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Effects of porcine-derived collagen hydrolysates on 24 h blood pressure profiles, markers for endothelial dysfunction and low-grade inflammation and the retinal vasculature in adults with overweight/obesity: a randomized, controlled trial

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In vitro and animal studies have shown promising effects of collagen hydrolysate on blood pressure (BP), serum lipid profiles, and plasma concentrations of endothelial and inflammatory markers. Therefore, we evaluated in humans the effects of a porcine-derived collagen hydrolysates on office and ambulatory blood pressure (ABP) profiles, retinal microvascular calibers, serum lipids, markers for endothelial dysfunction and low-grade inflammation. Given a possible link between peripheral vascular and brain vascular function, effects on cognitive performance were also explored. Therefore, in this randomized, placebo-controlled parallel trial, 56 middle-aged and older adults with overweight/obesity consumed 10 g porcine-derived collagen hydrolysates or placebo (erythritol) daily for four weeks after a 2-week run-in period. Measurements were performed at the end of the run-in and intervention periods. Collagen hydrolysates consumption did not significantly affect office systolic BP (SBP) (−1 mmHg, 95% CI: −3, 2; p = 0.529), or diastolic BP (DBP) (−1 mmHg, 95% CI: −2, 1; p = 0.449), nor 24-hour SBP (0 mmHg, 95% CI: −4, 4; p = 0.884) or DBP (−2 mmHg, 95% CI: −5, 1; p = 0.195). No significant changes were observed in mean arterial pressure, pulse pressure, nocturnal BP dipping, retinal microvascular calibers, serum lipids, and markers for endothelial dysfunction and low-grade inflammation. Cognitive performance remained unaffected, except for an unexpected increase in movement reaction time (29 ms, 95% CI: 553; p = 0.019). In conclusion, four-week porcine-derived collagen hydrolysate supplementation did not improve cardiometabolic risk markers and cognitive performance in middle-aged and older adults with overweight or obesity. This clinical trial was registered in November 2021 at ClinicalTrials.gov as NCT05282641.

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Introduction

Cardiovascular disease (CVD) remains the global leading cause of death, accounting for approximately 20.5 million deaths globally in 2021.[1] Major risk factors contributing to CVD risk include hypertension, dyslipidemia, type 2 diabetes, and being overweight or obese.[2,3] These risk factors are associated with endothelial dysfunction and chronic low-grade inflammation, which have been identified as predictors of future cardiovascular events.[4,5] Preventive strategies to target these risk factors are therefore crucial to reduce the incidence of CVD.

Recently, bioactive peptides derived from protein hydrolysates have received increasing attention to mitigate cardiovascular risk factors.[6–10] Amongst others, collagen hydrolysates have demonstrated promising results.[11] Collagen hydrolysates derived from marine and animal sources, including porcine, may be antihypertensive, possibly due to inhibiting angiotensin I-converting enzyme (ACE). ACE is an important regulator in the renin–angiotensin system, converting angiotensin into the active vasoconstrictor angiotensin 2 that increases the vascular tone and consequently blood pressure (BP).[12] *In vitro* studies and research with hypertensive rats have identified collagen hydrolysate peptides from bovine,[13] goat,[14] tilapia skin,[15] jellyfish,[16] chicken,[17] or porcine[18] as potential ACE inhibitors. However, human studies evaluating the effects of collagen hydrolysates on BP have yielded mixed results, with some reporting improvements,[19,20] while others have found no significant effects.[21,22]

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Besides BP, animal studies have also shown that collagen hydrolysates may lower serum total and low-density lipoprotein cholesterol (LDL-C) concentrations,[23,24] possibly by modulating the expression of proteins involved in fatty acid and cholesterol synthesis, and β-oxidation,[25] which could further reduce the risk of developing atherosclerosis.[26] Again, human studies are conflicting with some studies reporting reductions in total cholesterol, LDL-C, and triacylglycerol (TAG),[20] along with increases in high-density lipoprotein cholesterol (HDL-C),[20,27] while others showed no effects.[19,28,29] Finally, studies using cultured human endothelial cells or apolipoprotein E-deficient ($ApoE^{-/-}$) mice suggested that collagen hydrolysates reduced concentrations of markers reflecting low-grade inflammation or endothelial dysfunction such us interleukin-6 (IL-6), soluble intercellular adhesion molecule-1 (sICAM-1), and tumor necrosis factor-alpha (TNF-α).[30,31] However, the limited number of studies in humans did not show any significant effects on inflammatory markers,[32,33] while markers of endothelial dysfunction have not been evaluated so far. Changes in retinal arteriolar and venular calibers may indicate functional alterations in the retinal microvasculature that are associated with the risk of developing stroke and coronary heart diseases, even after adjusting for other well-established risk factors.[34] Finally, improvements in cardiovascular health are associated with better cognitive performance.[35] For instance, individuals with prediabetes, who have impaired endothelial function and consequently an increased CVD risk,[36] exhibit reduced cognitive function compared to normoglycemic individuals.[37] Specifically, impairments have been observed in the domains of attention and psychomotor speed, executive function, and memory.[38] Peripheral endothelial dysfunction has therefore been suggested as a potential mechanistic link between insulin resistance and cognitive decline.[39] Thus, improvements in CVD risk associated with collagen hydrolysate consumption may also lead to cognitive benefits.

Therefore, we here evaluated the effects of 4 weeks of daily consumption of 10 g of porcine-derived collagen hydrolysates on CVD risk markers, such as office and 24-hour BP profiles, retinal microvascular calibers, serum lipids and lipoproteins, and markers for endothelial dysfunction and chronic low-grade inflammation, as well as cognitive performance in men and women with overweight or obesity.

Methods

Subjects

Apparently healthy men and women with overweight or obesity (BMI 25–35 kg m^{-2}) and between 40–75 years old were recruited *via* posters on websites/social media or advertisements in local newspapers. Those who were interested were pre-screened *via* email or phone. Potential eligible participants were invited for two screening visits at the Metabolic Research Unit Maastricht (MRUM). For each visit, participants were instructed to fast overnight for 12 hours, to come by car or public transport, and to avoid vigorous physical activity and alcohol consumption for 48 and 24 hours, respectively. After explaining the study and discussing remaining questions, written informed consent was obtained before screening. Additionally, a medical questionnaire as well as the use of medication and supplements were documented. During screening, office BP, anthropometric measurements, and fasting blood samples were obtained as described in detail before.[40] Participants were eligible after meeting the inclusion criteria: serum total cholesterol < 8.0 mmol L^{-1}, triacylglycerol < 4.52 mmol L^{-1}, nonsmoker or smoking cessation > 1 year; no diabetes; no familial hypercholesterolemia; no abuse of drugs; no > 4 alcoholic consumptions on a day with a maximum of 21 per week; stable body weight (weight gain or loss < 3 kg in the past three months); no use of medication to treat hypertension, lipid or glucose metabolism; no severe medical conditions that might interfere with the study parameters such as epilepsy, asthma, kidney failure or renal insufficiency, chronic obstructive pulmonary disease, inflammatory bowel diseases, autoinflammatory diseases, or rheumatoid arthritis; no active cardiovascular diseases or events such as congestive heart failure, acute myocardial infarction, or cerebrovascular accidents; difficult to venipuncture; no blood donation within the past 12 weeks; engaged in less than 10 hours of sports per week; not working night shifts; no allergies to porcine-derived supplements and agreement to consume the study products. Ethical approval was obtained by the Ethical Committee of azM/UM (IDs NL72922.068.20/METC 20-007). The study was registered at ClinicalTrials.gov (identifier NCT05282641) and was performed according to the principles of the Declaration of Helsinki. The study was carried out between February 2021 and August 2023.

Study design

The study had a randomized, double-blind, placebo-controlled parallel design, and consisted of a two-week run-in period followed by 4-week of intervention (SI Fig. S1). After inclusion, participants were randomly allocated to the collagen hydrolysates or placebo group using a computer-generated block design by an independent researcher. During the study, participants visited the MRUM five times. The first three visits took place in the run-in period, one at the start (week 0), and twice at the end (both in week 2). Two follow-up visits (4 and 5) were in the last week of the intervention period (week 6). Participants completed two test days: one at the end of the run-in period (visit 3) and the second one at the end of the intervention period (visit 5). During the test day, various measurements were performed. Results on glycemic variability, fasting and postprandial glucose, insulin, and triacylglycerol concentrations, anthropometrical measurements, food intake, and physical activity profiles have already been published.[40] Here, we report the effects of 4 weeks of consuming the collagen hydrolysates on other cardiometabolic health parameters such as office BP, 24-hour BP profiles, characteristics of the retinal microvasculature, markers reflecting endothelial dysfunction and low-grade systemic inflammation, and cognitive performance.

Study product

Sachets from three different boxes labeled A, B, and C, were stored at 22 °C. An independent researcher performed the randomization procedure and coordinated supplement distribution together with an independent research assistant, who was responsible for packaging and providing the study products to the participants. For the two-week run-in period, sachets from box C were used, while for the 4-week intervention periods, the sachets from either boxes A or B were provided to the volunteers. All sachets had an identical appearance. The sachets containing the collagen hydrolysates consisted of 10 g of porcine-derived collagen hydrolysates (Rousselot, Someren, the Netherlands) plus 5 g of erythritol (Ingrizo, De Pinte, Belgium), while the placebo sachets, including the sachets from box C, contained only 5 g of erythritol (Ingrizo, De Pinte, Belgium). Both researchers as well as participants were blinded to the type of products they received. Every day, participants consumed the content of one sachet in 200 ml of water, preferably 15 min before breakfast. On visit days, participants consumed the product at home post-visit. The code of the study was broken after all statistics had been performed and conclusions had been formulated. Compliance was excellent: 98.8% ± 3.7 and 98.3% ± 3.2 in the collagen hydrolysate group, and 99.4% ± 3.0 and 97.5% ± 4.0 in the placebo group for the run-in and intervention periods, respectively.[40] Furthermore, participants were instructed to maintain regular levels of physical activity and to keep their dietary habits stable during the study, which was successful.[40]

Office BP and 24-hour ambulatory BP

Office BP was measured after 10 minutes of rest in a supine position using an Omron Intellisense M7 (Cemex Medische Techniek, Nieuwegein, the Netherlands) as already explained.[40] Moreover, an ambulatory BP monitor device (Mobil-O-Graph; I.E.M. Inc. Stolberg, Germany) was placed on the left arm to assess 24-hour BP profiles during the last weeks of the run-in period (week 2), and the intervention period (week 6). This device was placed at the end of visits 2 and 4. Participants removed the device themselves after 24 hours and returned it to the research team on the following test days for readout. During the 24-hour test days, BP was recorded every 15 min during the daytime (from 07:00 to 22:00) and every 30 min during the nighttime (from 22:00 to 07:00). The ambulatory BP profiles were calculated over 24 hours, as well as separately for daytime and nighttime. The first measurement was discarded in the analysis. Mean systolic BP (SBP), diastolic BP (DBP), mean arterial pressure (MAP), heart rate (HR), pulse pressure (PP), standard deviation (SD) profiles during 24 hours, daytime, and nighttime, as well as nocturnal dipping in SBP and DBP were calculated, as previously described.[41]

Retinal microvasculature

During the test days (visits 3 and 5), digital fundus images of the optic disc from the right eye were captured using a non-mydriatic retinal camera (TRC-NW300, Topcon Corporation, Tokyo, Japan). These images were used to assess changes in retinal vascular calibers. For this, digital images were analyzed using the IVAN software (University of Wisconsin, Wisconsin, USA), selecting the diameters of the three widest arterioles and the three widest venules, using exactly the same segments on the images from the run-in phase and the intervention phase within each participant. The Parr-Hubbard formula[42] was used to calculate the central retinal arteriolar equivalent (CRAE), the central retinal venular equivalent (CRVE), and the arteriolar-to-venular ratio (AVR).

Serum and plasma markers for endothelial dysfunction and low-grade inflammation

Fasting blood samples were collected at the end of the run-in period and at the end of the intervention period from the forearm using serum STT-II separator tubes (Becton, Dickson, and Company) and EDTA-coated vacutainer tubes (Becton, Dickson, and Company). Serum tubes were clotted at room temperature for 30 min and then centrifuged at 1300*g* for 10 min at 21 °C. EDTA tubes were kept on ice until centrifugation at 1300*g* for 10 min at 4 °C. Serum and EDTA plasma were portioned into aliquots, frozen in liquid nitrogen, and stored at −80 °C. All samples were analyzed in the same analytical run after completing the study. Serum samples were used to analyze total cholesterol (CHOD-PAP method; Roche Diagnostics, Manheim, Germany), HDL-C, (precipitation method, Roche Diagnostics, Mannheim, Germany), TAG (TRIGL method, Roche Diagnostics, Mannheim, Germany), and high-sensitive C-reactive protein concentrations (hs-CRP; immunoturbidimetric assay, Horiba ABX, Montpellier, France), while EDTA plasma samples were used to analyze concentrations of IL-6, IL-8, TNFα, soluble vascular adhesion molecule-1 (sVCAM-1), (sICAM-1), serum amyloid A protein (SAA), and soluble endothelial leukocyte adhesion molecule-1 (sE-Selectin) by ELISA (MesoScaleDiscovery, SECTOR Imager 2400, Gaithersburg, Maryland, USA). LDL-C concentrations were calculated using the Friedewald formula.[43] Results from serum samples obtained from the last two visits, 2 and 3 of the run period as well as the samples from the two visits, 4 and 5, at the end of the intervention period were averaged.

Cognitive function tests

During both test days, cognitive performance was evaluated using fully automated Cambridge neuropsychological test automated battery (CANTAB) software using an iPad (iPad 5th generation; Apple, California, USA). CANTAB is a validated computerized assessment tool to evaluate cognitive performance in different domains such as memory, psychomotor speed, and executive function. Participants were first familiarized with the software and the iPad through the motor screening task (MOT), which was not used in the study results. The working memory domain was assessed with the delayed matching to sample (DMS) test, and paired associates learning (PAL) test. Psychomotor speed was assessed with the reaction time (RTI) test. Finally, executive function was assessed *via* the multitasking test (MTT) and the spatial working memory (SWM) test, as previously described.[44,45]

Statistics

Results are presented as means ± SDs unless otherwise specified. The power calculation for the study was based on treatments effects on the interstitial glucose area under the curve during daytime and resulted in a sample size of 54 participants, as previously published.[40] Treatment effects were estimated using a one-factor ANCOVA, with the end-of-study values as the dependent variable, run-in values as covariate, and treatment as factor. When residuals did not follow a normal distribution, one-factor ANCOVA analyses were conducted on log10-transformed values. If the residuals remained not normally distributed as assessed with the Kolmogorov–Smirnov, treatment effects were estimated with Quade's ANCOVA with run-in values as a covariate. Two-sided p-values ≤ 0.05 were considered to be statistically significant. Analyses were performed using IBM SPSS Statistics Version 28.0 for Mac (IBM Corporation, Armonk, NY, United States).

Results

Study participants

Sixty-five participants were screened for eligibility, of which sixty-three were enrolled in the study and randomly assigned to either the collagen hydrolysates group (n = 32) or the placebo group (n = 31). As illustrated in the study flow chart (SI Fig. S2), seven participants discontinued the study. Three of these participants were originally randomized to the collagen hydrolysates group: two withdrew for personal reasons and one for health issues unrelated to the study. The other four participants were allocated to the placebo group: two of them declined for personal reasons, one for starting medication that could have interfered with the study outcomes, and another due to receiving the diagnosis of arthritis. Therefore, fifty-six participants were included in the statistical analysis, with 28 (women n = 16) in the collagen hydrolysates group and 28 (women n = 15) in the placebo group. Additionally, two participants were excluded from the retinal microvasculature analysis due to image capture failure. Moreover, fourteen participants were excluded from the ambulatory BP analysis: two participants declined to use the device, two participants encountered technical issues with the device, and ten participants were excluded due to insufficient data collected for analysis due to measurement errors. Finally, all participants completed the cognitive tests. Baseline characteristics of the participants (SI Table S1) have been reported before.[40]

Blood pressure outcomes and retinal microvascular calibers

Four weeks of collagen hydrolysate consumption showed no significant changes in office BP as compared to placebo. Likewise, there were no significant changes in mean BP profiles, heart rate, nocturnal BP dipping, and intra-individual BP variability during the 24-hour BP monitoring (Table 1). Results were similar when the daytime and nighttime data were considered separately (SI Table S2). Furthermore, no significant effects were found on CRAE (1 μm, 95% CI: −1 to 2; P = 0.550), CRVE (0 μm, 95% CI: −2 to 2; P = 0.841), and AVR (0 μm, 95% CI: 0 to 0; P = 0.799) in the collagen hydrolysate group as compared to placebo (Table 1).

Serum lipids and lipoproteins as well as markers for endothelial dysfunction and low-grade inflammation

Serum total cholesterol, HDL-C, and LDL-C concentrations remained unchanged after collagen hydrolysate consumption

Table 1 Effects of 10-gram collagen protein hydrolysates on retinal microvasculature, office blood pressure, and 24-hour blood pressure profiles at the end of the run-in period and at the end of the intervention period per group

	Collagen-hydrolysates group Run-in period	Collagen-hydrolysates group End of study	Placebo group Run-in period	Placebo group End of study	Treatment effect (95% CI)	p-Value
Office blood pressure						
SBP (mmHg)	130 ± 15	130 ± 15	127 ± 14	128 ± 15	−1 (−3, 2)	0.529
DBP (mmHg)	81 ± 7	82 ± 7	81 ± 6	83 ± 8	−1 (−2, 1)	0.449
24-Hour blood pressure						
SBP (mmHg)	128 ± 12	124 ± 10	124 ± 10	122 ± 9	0 (−4, 4)	0.844
DBP (mmHg)	74 ± 9	75 ± 8	77 ± 7	77 ± 6	−2 (−5, 1)	0.195
MAP (mmHg)	101 ± 9	97 ± 8	99 ± 8	98 ± 7	−1(−5, 2)	0.402
PP (mmHg)	50 ± 8	50 ± 7	46 ± 7	46 ± 7	−2 (−5, 1)	0.195
HR (beats per min)	65 ± 8	65 ± 7	67 ± 8	68 ± 10	0 (−3, 3)	0.919
Nocturnal dipping SBP (%)	9 ± 5	9 ± 6	11 ± 6	8 ± 8	2 (−2, 6)	0.398
Nocturnal dipping DBP (%)	13 ± 6	11 ± 8	15 ± 7	14 ± 9	−2 (−7, 3)	0.377
Retinal microvascular calibers						
CRAE (μm)	136 ± 17	136 ± 18	132 ± 13	131 ± 14	1 (−1, 2)	0.550
CRVE (μm)	226 ± 16	227 ± 16	231 ± 17	231 ± 17	0 (−2, 2)	0.841
AVR	0.60 ± 0.06	0.60 ± 0.06	0.57 ± 0.06	0.57 ± 0.05	0.00 (−0.01, 0.01)	0.799

Abbreviations: SBP systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; PP, pulse pressure; HR, heart rate; CRAE, central retinal arteriolar equivalent; CRVE, central retinal venular equivalent; AVR, arteriolar-venular-ratio. Values are mean ± SD. Treatment effects were evaluated with 1-factor ANCOVA with baseline value as a covariate. Office blood pressure: collagen hydrolysates group n = 28, placebo group n = 28. 24 h blood pressure: collagen hydrolysates group n = 19, placebo group n = 23. Retinal microvascular calibers: collagen hydrolysates group n = 27, placebo group n = 27.

Table 2 Effects of 10-gram collagen protein hydrolysates on biochemical parameters and markers for endothelial dysfunction and low-grade inflammation at the end of the run-in period and at the end of the intervention period per group

	Collagen-hydrolysates group Run-in period	Collagen-hydrolysates group End of study	Placebo group Run-in period	Placebo group End of study	Treatment effect (95% CI)	*p*-Value
Biochemical parameters						
Total cholesterol (mmol L^{-1})	5.68 ± 0.74	5.68 ± 0.62	5.54 ± 0.87	5.59 ± 0.84	−0.02 (−0.21, 0.17)	0.849
HDL-C (mmol L^{-1})	1.46 ± 0.33	1.45 ± 0.32	1.51 ± 0.29	1.54 ± 0.29	−0.03 (−0.08, 0.03)	0.281
LDL-C (mmol L^{-1})	3.52 ± 0.61	3.53 ± 0.56	3.45 ± 0.71	3.44 ± 0.70	0.02 (−0.14, 0.18)	0.803
Markers for endothelial dysfunction						
sVCAM-1 (ng ml^{-1})	402 ± 81	434 ± 83	407 ± 87	430 ± 121	6 (−31, 43)	0.754
sICAM-1 (ng ml^{-1})	327 ± 80	357 ± 80	308 ± 65	320 ± 81	18 (−7, 43)	0.161
sE-Selectin (ng ml^{-1})	43 ± 18	41 ± 18	42 ± 21	41 ± 19	−1 (−3, 2)	0.563
Markers for low-grade inflammation						
IL-6 (pg ml^{-1})[a]	0.72 (0.35. 3.32)	0.74 (0.41, 1.53)	0.71 (0.38, 3.44)	0.66 (0.31, 3.96)		0.673
IL-8 (pg ml^{-1})	5.19 ± 2.0	4.77 ± 1.9	4.47 ± 1.30	4.48 ± 1.18	−0.09 (−0.78, 0.60)	0.798
TNFα (pg ml^{-1})	1.33 ± 0.44	1.24 ± 0.36	1.36 ± 0.39	1.29 ± 0.34	0.03 (−0.13, 0.07)	0.526
SAA (μg ml^{-1})[a]	2.54 (0.73, 89.3)	2.28 (0.96, 35.2)	3.08 (0.72, 20.0)	3.04 (0.86, 21.7)		0.444
hs-CRP (ng mL^{-1})[a]	1.81 (0.06, 9.07)	1.42 (0.06, 9.94)	0.73 (0.17, 21.9)	0.70 (0.10, 17.1)		0.625

Abbreviations: HDL-c, high-density lipoprotein cholesterol; LDL-c, low-density lipoprotein cholesterol; sVCAM-1, soluble vascular cell adhesion molecule-1; sVCAM-1, soluble intercellular adhesion molecule-1; sE-Selectin, soluble endothelial leukocyte adhesion molecule-1; IL-6, interleukin-6; IL-8, interleukin-8; TNFα Tumor necrosis factor-alpha; SAA, serum amyloid A protein; hs-CRP, high sensitive C-reactive protein. Values are mean ± SD or median (minimum and maximum values). Treatment effects were evaluated with 1-factor ANCOVA with baseline value as a covariate. [a] When residuals were not normally distributed, treatment effect changes were evaluated with Quade's ANCOVA with baseline value as a covariate. In bold *p*-value < 0.05. Collagen hydrolysates group $n = 28$, placebo group $n = 28$. IL-6: collagen hydrolysates group $n = 27$, placebo group $n = 28$.

as compared to placebo. Furthermore, markers for endothelial dysfunction (sVCAM-1, sICAM-1, and sE-selectin) or low-grade inflammation (IL-6, IL-8, TNFα, SAA, and hs-CRP) did not significantly change (Table 2).

Cognitive function

In the psychomotor speed domain, collagen hydrolysates consumption significantly increased RTI movement time by 29 ms (95% CI: 5 to 53; $P = 0.019$) as compared to placebo, whereas no significant differences were observed in RTI reaction time (10 ms, 95% CI: −5 to 25; $P = 0.206$) (Table 3). Additionally, no significant changes were observed between the collagen hydrolysates and the placebo group for tests related to memory or executive function domains (Table 3).

Discussion

In this randomized controlled trial including men and women with overweight or obesity, we found that 10 g of daily porcine-collagen hydrolysate consumption did not significantly affect office and 24-hour BP profiles, serum lipids and lipoproteins, markers for low-grade chronic inflammation and endothelial dysfunction, and retinal vascular calibers. For cognitive performance, we observed an unexpected increase in RTI movement time, while no other significant changes were observed in any of the different cognitive outcomes.

In line with other protein hydrolysates, collagen peptides have been suggested to reduce BP by inhibiting ACE.[11] However, we here showed that the consumption of porcine-derived collagen hydrolysates for 4 weeks did not improve office or ambulatory BP profiles. This is in contrast with a recent meta-analysis, which concluded that collagen hydrolysates supplementation reduced SBP by 5 mmHg, but not DBP, based on moderate- and very low-quality evidence, respectively. Moreover, sub-group analyses indicated that this reduction in SBP was limited to unhealthy populations (−8 mmHg)[11] with diabetes and/or with mild or moderate hypertension,[19,20,46] whereas no effects were found in healthy individuals.[11] This was also supported by a recent study using fish-derived collagen hydrolysates in healthy populations.[22] Consistent with these results for porcine-collagen hydrolysates, long-term egg protein hydrolysate consumption also did not improve ambulatory BP profiles in a normotensive population.[47] In contrast, the consumption of the same egg protein hydrolysates,[48] of shrimp-derived,[49] and of hemp seed-derived protein hydrolysates[50] significantly reduced ambulatory SBP and DBP in populations with mild or moderate hypertension. In addition, a meta-analysis showed that lactotripeptide intake, peptides derived from milk casein hydrolysis (Isoleucine-Proline-Proline and Valine-Proline-Proline), exhibited a modest BP-lowering effect, which was more pronounced in hypertensive than in normotensive individuals.[51] Overall, the beneficial effects of hydrolysates from collagen or other protein sources on BP profiles have been observed mainly in mild and moderate hypertensive populations. Therefore, it is tempting to suggest that the potential BP-lowering effects of collagen hydrolysates may be restricted to hypertensive individuals, which may explain the absence of an effect in the current study.

Collagen hydrolysates have also been suggested to improve lipid metabolism by modulating the expression of proteins involved in fatty acid and cholesterol synthesis, as well as for

Table 3 Effects of 10-gram collagen protein hydrolysate on cognitive performance at the end of the run-in period and at the end of the intervention period per group

	Collagen-hydrolysates group Run-in period	Collagen-hydrolysates group End of study	Mean difference	Placebo group Run-in period	Placebo group End of study	Mean difference	Treatment effect (95% CI)	*p*-Value
Memory								
DMS (% correct)	78 ± 10	83 ± 13	4 ± 14	81 ± 10	84 ± 10	4 ± 9	−1 (−6, 5)	0.779
PAL (1st attempt memory score)	11 ± 4	11 ± 4	0 ± 4	12 ± 4	12 ± 4	0 ± 5	−1 (−3, 2)	0.274
PAL (total error)	22 ± 15	20 ± 14	−1 ± 5	17 ± 13	15 ± 12	−2 ± 9	2 (−3, 7)	0.359
Psychomotor speed								
RTI movement time (ms)	321 ± 68	328 ± 68	7 ± 35	323 ± 93	300 ± 62	−23 ± 70	29 (5, 53)	**0.019**
RTI reaction time (ms)	392 ± 36	400 ± 45	8 ± 27	393 ± 36	391 ± 35	−1 ± 30	10 (−5, 25)	0.206
Executive function								
MTT incongruency cost (ms)	117 ± 63	116 ± 50	0 ± 63	97 ± 59	87 ± 48	−10 ± 65	24 (−1, 49)	0.063
MTT multitasking cost (ms)	191 ± 163	204 ± 109	13 ± 175	245 ± 165	223 ± 130	−22 ± 177	−8 (−72, 55)	0.795
MTT reaction latency (ms)	767 ± 128	762 ± 127	−5 ± 99	759 ± 97	740 ± 115	−18 ± 79	16 (−31, 62)	0.505
MTT (total incorrect)	13 ± 14	7 ± 10	−6 ± 10	11 ± 13	6 ± 9	−5 ± 8	0 (−3, 3)	0.984
SWM (between errors)	32 ± 11	32 ± 10	−1 ± 13	32 ± 13	31 ± 14	−1 ± 14	0 (−6, 6)	0.913
SWM (strategy score)	9 ± 2	7 ± 2	−1 ± 2	8 ± 2	7 ± 3	−1 ± 2	0 (−1, 1)	0.787
SWM (total errors)	33 ± 12	33 ± 10	−1 ± 14	33 ± 14	33 ± 16	0 ± 16	0 (7, 7)	0.976

Abbreviations: DMS, delayed matching to sample; PAL, paired associates learning; RTI, reaction time; MTT, multitasking test; SWM, spatial working memory. Values are mean ± SD. Treatment effect changes were evaluated with 1-factor ANCOVA with baseline value as a covariate. Collagen hydrolysates group $n = 28$, placebo group $n = 28$.

β-oxidation.[25] However, in the present study, porcine-derived collagen hydrolysate supplementation had no significant effect on serum total cholesterol, LDL-C, or HDL-C concentrations. This contrasts a recent meta-analysis, which suggested a modest but significant reduction in serum LDL-C concentrations (−0.11 mmol L^{-1}), without significant changes in HDL-C and total cholesterol concentrations, although the quality of evidence was considered to be very low.[11] However, further research is warranted, as several other studies that were not included in the meta-analysis found, consistent with our findings, no effects on serum total cholesterol[19,27,29] and LDL-C[27] concentrations. Finally, as previously reported and discussed, no significant changes were observed in TAG concentrations.[40]

To our knowledge, this is the first study to examine the effects of collagen hydrolysate consumption on markers for endothelial dysfunction. We observed no significant effects on sVCAM-1, sICAM-1, or sE-Selectin, which aligns with observations using hydrolyzed[52] or whole protein intake[53] in healthy populations. In contrast, protein supplementation has been shown to reduce sVCAM-1[54] and sICAM-1[54,55] in adults with prehypertension and mild hypertension. This might be explained by the fact that hypertensive individuals exhibit higher expression of adhesion molecules and E-selectin[56] than normotensive individuals, due to endothelial activation induced by inflammatory mediators,[57] thereby providing more room for improvement. Similarly, we did not observe significant changes in IL-6, IL-8, TNFα, SAA, and hs-CRP, markers for low-grade chronic inflammation. These findings are consistent with two previous studies providing 10 g of bovine-collagen hydrolysates for 12 weeks[32] or 15 g for 7 days.[33] Additionally, other studies did not report significant reductions in hs-CRP following collagen hydrolysate intake.[20,58,59] Overall, it can be concluded that collagen hydrolysates may not improve markers for low-grade inflammation, but studies in patients with elevated concentrations of these markers at baseline are missing. The absence of effects on BP, lipids, and lipoproteins, and markers for endothelial dysfunction and low-grade inflammation might explain the lack of effects on microvascular calibers in the retina after consuming the porcine-collagen hydrolysates.

Finally, for cognitive performance, we observed an unexpected – but significant – increased movement time during the psychomotor speed test. In contrast, a study investigating the effect of 15 g of bovine-based collagen hydrolysates supplementation for 7 days before bedtime showed no significant effect on a simple reaction time and a choice test.[33] Additionally, other protein hydrolysates have yielded mixed effects on reaction time tests. For instance, a tryptophan-rich protein hydrolysate reduced both simple and complex reaction time in women,[60] while other protein hydrolysates found no significant effects,[44,61–63] Furthermore, the reaction time during the RTI test did not change. Thus, our findings indicate no changes in attention, and processing speed from the onset of a stimulus to the initiation of a response. However, a slower movement time was noted. The 29 ms increased RTI movement time falls within the suggested range of normal test-retest variability for this test (90% Reliable Change Index: 50.36 to −54.01 ms) in a healthy population.[64] This might indicate that our observed increase in RTI movement time could be a chance finding.

The strengths and limitations of this study with respect to the potential impact of timing, dosage, and preparation methods on the outcomes have previously been discussed.[40]

An additional strength of the study results reported here is the use of an ambulatory BP device, which is a better predictor for cardiovascular disease risk as compared to traditional office BP measurements.[65] A limitation, however; is that 25% of the ambulatory blood pressure data could not be included in the analysis. Furthermore, although we measured markers of endothelial dysfunction, we did not include vascular function measurements in our study. Collagen hydrolysates may improve arterial stiffness by lowering brachial-ankle pulse wave velocity after 12 months,[21,46] while no studies have yet evaluated changes in endothelial dysfunction measured through flow-mediated dilatation. Therefore, it can be speculated that longer intervention periods are needed to detect potential benefits of collagen hydrolysate consumption on cardiometabolic parameters. Furthermore, combining collagen hydrolysate supplementation with exercise may result in more pronounced improvements in cardiometabolic parameters.[66]

Conclusion

In conclusion, we here show that daily consumption of 10 g of porcine-derived collagen for four weeks did not significantly change office and ambulatory BP profiles, serum lipids and lipoproteins, retinal vascular calibers, and markers for endothelial dysfunction and low-grade chronic inflammation in apparently healthy middle-aged and older adults with overweight or obesity. In addition, cognitive performance was not affected. However, its effects on individuals at higher cardiovascular risk, such as those with hypertension or diabetes, are uncertain and require further research.

Author contributions

MACA, RPM, PJJ and JP contributed intellectually and directly to the manuscript. Data collection was conducted by MACA.

Conflicts of interest

The authors declare no conflicts of interest.

Abbreviations

CVD	Cardiovascular diseases
ACE	Angiotensin I-converting enzyme
SBP	Systolic blood pressure
DBP	Diastolic blood pressure
LDL-C	Low-density lipoprotein cholesterol
TAG	Triacylglycerol
HDL-C	High-density lipoprotein cholesterol
IL-6	Interleukin-6
sICAM-1	Soluble intercellular adhesion molecule-1
(TNF-α)	Tumor necrosis factor-alpha
MAP	Mean arterial pressure
HR	Heart rate
PP	Pulse pressure
SD	Standard deviation
CRAE	Central retinal arteriolar equivalent
CRVE	Central retinal venular equivalent
AVR	Arteriolar-to-venular ratio
IL-8	Interleukin-8
hs-CRP	High-sensitive C reactive protein
sVCAM-1	Soluble vascular adhesion molecule-1
SAA	Serum amyloid A protein
sE-Selectin	Soluble endothelial leukocyte adhesion molecule-1
CANTAB	Cambridge neuropsychological test automated battery
MOT	Motor screening task
DMS	Delayed matching to sample
PAL	Paired associates learning
RTI	Reaction time
MTT	Multitasking test
SWM	Spatial working memory

Data availability

The datasets used and/or analyzed during the study are available from the corresponding author on reasonable request.

Supplementary information (SI) is available. See DOI: https://doi.org/10.1039/d5fo01261a.

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