Research Article

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

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

Abstract

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

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

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

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

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

Introduction

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

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

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

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

Materials and Methods

Study population

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

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

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

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

Study design

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

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

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

Study treatments

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

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

Study visits

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

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

BP

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

Central BP and assessment of arterial stiffness index

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

Anthropometry

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

Blood sampling

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

158 processed and immediately stored at -80°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,

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

Results

Study participation

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

Baseline characteristics

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

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

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

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

Effect of egg-protein hydrolysate supplementation on biomarkers of cardiovascular risk

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

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

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

Discussion

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

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

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

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

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

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

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

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

Concluding remarks

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

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

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

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

[a] 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* [a]
Age (y)	56.9 ± 5.2	57.1 ± 5.4	56.6 ± 4.4	0.734
Systolic BP (mmHg) *(Office)*	136.3 ± 12.1	140.1 ± 11.9	131.2 ± 10.6	0.003
Diastolic BP (mmHg) *(Office)*	88.9 ± 6.4	89.3 ± 6.0	88.3 ± 6.9	0.562
Systolic BP (mmHg) *(Central)*	135.1 ± 11.9	135.1 ± 12.0	134.9 ± 11.9	0.953
Diastolic BP (mmHg) *(Central)*	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^2)	28.3 ± 3.5	28.2 ± 3.3	28.3 ± 3.8	0.930
Body fat (%)[b]	34.5 ± 7.8	29.5 ± 5.6	40.9 ± 5.1	<0.001
Fat free mass (kg)[b]	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
[a] Independent student's t test
[b] 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 (*n* 65)				Placebo (*n* 65)					
	Baseline	Endpoint	Mean change[a] (95% CI)	*P*[b]	Baseline	Endpoint	Mean change[a] (95% CI)	*P*[b]	*P*[c]	*P*[d]
Systolic BP[*] (mmHg) *(Office)*	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) *(Office)*	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) *(Central)*	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) *(Central)*	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

[a] Mean change (Post-intervention – baseline)

[b] *P* for Intra (within) -group comparison (Paired student's t test)

[c] *P* for Inter (between) -group comparison (Independent student's t test)

[d] *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[a] (95% CI)	*P*[b]	Baseline	Endpoint	Mean change[a] (95% CI)	*P*[b]	*P*[c]	*P*[d]
Normotensive[e]		*n* 15				*n* 13				
SBP (mmHg) *(Office)*	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) *(Office)*	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) *(Central)*	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) *(Central)*	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
Hypertensive[f]		*n* 18				*n* 19				
SBP (mmHg) *(Office)*	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) *(Office)*	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) *(Central)*	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) *(Central)*	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

[a] Mean change (Post-intervention – baseline)

[b] *P* for Intra (within) -group comparison (Paired student's t test)

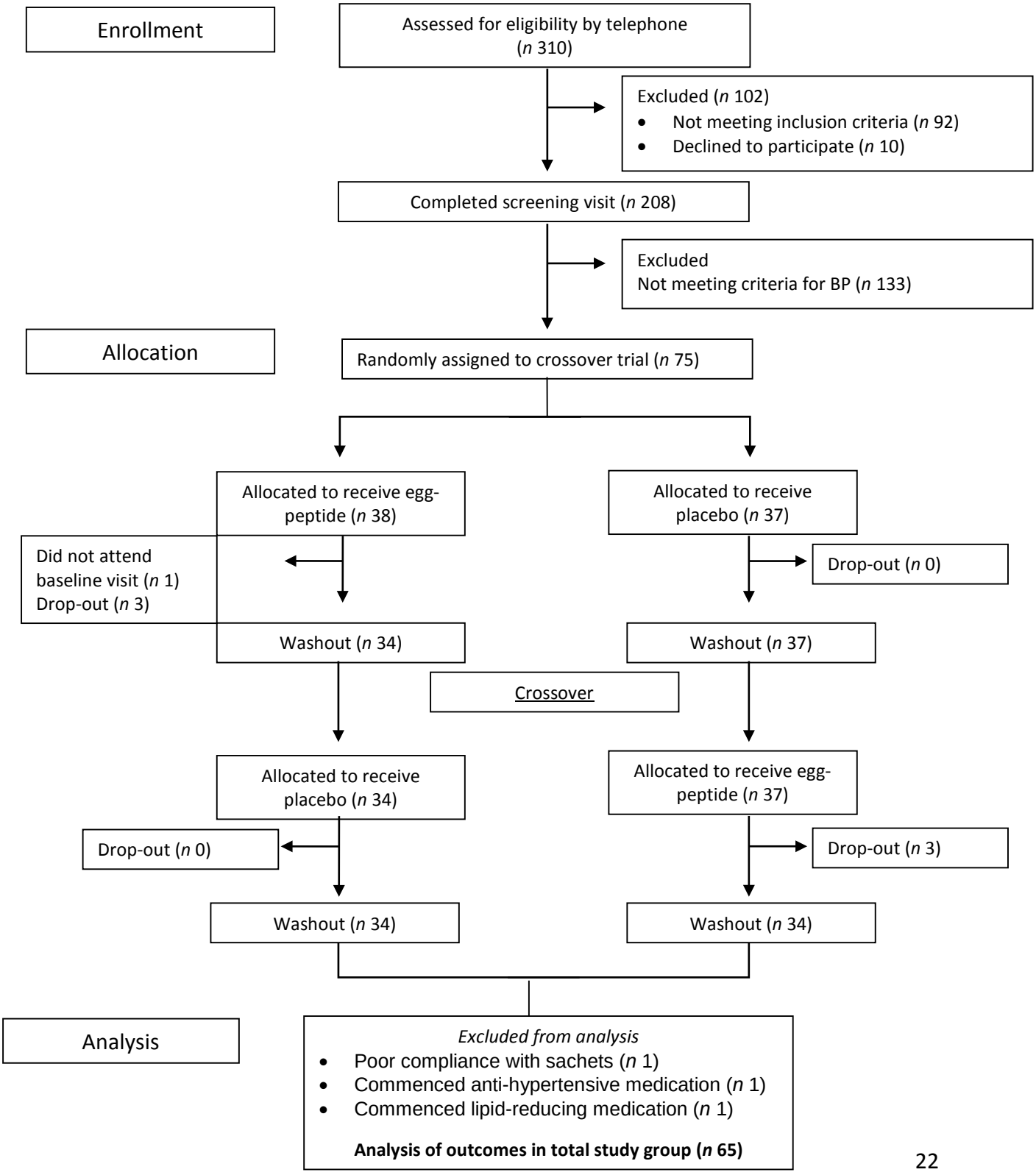
[c] *P* for Inter (between) -group comparison (Independent student's t test)

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

[e] SBP ≤ 140mm Hg and/or DBP ≤ 90 mmHg (17)

[f] SBP > 140mm Hg and/or DBP > 90 mmHg (17)

Figure 1 CONSORT Flow diagram of participants

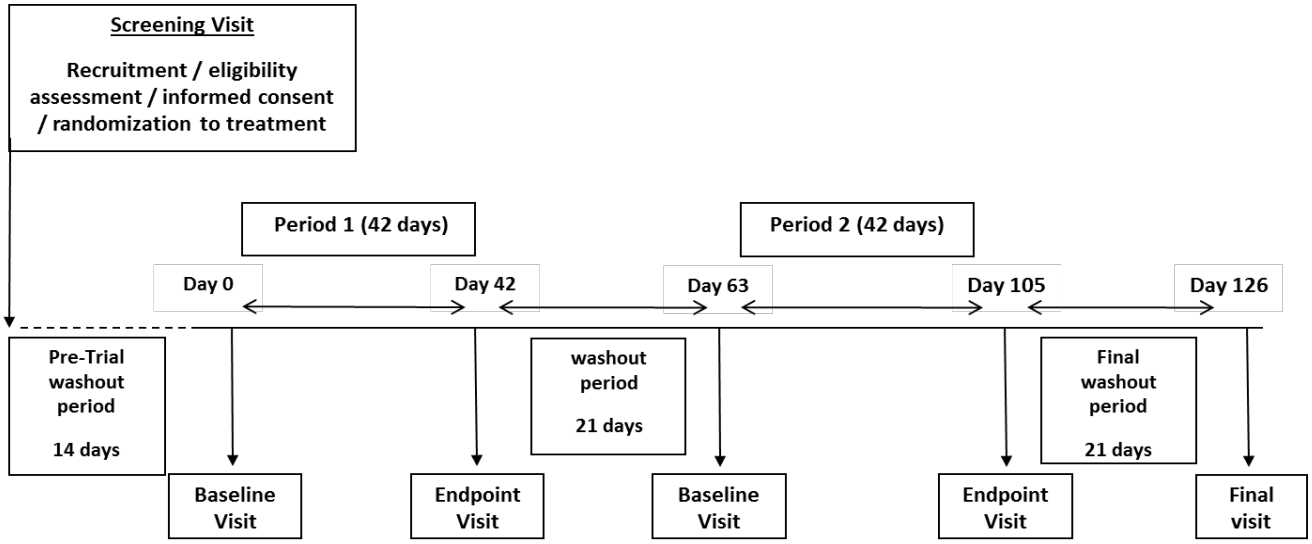


Online Resources (Supplemental Information)

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 (*n* 74)				Placebo (*n* 74)					
	Baseline	Endpoint	Mean change[a] (95% CI)	P^b	Baseline	Endpoint	Mean change[a] (95% CI)	P^b	P^c	P^d
SBP (mmHg) *(Office)*	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) *(Office)*	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) *(Central)*	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) *(Central)*	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

[a] Mean change (Post-intervention – baseline)

[b] *P* for Intra (within) -group comparison (Paired student's t test)

[c] *P* for Inter (between) -group comparison (Independent student's t test)

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