study, rounded up to 33 per group. Anticipating a 20% dropout rate,  
173 we aimed to randomly allocate 78 participants.

174

### 175 **Statistical analysis**

176 Statistical analyses were performed using IBM SPSS statistical software package (Version 23). Analyses are  
177 presented on a per-protocol basis; intention-to-treat (ITT) analysis is included as a Review Table for informational  
178 purposes (Online resource 3). Findings were similar between both analyses. Variables were tested using Shapiro  
179 Wilks test to establish whether data followed a parametric or non-parametric distribution. Plasma HDL  
180 cholesterol and triglyceride concentrations assumed a parametric distribution once log-transformed.

181 As no treatment carryover effects were observed between the two study periods, data were subsequently  
182 analysed as a total group. Differences between sexes at baseline were tested using an independent student's t test.  
183 Similarly, differences in baseline values between treatment groups were compared using an independent student's  
184 t test. Intra-group differences (baseline v. endpoint) were performed using a paired student's t test. Inter-group  
185 comparisons (changes during egg-protein hydrolysate treatment v. changes during placebo treatment) of normally  
186 distributed data were performed using an independent student's t test and were also evaluated using a general  
187 linear model (GLM) ANCOVA, with mean change (post-minus-baseline) in the variable as the dependent factor,



188 study treatment as the fixed factor. Potential confounding factors including the baseline values of the dependent  
189 variable, age, gender, and waist circumference were added to the model. A  $P$ -value  $\leq 0.05$  was considered as  
190 statistically significant.

191

## 192 **Results**

### 193 **Study participation**

194 Of the 75 volunteers enrolled onto the study, 68 completed the protocol (Fig 1). One participant failed to  
195 commence the study. Six study participants withdrew from the study for a variety of reasons: one participant had  
196 elevated BP measures during their visit and were advised to attend their Physican; one participant developed  
197 urticaria and was advised by their Physican to withdraw from the study; two participants did not attend visits and  
198 were uncontactable, and the remainder withdrew for personal reasons. There were two potential AEs, although  
199 not confirmed to be associated with the intervention. While 68 participants completed the study, three participants  
200 were subsequently excluded from the analysis: one commenced anti-hypertensive therapy, another commenced a  
201 cholesterol-lowering medication, and a third participant was removed due to poor compliance. This analysis  
202 includes data from 65 participants (37 male, 28 female). Overall, counts of returned sachets indicated a 94.1 (SD  
203 11)% and 93.0 (SD 11.0)% compliance with egg-protein hydrolysate and placebo consumption, respectively.  
204 Specifically, in period 1, counts of returned sachets indicted a 95.1 (SD 8)% and 95.2 (SD 10)% compliance for  
205 egg-protein hydrolysate and placebo consumption, respectively, and in period 2, a 93.2 (SD 12)% and 90.6 (SD  
206 11)% compliance for egg-protein hydrolysate and placebo consumption, respectively.

207

### 208 **Baseline characteristics**

209 Baseline characteristics after the 2-week run-in period are summarised in Table 2. Gender differences for office  
210 systolic BP, cfPWV, body weight, height, fat free mass and waist circumference were observed, where men had  
211 significantly higher values ( $P < 0.05$ ) compared to women. Women had significantly higher ( $P < 0.05$ ) values for  
212 PWA augmentation index (%) and concentrations of HDL-cholesterol compared to men. No significant  
213 differences in baseline values were observed for any variable between the egg-protein hydrolysate and placebo  
214 groups ( $P > 0.05$ ).

215

### 216 **Effect of egg-protein hydrolysate supplementation on BP and arterial stiffness index**

217 Office and central systolic BP and diastolic BP and cfPWV did not significantly change over time (Table 3). PWA  
218 augmentation index (%) increased slightly in the placebo group ( $P = 0.056$ ). No significant differences for mean  
219 changes in office or central BP, cfPWV or PWA were observed between the egg-protein hydrolysate and the  
220 placebo groups accounting for baseline levels of the variable, age, gender and waist circumference. A sub-group  
221 analysis stratified by gender also indicated no differences in the response of men and women to the treatments  
222 (data not shown).

223 Due to the variability in BP over the course of the study, a sub-group analysis stratifying participants  
224 according to their baseline BP values (group 1: normotensive participants with a systolic BP < 140 mm Hg and a  
225 diastolic BP < 90 mm Hg and group 2: hypertensive participants with a systolic BP  $\geq$ 140 mm Hg and /or a diastolic  
226 BP  $\geq$ 90 mm Hg) is presented in Table 4. There was no effect of treatment for systolic BP, PWA or cfPWV in the  
227 normotensive or hypertensive group. In the egg-protein hydrolysate group, hypertensive participants showed a  
228 non-significant reduction in systolic BP ( $\sim$ 2.2 mm Hg;  $P > 0.1$ ); while in normotensive participants, systolic BP  
229 appeared to increase (+3.6 mm Hg,  $P < 0.05$ ).

230

### 231 **Effect of egg-protein hydrolysate supplementation on biomarkers of cardiovascular risk**

232 In the egg-protein hydrolysate and placebo groups, concentrations of fasting glucose, triglycerides and LDL-  
233 cholesterol (all secondary outcomes) did not significantly change over time (Table 3). In the placebo group, but  
234 not in the egg-protein hydrolysate group, concentrations of HDL- ( $P = 0.001$ ) and total-cholesterol ( $P = 0.032$ )  
235 increased significantly over the 6-weeks. No significant differences for the mean changes in concentrations of  
236 fasting glucose, total and LDL-cholesterol and triglycerides between the egg-protein hydrolysate and the placebo  
237 groups were observed adjusting for the baseline value, age, gender and waist circumference.

238

### 239 **Effect of egg-protein hydrolysate supplementation on body weight and waist circumference**

240 A modest increase in body weight and BMI ( $P < 0.05$ ) was observed in the egg-protein hydrolysate group over the  
241 6-week intervention (mean increase in body weight: 0.4 [95% CI: 0.1, 0.7] kg and mean increase in BMI: 0.1  
242 [95%CI: 0.01, 0.2] kg/m<sup>2</sup>). However, these increases were not significantly different ( $P > 0.3$ ) from the smaller  
243 and non-significant increases observed for the placebo group.

244

245

246

247 **Discussion**

248 We investigated for the first time the effects of an egg white, ovalbumin-derived protein hydrolysate on BP,  
249 arterial stiffness and markers of CVD risk in adults with a mildly elevated systolic BP. Our findings indicate that  
250 supplementation with 3g/d of an ovalbumin protein hydrolysate fraction for 6-weeks does not lower BP, improve  
251 arterial stiffness or modify biomarkers of cardiovascular risk. In contrast, to the best of our knowledge, the only  
252 other dietary intervention study in humans investigated the effects of a different egg-white derived peptide from  
253 the protein lysozyme on BP [13]. This dose-finding study demonstrated significant reductions in daytime SBP  
254 and DBP in participants with mild hypertension but no effect on BP was observed in participants with a normal  
255 or high-normal BP [13]. In the current study, participants were not hypertensive with a mean (SD) baseline SBP  
256 of 136 (12) mm Hg and DBP of 89 (6) mm Hg, respectively.

257 Recent systematic reviews and meta-analyses have demonstrated positive effects of bioactive peptides  
258 particularly lactotriptides on BP [7-8; 24-25]. These effects appear dependent on baseline BP [7] and ethnicity,  
259 with Asian subjects demonstrating a greater response for BP when compared to caucasian subjects [7, 26]. In the  
260 current study, the principal screening criterion was a mean office systolic BP 130 – 149 mmHg. While we aimed  
261 to rule-out “white-coat” hypertension by conducting measurements in accordance with best practice guidelines  
262 [21]; readings for systolic BP <130 mmHg were observed at baseline in some participants. The inclusion of  
263 repeated screenings for BP may have reduced the variability for BP in our sample [21] and the likelihood of  
264 including normotensive participants. Trials with dairy peptides reporting reductions in systolic BP have generally  
265 included adults with higher baseline BP values compared to the current study [27 – 30] which may partially  
266 explain the lack of effect observed. Potential acute effects of this ovalbumin-derived protein hydrolysate fraction  
267 on BP were not measured in the current study.

268 A strength of the current study was the measurement of both peripheral (office) and central BP which  
269 was assessed by PWA. Data indicate that aortic or central pressure is more strongly related to future  
270 cardiovascular events than peripheral pressure [31, 32]. We did not observe an effect of this egg protein  
271 hydrolysate fraction on arterial stiffness (PWA augmentation index or PWV). While improvements in PWV  
272 measures after 6 weeks of lactotriptide consumption have been reported [33]; Jauhiainen et al. [34] observed  
273 reductions in PWA augmentation index after lactotriptide consumption in a 12-week trial. This may indicate  
274 that an intervention period greater than 6-weeks is required to observe significant changes in arterial stiffness,  
275 however, considering the crossover nature of the current trial, maintaining participant compliance for longer  
276 periods may have become an issue.

277

278           Animal and *in vitro* evidence have postulated three potential mechanisms by which the ovalbumin –  
279 derived protein hydrolysate fractions may exert their BP lowering effects; angiotensin-converting enzyme (ACE)-  
280 inhibition [9, 10; 19, 35], nitric oxide (NO)-mediated vasodilation [11, 36] and increased antioxidant capacity  
281 [37]. The clinical efficacy of peptides depends on two critical factors: their resistance to degradation by gastro-  
282 intestinal peptidases and their absorption into the blood stream in sufficient quantities [6, 38]. In the current trial,  
283 the bioactivity of this egg ovalbumin-derived protein hydrolysate fraction, in terms of anti-hypertensive activity  
284 was confirmed prior to study commencement. Furthermore, *in vitro* research describing a methodology for  
285 quantifying these egg-protein hydrolysates and the application to quantify the survival of the ACE-inhibitory  
286 peptides in these protein hydrolysate fractions in the gastro-intestinal tract were undertaken as part of this study  
287 [18]. Gastro-intestinal digestion is not always sufficient for optimal release and stability of bioactive fragments  
288 from the original protein structure [38]. In this context, the interference of food matrix constituents with bioactive  
289 peptide release and bioavailability are also important considerations, although this aspect is generally overlooked  
290 when developing peptide-containing hydrolysate based functional foods [18]. We were unable to establish a  
291 reliable biomarker of exposure to assess compliance with study treatments due to the established short half-lives of  
292 these peptides [39]. This is partially attributable to the limited availability of analytical protocols for the  
293 quantification of functional peptides in complex foods and physiologically relevant matrices.

294           This ovalbumin-derived protein hydrolysate fraction did not significantly modify concentrations of  
295 plasma total-, HDL- or LDL-cholesterol, triglycerides or plasma glucose. Animal and *in vitro* data have indicated  
296 potential benefits of soy, lupin and milk peptides for reducing LDL-cholesterol [6]. While studies investigating  
297 the effects of egg-peptides on blood lipids are limited, Manso et al. [37] observed significant reductions in total-  
298 cholesterol and triglyceride concentrations in spontaneously hypertensive rats fed egg-white hydrolysates. While  
299 modest increases in HDL- and total-cholesterol were observed in the placebo group, there were no corresponding  
300 changes in body weight.

301           The current study benefitted from its robust study design (a randomised double-blind, placebo-controlled  
302 crossover trial) that was suitably-powered with a six-week treatment duration; other studies to date have included a  
303 four-week treatment duration [15, 27, 28]. Considering the crossover nature, dropout rates were low and  
304 compliance with the treatments was good. The absence of dietary data, in particular for protein intake, to inform  
305 on background habitual diet is a limitation of the current study. While a supplemental dose of 3g protein per day  
306 was provided as the study treatment to participants, this quantity of protein could be deemed as modest when

307 reflected in the context of total protein intake and should not influence total dietary intake. Findings from a sub-  
308 analysis stratified by gender did not differ from the findings of the whole-group. Similarly, other interventions  
309 with bioactive peptides have reported no difference in treatment responses between men and women [15, 27].

310 The appropriate selection of a food matrix for the delivery of study treatments is crucial. Participants  
311 mixed their study treatments with a fruit juice (predominantly orange juice). Sensory analysis prior to the study  
312 indicated that fruit juice was a suitable matrix for masking the bitter taste associated with the ovalbumin-derived  
313 protein hydrolysate. While a separate assessment of the bioavailability of this protein hydrolysate within a fruit  
314 juice matrix was not conducted; findings from other trials where dairy protein hydrolysates were incorporated  
315 with fruit juices have also indicated a lack of effect on clinical outcomes, particularly for BP [14], which questions  
316 the suitability of fruit juice as a food vehicle for bioactive peptides.

317

### 318 **Concluding remarks**

319 Increasing consumer knowledge of the link between diet and health has raised the demand for food ingredients  
320 with scientifically proven health benefits. A reduction of 3 mm Hg in systolic BP has been estimated to reduce  
321 coronary heart disease by 5-9%, stroke by 8-14%, and all-cause mortality by 4% [40], thus, bioactive peptides  
322 with proven anti-hypertensive activity, consumed as part of a healthy diet, may be of functional interest in both  
323 the treatment and prevention of hypertension. This is the first study to investigate and show that supplementation  
324 with an egg ovalbumin-derived protein hydrolysate fraction for 6-weeks does not lower BP or improve markers  
325 of CVD risk. Well-designed dietary interventions which consider not only the variability in baseline BP values  
326 and ethnicity; but also the suitability of the food matrix and timing of delivery are warranted to further elucidate  
327 the biological functions of egg-derived components.

328

329 *Author contributions: MEK, AJL and PAK designed the study; MEK and AJL monitored the study, CH, EM and*  
330 *AJL conducted study visits, biochemical analysis and data-entry. AJL and MEK analyzed and interpreted the*  
331 *data and wrote the manuscript. PAK modified the writing of the manuscript and read and approved the final*  
332 *manuscript.*

333

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338

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345

346 **References**

- 347 1. Mozaffarian D, Benjamin EJ, Go AS, Arnett DK et al. (2016) Heart Disease and Stroke Statistics—2016  
348 Update. *Rep Am Heart Assoc.* 133: e38-e360.
- 349 2. Landsberg L, Aronne LJ, Beilin LJ, Burke V, et al. (2013) Obesity-Related Hypertension: Pathogenesis,  
350 Cardiovascular Risk, and Treatment: A Position Paper of The Obesity Society and the American Society of  
351 Hypertension. *J Clin Hypertens* 15: 14-33.
- 352 3. Appel LJ, Moore TJ, Obarzanek E, Vollmer WM, et al. (1997) A Clinical Trial of the Effects of Dietary  
353 Patterns on Blood Pressure. *New Engl J Med* 336: 1117-1124.
- 354 4. Saneei P, Salehi-Abargouei A, Esmailzadeh A, Azadbakht L. (2014) Influence of Dietary Approaches to  
355 Stop Hypertension (DASH) diet on blood pressure: A systematic review and meta-analysis on randomized  
356 controlled trials. *Nutr Metab Cardiovas* 24: 1253-1261.
- 357 5. Hernández-Ledesma B, Del Mar Contreras M, Recio, I. (2011) Antihypertensive peptides: Production,  
358 bioavailability and incorporation into foods. *Adv Colloid Interface Sci* 165: 23-35.
- 359 6. Cicero AFG, Fogacci F, Colletti A. (2017) Potential role of bioactive peptides in prevention and treatment of  
360 chronic diseases: a narrative review. *Br J Pharmacol.* 174: 1378-1394.
- 361 7. Cicero AFG, Gerocarni B, Laghi L, Borghi C. (2011) Blood pressure lowering effect of lactotripeptides  
362 assumed as functional foods: A meta-analysis of current available clinical trials. *J Hum Hypertens* 25: 425-  
363 436.
- 364 8. Pripp AH (2008) Effect of peptides derived from food proteins on blood pressure: A meta-analysis of  
365 randomized controlled trials. *Food Nutr Res* 52: 10.3402/fnr.v52i0.1641
- 366 9. Miguel M, Manso MA, Martín-Álvarez PJ, Aleixandre A et al. (2007) Angiotensin-converting enzyme  
367 activity in plasma and tissues of spontaneously hypertensive rats after the short- And long-term intake of  
368 hydrolysed egg white. *Mol Nutr Food Res* 51: 555-563.
- 369 10. Miguel M, López-Fandiño R, Ramos M, Aleixandre A. (2006) Long-term intake of egg white hydrolysate  
370 attenuates the development of hypertension in spontaneously hypertensive rats. *Life Sci* 78: 2960-2966.
- 371 11. Garcia-Redondo AB, Roque FR, Miguel M, López-Fandiño R. et al. (2011) Vascular effects of egg white-  
372 derived peptides in resistance arteries from rats. Structure-activity relationships. *J Sci Food Agr* 90: 1988-  
373 1993.
- 374 12. Miguel M, López-Fandiño R, Ramos M, Aleixandre A. (2005) Short-term effect of egg-white hydrolysate  
375 products on the arterial blood pressure of hypertensive rats. *Br J Nutr* 94: 731-737.

- 376 13. Plat J, Severins N, Morrison S, Mensink RP (2017) Effects of NWT-03, an egg-protein hydrolysate, on blood  
377 pressure in normotensive and mildhypertensive men and women: a dose-finding study. *Br J Nutr* 117: 942-  
378 950.
- 379 14. Cicero AF, Rosticci M, Veronesi M, Bacchelli S, et al., (2010) Hemodynamic effects of lactotriptides from  
380 casein hydrolysate in Mediterranean normotensive subjects and patients with high-normal blood pressure: a  
381 randomized, double-blind, crossover clinical trial. *J Med Food* 13: 1363-1368.
- 382 15. Van Mierlo LA, Koning MMG, Van Zander KD, Draijer R. (2009) Lactotriptides do not lower ambulatory  
383 blood pressure in untreated whites: Results from 2 controlled multicenter crossover studies. *Am J Clin Nutr*  
384 89: 617-623.
- 385 16. Miguel M, Recio I, Gomez-Ruiz JA, Ramos M, Lopez-Fandino R. (2004) Angiotensin I-converting enzyme  
386 inhibitory activity of peptides derived from egg white proteins by enzymatic hydrolysis. *J Food Prot.* 67:  
387 1914-1920.
- 388 17. Davalos A, Miguel M, Bartolome B, Lopez-Fandino R. (2004) Antioxidant activity of peptides derived from  
389 egg white proteins by enzymatic hydrolysis. *J Food Prot* 67:1939-44.
- 390 18. Grootaert CJG, Matthijs B, Pitart J, Baggerman G. et al. (2017) Quantificant of egg ovalbumin hydrolysate-  
391 derived anti-hypertensive peptides in an in vitro model combining luminal digestion with intestinal Caco-2  
392 cell transport. *Food Res Int* 99: 513-541.
- 393 19. Miguel M, Aleixandre A. (2006) Antihypertensive peptides derived from egg proteins. *J Nutr* 136: 1457-  
394 1460.
- 395 20. Miguel M, Manso M, Aleixandre A, Alonso MJ, et al. (2007) Vascular effects, angiotensin I-converting  
396 enzyme (ACE)-inhibitory activity, and antihypertensive properties of peptides derived from egg white. *J Agr*  
397 *Food Chem* 55: 10615-10621.
- 398 21. Mancia G, Fagard R, Narkiewicz K, Redon J, et al. (2014) 2013 ESH/ESC Practice Guidelines for the  
399 Management of Arterial Hypertension. *Blood Pressure* 23: 3-16.
- 400 22. Laurent S, Marais L, Boutouyrie P. (2016) The Noninvasive Assessment of Vascular Aging. *Can J Cardiol*  
401 32: 669-679.
- 402 23. Friedewald WT, Levy RI, Fredrickson DS. (1972) Estimation of the Concentration of Low-Density  
403 Lipoprotein Cholesterol in Plasma, Without Use of the Preparative Ultracentrifuge. *Clin Chem* 18: 499-502.



- 404 24. Cicero AFG, Aubin F, Azais-Braesco V, Borghi C. (2013) Do the Lactotripeptides Isoleucine–Proline–  
405 Proline and Valine–Proline–Proline Reduce Systolic Blood Pressure in European Subjects? A Meta-Analysis  
406 of Randomized Controlled Trials. *Am J Hypertens* 26: 442-449.
- 407 25. Fekete ÁA, Givens DI, Lovegrove JA. (2015) Casein-derived lactotripeptides reduce systolic and diastolic  
408 blood pressure in a meta-analysis of randomised clinical trials. *Nutrients* 7: 659-681.
- 409 26. Fekete ÁA, Givens DI, Lovegrove JA. (2016) Can milk proteins be a useful tool in the management of  
410 cardiometabolic health? An updated review of human intervention trials. *Proc Nutr Soc* 75: 328-341.
- 411 27. Boelsma E, Kloek J. (2010) IPP-rich milk protein hydrolysate lowers blood pressure in subjects with stage 1  
412 hypertension, a randomized controlled trial. *Nutr J*. 9:52.
- 413 28. Cicero AFG, Colletti A, Rosticci M, Cagnati M, et al. (2016) Effect of Lactotripeptides (Isoleucine-Proline-  
414 Proline/Valine-Proline-Proline) on Blood Pressure and Arterial Stiffness Changes in Subjects with  
415 Suboptimal Blood Pressure Control and Metabolic Syndrome: A Double-Blind, Randomized, Crossover  
416 Clinical Trial. *Metab Syndr Rel Disord* 14: 161-166.
- 417 29. Seppo L, Jauhiainen T, Poussa T, Korpela R. (2003) A fermented milk high in bioactive peptides has a blood  
418 pressure-lowering effect in hypertensive subjects. *Am J Clin Nutr* 77: 326-330.
- 419 30. Turpeinen AM, Ehlers PI, Kivimäki AS, Järvenpää S. et al. (2011) Ile-Pro-Pro and Val-Pro-Pro tripeptide-  
420 containing milk product has acute blood pressure lowering effects in mildly hypertensive subjects. *Clin Exp*  
421 *Hypertens* 33: 388-396.
- 422 31. McEniery CM, Cockcroft JR, Roman MJ, Franklin SS, et al. (2014) Central blood pressure: current evidence  
423 and clinical importance. *Eur Heart J* 35: 1719-1725.
- 424 32. Roman MJ, Devereux RB, Kizer JR, Okin PM, et al. (2009) High Central Pulse Pressure Is Independently  
425 Associated With Adverse Cardiovascular Outcome: The Strong Heart Study. *J Am Coll Cardiol* 54: 1730-  
426 1734.
- 427 33. Cicero AFG, Rosticci M, Gerocarni B, Bacchelli S, et al. (2011) Lactotripeptides effect on office and 24-h  
428 ambulatory blood pressure, blood pressure stress response, pulse wave velocity and cardiac output in patients  
429 with high-normal blood pressure or first-degree hypertension: a randomized double-blind clinical trial.  
430 *Hypertens Res.* 34: 1035-1040.
- 431 34. Jauhiainen T, Ronnback M, Vapaatalo H, Wuolle K, et al. (2010) Long-term intervention with *Lactobacillus*  
432 *helveticus* fermented milk reduces augmentation index in hypertensive subjects. *Eur J Clin Nutr* 64: 424-431.

- 433 35. Miguel M, Alonso MJ, Salaices M, Aleixandre A, et al. (2007) Antihypertensive, ACE-inhibitory and  
434 vasodilator properties of an egg white hydrolysate: Effect of a simulated intestinal digestion. *Food Chem* 104:  
435 163-168.
- 436 36. Miguel M, Alvarez Y, López-Fandiño R, Alonso MJ, et al. (2007) Vasodilator effects of peptides derived  
437 from egg white proteins. *Regul Peptides* 140: 131-135.
- 438 37. Manso MA, Miguel M, Even J, Hernández R, et al. (2008) Effect of the long-term intake of an egg white  
439 hydrolysate on the oxidative status and blood lipid profile of spontaneously hypertensive rats. *Food Chem*  
440 109: 361-367.
- 441 38. Liu YF, Oey I, Bremer P, Carne A, Silcock P. (2017) Bioactive peptides derived from egg proteins: A review.  
442 *Crit Rev Food Sci* 13:1-23.
- 443 39. Sato K, Urado D. (2015) in *Detection and Identification of Food-Derived Peptides in Human Blood: Food-*  
444 *Derived Short Chain Peptidomes in Human Blood, in Genomics, Proteomics and Metabolomics in*  
445 *Nutraceuticals and Functional Foods: Second Edition*, pp. 441-452.
- 446 40. Rosendorff C, Black CP, Cannon BJ, Gersh, et al. (2007) Treatment of hypertension in the prevention and  
447 management of ischemic heart disease: A scientific statement from the American Heart Association council  
448 for high blood pressure research and the councils on clinical cardiology and epidemiology and prevention.  
449 *Circulation* 115: 2761-2788.
- 450

**Table 1:** Key peptides present in egg ovalbumin-derived protein hydrolysate fraction.

<b>Peptides present</b>	<b>Antihypertensive activity</b>	<b>Antioxidant activity (by ORAC-Fl assay)</b>	<b>References</b>
Whole hydrolysate (Egg ovalbumin-derived peptide digestate fraction used in study)	ACE-inhibitory activity	<i>In vitro</i> radical scavenging	In house data produced by BioActor
FRADHPFL <sup>a</sup>	ACE-inhibitory activity	<i>In vitro</i> radical scavenging	Miguel et al. [16, 35] Dávalos et al. [17]
RADHPFL <sup>a</sup>	ACE-inhibitory activity	<i>In vitro</i> radical scavenging	Miguel et al. [16, 35] Dávalos et al. [17] Grootaert et al. [18]
YAEERYPIL <sup>a</sup>	ACE-inhibitory activity	<i>In vitro</i> radical scavenging	Miguel et al. [16, 35] Dávalos et al. [17] Grootaert et al. [18]

<sup>a</sup> Bioactivity of peptides was determined based on previously published studies

**Table 2.** Participant characteristics at the baseline visit during period 1

	Total (n 65)	Men (n 37 )	Women (n 28)	P <sup>a</sup>
Age (y)	56.9 ± 5.2	57.1 ± 5.4	56.6 ± 4.4	0.734
Systolic BP (mmHg) ( <i>Office</i> )	136.3 ± 12.1	140.1 ± 11.9	131.2 ± 10.6	0.003
Diastolic BP (mmHg) ( <i>Office</i> )	88.9 ± 6.4	89.3 ± 6.0	88.3 ± 6.9	0.562
Systolic BP (mmHg) ( <i>Central</i> )	135.1 ± 11.9	135.1 ± 12.0	134.9 ± 11.9	0.953
Diastolic BP (mmHg) ( <i>Central</i> )	75.5 ± 6.4	76.3 ± 6.1	74.3 ± 6.6	0.215
PWV (m/s)	9.1 ± 1.3	9.4 ± 1.4	8.7 ± 1.2	0.035
PWA (AI %)	27.6 ± 6.2	25.2 ± 5.5	30.7 ± 5.7	<0.001
Height (m)	1.7 ± 0.1	1.7 ± 0.1	1.6 ± 0.1	<0.001
Body weight (kg)	81.1 ± 13.0	86.6 ± 12.3	73.8 ± 10.0	<0.001
BMI (kg/m <sup>2</sup> )	28.3 ± 3.5	28.2 ± 3.3	28.3 ± 3.8	0.930
Body fat (%) <sup>b</sup>	34.5 ± 7.8	29.5 ± 5.6	40.9 ± 5.1	<0.001
Fat free mass (kg) <sup>b</sup>	51.0 ± 10.1	58.3 ± 6.6	41.6 ± 4.2	<0.001
Waist circumference (cm)	94.0 ± 10.2	97.3 ± 9.6	89.7 ± 9.4	0.002
Total cholesterol (mmol/L)	5.57 ± 0.9	5.48 ± 0.9	5.69 ± 0.9	0.408
HDL- cholesterol (mmol/L)	1.47 ± 0.6	1.33 ± 0.6	1.67 ± 0.7	0.035
LDL-cholesterol (mmol/L)	3.58 ± 0.9	3.57 ± 0.9	3.59 ± 0.9	0.919
Triglycerides (mmol/L)	1.29 ± 1.5	1.56 ± 1.9	0.95 ± 0.4	0.119
Glucose (mmol/L)	5.49 ± 0.6	5.59 ± 0.6	5.35 ± 0.7	0.155

Data are presented as Mean ± SD (all such values)

BP, blood pressure; PWV, pulse wave velocity; PWA, pulse wave analysis; AI, augmentation index

<sup>a</sup> Independent student's t test

<sup>b</sup> Measured by DXA scan during one study visit

**Table 3.** Measures of blood pressure and cardiovascular risk stratified by treatment allocated in 65 men and women

	Ovalbumin egg-protein hydrolysate ( <i>n</i> 65)				Placebo ( <i>n</i> 65)					
	Baseline	Endpoint	Mean change <sup>a</sup> (95% CI)	<i>P</i> <sup>b</sup>	Baseline	Endpoint	Mean change <sup>a</sup> (95% CI)	<i>P</i> <sup>b</sup>	<i>P</i> <sup>c</sup>	<i>P</i> <sup>d</sup>
Systolic BP* (mmHg) ( <i>Office</i> )	132.5 ± 12.6	132.8 ± 12.1	-0.2 (-2.8, 2.3)	0.867	134.4 ± 10.7	131.6 ± 11.5	-1.3 (-3.9, 1.4)	0.344	0.472	0.471
Diastolic BP* (mmHg) ( <i>Office</i> )	87.7 ± 7.0	88.3 ± 7.9	0.6 (-0.9, 2.0)	0.447	88.1 ± 8.0	87.0 ± 8.1	-0.5 (-2.2, 1.1)	0.528	0.328	0.309
Systolic BP*(mmHg) ( <i>Central</i> )	135.5 ± 12.3	134.8 ± 10.6	-0.8 (-3.7, 2.1)	0.478	133.8 ± 10.6	132.7 ± 10.7	-0.5 (-3.3, 2.2)	0.679	0.820	0.660
Diastolic BP* (mmHg) ( <i>Central</i> )	75.9 ± 6.3	75.9 ± 6.2	-0.4 (-1.9, 1.1)	0.885	76.1 ± 7.0	75.4 ± 8.6	-0.6 (-2.5, 1.2)	0.446	0.933	0.857
PWV (m/s)	9.1 ± 1.5	9.2 ± 1.3	0.04 (-0.3, 0.4)	0.570	9.3 ± 1.2	9.3 ± 1.4	0.06 (-0.3, 0.4)	0.522	0.888	0.968
PWA (AI %)	27.8 ± 6.7	27.8 ± 7.8	-0.02 (-1.6, 1.5)	0.955	26.8 ± 6.3	28.9 ± 8.5	1.8 (0.01, 3.6)	0.056	0.142	0.093
Total cholesterol (mmol/L)	5.67 ± 1.1	5.69 ± 1.0	0.03 (-0.2, 0.2)	0.747	5.65 ± 1.0	5.91 ± 1.1	0.3 (0.02, 0.5)	0.032	0.146	0.147
HDL-cholesterol (mmol/L)	1.54 ± 0.6	1.56 ± 0.6	0.02 (-0.1, 0.1)	0.703	1.48 ± 0.6	1.66 ± 0.6	0.2 (0.1, 0.2)	0.001	0.077	0.076
LDL-cholesterol (mmol/L)	3.65 ± 0.9	3.58 ± 0.9	-0.1 (-0.3, 0.1)	0.364	3.62 ± 0.9	3.71 ± 0.9	0.1 (-0.1, 0.3)	0.361	0.281	0.288
Triglycerides (mmol/L)	1.10 ± 0.6	1.19 ± 0.6	-0.02 (-0.3, 0.2)	0.841	1.21 ± 0.6	1.18 ± 0.5	-0.03 (-0.2, 0.1)	0.712	0.982	0.955
Glucose (mmol/L)	5.55 ± 0.8	5.61 ± 0.8	0.04 (-0.2, 0.2)	0.710	5.67 ± 0.8	5.62 ± 0.9	-0.04 (-0.3, 0.2)	0.766	0.642	0.533

Mean ± SD (all such values); \*Primary outcome BP: BP, blood pressure; PWV, pulse wave velocity; PWA, pulse wave analysis; AI, augmentation index

<sup>a</sup> Mean change (Post-intervention – baseline)

<sup>b</sup> *P* for Intra (within) -group comparison (Paired student's *t* test)

<sup>c</sup> *P* for Inter (between) -group comparison (Independent student's *t* test)

<sup>d</sup> *P* for Inter-group comparison (GLM ANCOVA model adjusted for baseline value for the dependent variable, gender, age and waist circumference)

**Table 4.** Measures of blood pressure, pulse wave velocity and pulse wave analysis stratified by treatment allocated and the presence or absence of hypertension

	Ovalbumin egg-protein hydrolysate				Placebo					
	Baseline	Endpoint	Mean change <sup>a</sup> (95% CI)	<i>P</i> <sup>b</sup>	Baseline	Endpoint	Mean change <sup>a</sup> (95% CI)	<i>P</i> <sup>b</sup>	<i>P</i> <sup>c</sup>	<i>P</i> <sup>d</sup>
<i>Normotensive<sup>e</sup></i>	<i>n</i> 15				<i>n</i> 13					
SBP (mmHg) ( <i>Office</i> )	122.5 ± 5.5	125.5 ± 8.4	3.6 (0.2, 7.0)	0.041	125.7 ± 5.8	125.1 ± 8.9	-1.7 (-6.9, 3.5)	0.505	0.191	0.164
DBP (mmHg) ( <i>Office</i> )	83.4 ± 4.0	84.9 ± 5.8	1.7 (-0.5, 4.0)	0.126	81.9 ± 5.5	83.2 ± 5.8	0.5 (-2.0, 3.1)	0.672	0.487	0.436
SBP (mmHg) ( <i>Central</i> )	130.7 ± 12.5	132.8 ± 12.0	2.0 (-2.9, 7.1)	0.558	127.3 ± 10.2	127.9 ± 8.3	0.6 (-3.8, 4.9)	0.641	0.481	0.377
DBP (mmHg) ( <i>Central</i> )	72.0 ± 5.7	72.7 ± 3.7	0.6 (-1.5, 2.7)	0.262	73.1 ± 5.2	71.5 ± 9.1	-1.5 (-6.2, 3.1)	0.502	0.059	0.055
PWV (m/s)	9.2 ± 1.2	9.0 ± 1.4	-0.2 (-0.6, 0.3)	0.716	8.9 ± 0.9	9.1 ± 1.5	0.2 (-0.5, 1.0)	0.430	0.504	0.409
PWA (AI %)	28.7 ± 4.4	27.6 ± 5.7	-1.1 (-3.6, 1.3)	0.255	29.6 ± 3.9	30.7 ± 7.3	1.1 (-2.1, 4.3)	0.386	0.242	0.201
<i>Hypertensive<sup>f</sup></i>	<i>n</i> 18				<i>n</i> 19					
SBP (mmHg) ( <i>Office</i> )	137.5 ± 11.7	136.5 ± 11.6	-2.2 (-5.6, 1.2)	0.201	138.5 ± 9.9	135.3 ± 11.1	-1.0 (-4.2, 2.1)	0.508	0.829	0.828
DBP (mmHg) ( <i>Office</i> )	89.6 ± 7.2	89.8 ± 8.1	-0.3 (-2.0, 1.9)	0.971	90.9 ± 7.1	88.6 ± 8.4	-1.1 (-3.3, 1.1)	0.333	0.483	0.475
SBP (mmHg) ( <i>Central</i> )	138.1 ± 11.0	136.2 ± 10.1	-2.1 (-5.8, 1.6)	0.214	137.2 ± 8.8	135.6 ± 10.6	-1.0 (-4.6, 2.5)	0.468	0.781	0.895
DBP (mmHg) ( <i>Central</i> )	77.9 ± 5.5	77.0 ± 6.8	-0.9 (-3.0, 1.1)	0.460	77.3 ± 7.2	77.2 ± 7.4	-0.2 (-2.1, 1.7)	0.711	0.099	0.126
PWV (m/s)	9.0 ± 1.6	9.2 ± 1.2	0.1 (-0.3, 0.6)	0.412	9.4 ± 1.3	9.4 ± 1.2	-0.1 (-0.5, 0.4)	0.864	0.613	0.471
PWA (AI %)	27.3 ± 7.4	27.9 ± 8.4	0.5 (-1.5, 2.5)	0.504	25.5 ± 6.7	27.8 ± 8.9	2.2 (-0.1, 4.4)	0.092	0.328	0.241

Mean ± SD (all such values). SBP, systolic blood pressure; DBP, diastolic blood pressure; PWV, pulse wave velocity; PWA, pulse wave analysis; AI, augmentation index

<sup>a</sup> Mean change (Post-intervention – baseline)

<sup>b</sup> *P* for Intra (within) -group comparison (Paired student's t test)

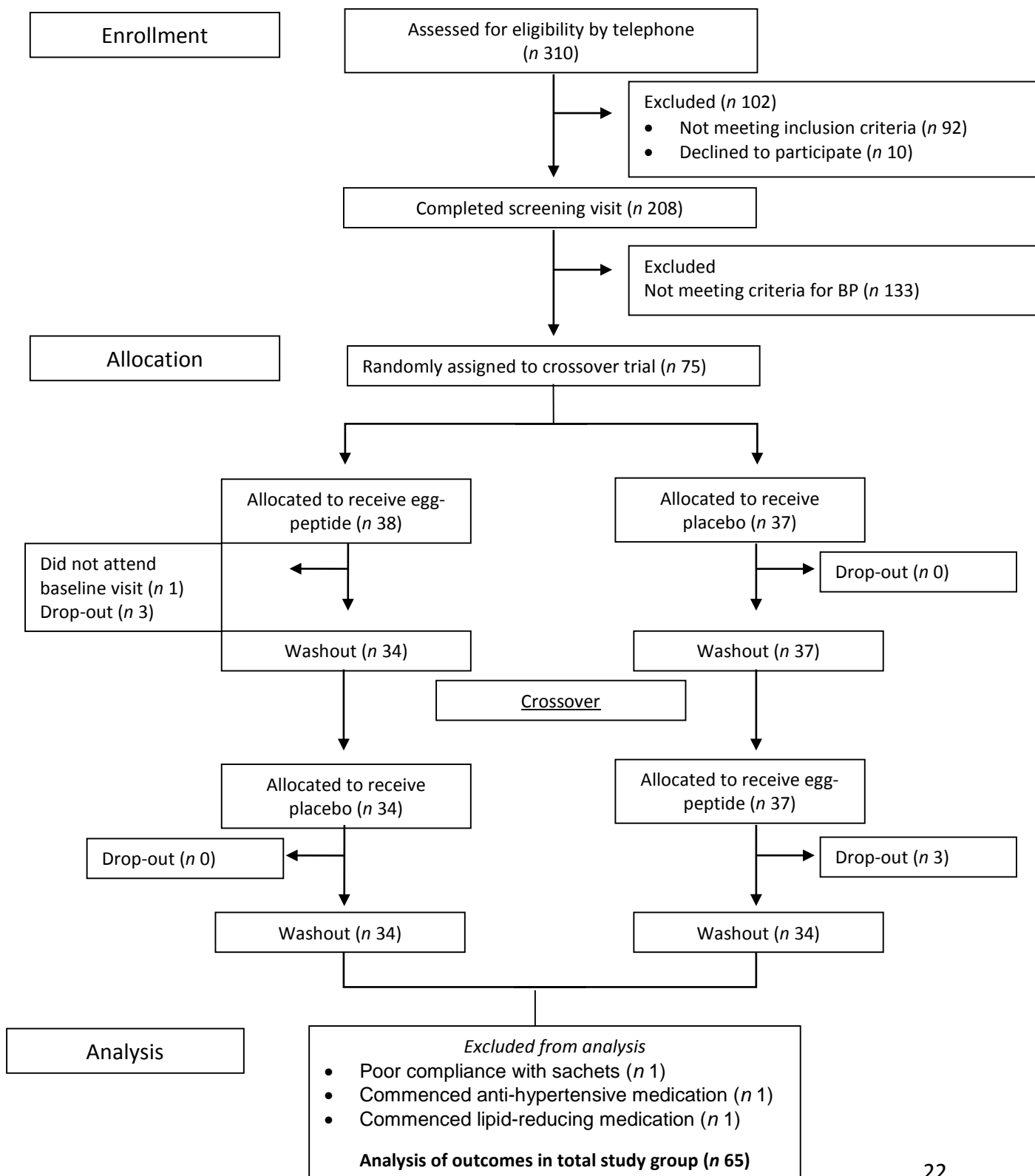
<sup>c</sup> *P* for Inter (between) -group comparison (Independent student's t test)

<sup>d</sup> *P* for Inter-group comparison (Univariate ANCOVA model adjusted for baseline value for the dependent variable, gender, age and waist circumference)

<sup>e</sup> SBP ≤ 140mm Hg and/or DBP ≤ 90 mmHg (17)

<sup>f</sup> SBP > 140mm Hg and/or DBP > 90 mmHg (17)

**Figure 1** CONSORT Flow diagram of participants



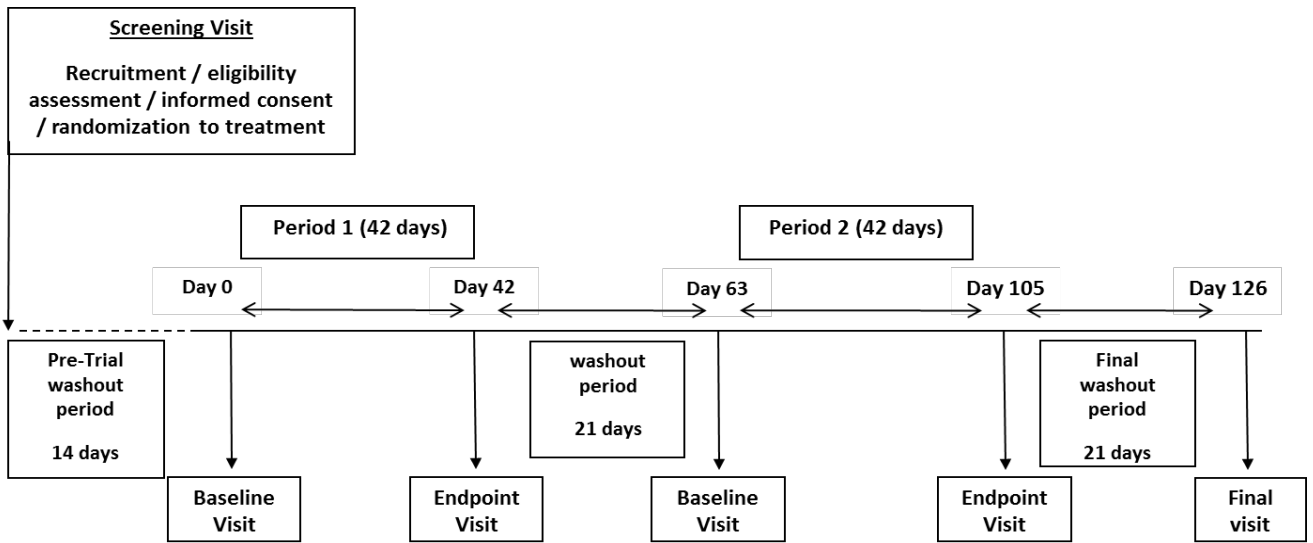
### Online Resources (Supplemental Information)

**Manuscript Title:** Effect of an egg ovalbumin-derived protein hydrolysate on blood pressure and cardiovascular risk in adults with a mildly elevated blood pressure: a randomized placebo-controlled crossover trial

**Online Resource 1** The nutrient composition of study treatments (one sachet = 3g).

Nutritional information per 3g sachet	Ovalbumin-derived peptide digestate fraction	Placebo (Maltodextrin)
Energy (kJ)	47.5	52.7
Protein (g)	2.8	<1.0
Total carbohydrate (g)	<1.0	3.0
Fat (g)	<1.0	<1





**Online Resource 2** Flow diagram of study visits

For review purposes: Online Resource 3 reports intention-to-treat analysis

Online Resource 3 Measures of blood pressure and cardiovascular risk stratified by treatment allocated

	Ovalbumin egg-peptide ( <i>n</i> 74)				Placebo ( <i>n</i> 74)					
	Baseline	Endpoint	Mean change <sup>a</sup> (95% CI)	<i>P</i> <sup>b</sup>	Baseline	Endpoint	Mean change <sup>a</sup> (95% CI)	<i>P</i> <sup>b</sup>	<i>P</i> <sup>c</sup>	<i>P</i> <sup>d</sup>
SBP (mmHg) ( <i>Office</i> )	132.8 ±12.8	133.5 ±12.3	-0.6 (-3.4, 2.2)	0.654	134.9 ± 10.9	132.4 ± 11.9	-0.8 (-3.4, 1.8)	0.436	0.743	0.471
DBP (mmHg) ( <i>Office</i> )	88.1 ± 7.2	88.8 ± 8.2	0.3 (-1.3, 1.9)	0.708	88.0 ± 7.9	87.2 ± 8.1	-0.2 (-1.9, 1.4)	0.760	0.631	0.309
SBP (mmHg) ( <i>Central</i> )	135.9 ±12.2	135.7 ±11.3	-0.4 (-3.2, 2.5)	0.666	134.3 ± 10.7	133.7 ± 11.7	-0.1 (-2.8, 2.6)	0.690	0.556	0.660
DBP (mmHg) ( <i>Central</i> )	76.3 ± 6.5	76.1 ± 6.2	-0.6 (-2.1, 0.9)	0.929	75.7 ± 7.5	75.4 ± 8.6	-0.2 (-2.1, 1.7)	0.730	0.441	0.857
PWV (m/s)	9.1 ± 1.5	9.2 ± 1.4	0.06 (-0.3, 0.4)	0.473	9.3 ± 1.2	9.2 ± 1.3	0.06 (-0.3, 0.4)	0.341	0.797	0.968
PWA (AI %)	27.9 ± 6.9	27.6 ± 7.7	-0.2 (-1.8, 1.5)	0.909	26.9 ± 6.2	28.8 ± 8.4	1.6 (-0.2, 3.3)	0.083	0.159	0.093
Total-Cholesterol (mmol/L)	5.68 ± 1.1	5.75 ± 1.1	0.03 (-0.2, 0.2)	0.790	5.67 ± 1.0	5.91 ± 1.1	0.2 (-0.01, 0.5)	0.048	0.146	0.147
HDL-Cholesterol (mmol/L)	1.52 ± 0.6	1.56 ± 0.6	0.02 (-0.1, 0.1)	0.707	1.48 ± 0.7	1.64 ± 0.6	0.2 (0.1, 0.2)	0.001	0.077	0.076
LDL-Cholesterol (mmol/L)	3.66 ± 0.9	3.61 ± 0.9	-0.1 (-0.3, 0.1)	0.413	3.65 ± 0.9	3.72 ± 0.9	0.1 (-0.1, 0.3)	0.495	0.281	0.288
Triglycerides (mmol/L)	1.32 ± 1.3	1.22 ± 0.7	-0.1 (-0.3, 0.2)	0.513	1.36 ± 1.5	1.33 ± 1.2	-0.03 (-0.2, 0.1)	0.690	0.982	0.955
Glucose (mmol/L)	5.62 ± 0.9	5.62 ± 0.8	0.04 (-0.2, 0.2)	0.638	5.68 ± 0.8	5.69 ± 0.9	-0.03 (-0.3, 0.2)	0.939	0.642	0.533

Mean ± SD (all such values); BP, blood pressure; PWV, pulse wave velocity; PWA, pulse wave analysis; AI, augmentation index

<sup>a</sup> Mean change (Post-intervention – baseline)

<sup>b</sup> *P* for Intra (within) -group comparison (Paired student's t test)

<sup>c</sup> *P* for Inter (between) -group comparison (Independent student's t test)

<sup>d</sup> *P* for Inter-group comparison (GLM ANCOVA model adjusted for baseline value for the dependent variable, gender, age and waist circumference)

