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NOTE

A double-blind, placebo-controlled, randomised clinical study of the effect of pork collagen peptide supplementation on atherosclerosis in healthy older individuals

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ABSTRACT

We examined whether baPWV could be affected by pork collagen peptide (CP) ingestion. Seventy subjects were randomized into two groups (2.5 g/day CP and 2.5 g/day placebo). A significant reduction in baPWV was observed in the CP group compared to the placebo group. This study demonstrated that pork CP may contribute to the prevention of atherosclerosis in elderly.

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Pork collagen peptide; brachial-ankle pulse wave velocity; a double-blind placebo-controlled randomised trial; multiple regression analysis

Atherosclerotic disease, including coronary artery disease (CAD) and cerebrovascular disease (CVD), are the most common cause of death in many countries. Reducing risk factors for atherosclerosis is important to improving and maintaining quality of life. Atherosclerosis can remain asymptomatic until it is quite advanced. Therefore, risk reduction for atherosclerotic diseases is vital, and depends on the identification of early risk factors. Arterial stiffness is a good marker for atherosclerosis, predicting the risk of future CAD and CVD events [1,2].

Non-invasive measurements of arterial stiffness are now available to detect early vascular changes in asymptomatic people. The brachial-ankle pulse wave velocity (baPWV) is well-known for predicting cardiovascular mortality and morbidity [1].

Collagen peptide (CP) has been using as functional food ingredient. The various beneficial effects of ingesting CP are believed to be attributable to the physiological properties of circulating dipeptides [3]. Here, we examined whether this marker of arterial stiffness, baPWV, could be affected by pork CP ingestion.

Subjects were participants in a medical check-up program at Ehime University Hospital Anti-aging Centre (AAC), which is specifically designed to evaluate for atherosclerosis [4]. To rule out the impact of disease or medications, we excluded participants with a history of symptomatic cardiovascular events and those receiving medications for diabetes, dyslipidaemia, or hypertension. Enrolment was conducted over a 4-month period starting in April 2016. A total of 70 subjects (mean age was 73 ± 7) were enrolled. Subjects were randomized by a computerized random number generator into two groups, one receiving a test meal containing 2.5 g/day pork CP and the other receiving a placebo containing 2.5 g/day whey powder, both from Nitta Gelatin Inc. (Osaka, Japan) (Table 1).

The study was conducted after acceptance by the institutional review board (IRB) for clinical trial services at Ehime University Graduate School of Medicine and was performed under inspection by the study investigators. Written informed consent was obtained from all participants before examination. The study was conducted in accordance with the ethical principles laid out in the current version of the Declaration of Helsinki and was registered (UMIN000023388). During the study, the subjects were advised to consume the test meal orally as 2.5 g dissolved in 250 mL hot water followed by routine breakfast.

Each participant was interviewed by a doctor two times (at baseline and 12 weeks) to look for such symptoms and adverse events as dry cough, headache, dizziness, or gastrointestinal problems including diarrhoea, eruptions, skin itching, or taste disorders.

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Table 1. Composition of the pork cp and placebo.

Components	Pork CP*	Placebo (whey powder)
Protein (g/100 g)	89.4	81.9
Fat (g/100 g)	0.0	4.6
Ash (g/100 g)	0.4	2.6
Water (g/100 g)	6.1	5.6
Carbohydrate (g/100 g)	4.1	5.3
Amino acid** (g/100 g)		
GLY	23.4	
PRO	13.0	
HYP	11.3	
ALA	8.9	
GLU	10.1	
ARG	7.9	
ASP	5.5	
LYS	3.6	
SER	3.3	
LEU	2.7	
VAL	2.3	
PHE	1.9	
THR	1.7	
ILE	1.2	
MET	0.9	
HYL	1.0	
HIS	0.7	
TYR	0.6	
CYS	0.0	
TRP	ND	

*Dipeptide-rich Pro-Hyp and Hyp-Gly >3000 ppm porcine skin type I collagen hydrolysate powder. The average molecular weight is 1 kDa.; **The amino acid composition after hydrolysis.

Table 2. Change in SBP, DBP, PR and baPWV during treatment.

	Baseline	12 week	Change (%), week 12
SBP, mmHg			
Pork CP ($n = 30$)	129 ± 15	125 ± 16	−3.1 ± 9.6
Placebo ($n = 34$)	130 ± 17	131 ± 17	1.1 ± 11.0
DBP, mmHg			
Pork CP ($n = 30$)	74 ± 10	72 ± 11	−2.4 ± 11.7
Placebo ($n = 34$)	76 ± 11	76 ± 11	0.6 ± 11.9
PR, bpm			
Pork CP ($n = 30$)	69 ± 7	69 ± 10	0.3 ± 11.8
Placebo ($n = 34$)	70 ± 8	71 ± 9	1.3 ± 7.8
baPWV, cm/s			
Pork CP ($n = 30$)	1709 ± 248	1611 ± 221	−5.4 ± 6.5*
Placebo ($n = 34$)	1670 ± 266	1646 ± 211	−0.7 ± 8.7

Values are expressed as the mean ± standard deviation.
CP, collagen peptide; SBP, systolic blood pressure; DBP, diastolic blood pressure; PR, pulse rate; baPWV, brachial–ankle pulse wave velocity.
*Intergroup comparison ($p < 0.01$, vs. placebo group).

Table 3. Simple regression and multiple regression analyses of variables that independently predict delta baPWV in the whole study population.

	Univariate		Multivariate	
	r	*P*	*β*	*P*
Age	−0.048	0.707		
Sex (male)	0.099	0.437		
SBP (baseline)	−0.166	0.191		
baPWV (baseline)	−0.462	< 0.001	−0.428	<0.001
baPWV (12 weeks)	0.071	0.578		
Pork CP	5.682	0.007	0.279	0.013

SBP, systolic blood pressure; baPWV, brachial–ankle pulse wave velocity; CP, collagen peptide.

Blood pressure, pulse rate, hematological examinations, and baPWV examinations were also conducted at baseline and after 12 weeks. As for blood pressure and pulse rate, the mean value of two measurements was recorded. Blood samples for hematological examinations were collected between 9:00 and 10:00 am from the cubital vein following an overnight fast. Routine biochemical parameters were determined in fresh samples. Low-density lipoprotein (LDL) levels were calculated using the Friedewald formula [5]. Estimated GFR (eGFR, mL/min/1.73 m^2) was calculated using the Cockcroft–Gault formula [6]. BaPWV was measured with a volume plethysmographic apparatus (FORM/ABI; Omron Colin Co. Ltd., Komaki, Japan) as previously described [7]. Measurements were reported as the mean value for bilateral measurements.

All continuous variables are expressed as mean ± standard deviation (SD), unless otherwise indicated. Comparisons between the two groups were assessed using the unpaired t-test for parametric variables and Mann-Whitney U test for nonparametric variables. The Wilcoxon signed-rank test was used to assess significant differences between baseline and 12 weeks. Correlations between variables were evaluated using Pearson's correlation coefficient. Multiple regression analysis was further performed for baPWV and other variables, including pork CP consumption. In all comparisons, $p < 0.05$ was considered statistically significant. Analyses were performed using the SPSS software package for Windows version 17 (SPSS, Chicago IL, USA).

Seventy subjects were randomly assigned to receive either pork CP (34 subjects) or placebo (36 subjects). Of the 70 subjects, one participant experienced a mild adverse event of diarrhoea. After he quit the study, this symptom resolved spontaneously. In addition, five participants did not complete the study because of moving residence ($n = 1$), and taste preference ($n = 4$). As a result, a total 64 subjects were included in the final analysis (Participant demographics are shown in Supplement data 1.). The mean age of subjects was 73 years old and 14 (22%) of the subjects were men.

Changes in systolic blood pressure (SBP), diastolic blood pressure (DBP), pulse rate (PR) and baPWV are shown in Table 2. There were no significant changes in SBP, DBP or PR between the pork CP and placebo groups. The baPWV values after 12 week in the pork CP group were significantly lower than at week 0 ($p = 0.003$). There were no significant differences in the placebo group at week 12 when compared with the value at week 0. In addition, changes in baPWV values in the pork CP group significantly decreased compared to the placebo group ($p = 0.007$). A positive correlation was also observed between delta baPWV and baseline mean baPWV. Multiple regression analysis demonstrated a significant independent relationship between mean delta baPWV and baseline baPWV and pork CP ingestion in the whole study population (Table 3).

None of the biochemical parameters showed any significant variation during the study period. These findings demonstrate the safety of pork CP in humans. (The

results of the baseline and 12-week laboratory examinations are shown in Supplement data 2.).

In this study, we firstly demonstrated a significant decrease in baPWV in healthy participants orally administered pork CP compared to those administered placebo by a double-blind, placebo-controlled, randomised clinical study. Our data also showed that a significant independent relationship between delta baPWV and pork CP ingestion in the whole study population. Tomosugi et al. [8] previously published a similar study in middle-aged and older individuals. In their open-label, single-dose trial of pork CP in healthy subjects, they also showed that arterial stiffness was significantly decreased in all subjects from baseline to 24 weeks. They suggested that pork CP directly act on the vascular wall.

In contrast to our data, Kouguchi et al. [9] showed that a lactic acid drink with chicken CP significantly decreased baPWV compared to placebo, dependent of SBP. The discrepancy could be due, in part, to differences in ACE-inhibitory activity between chicken CP and pork CP. Saiga et al. [10] showed that chicken CP possesses strong ACE-inhibitory activity and lowered BP in spontaneously hypertensive rats. A parallel study using laboratory-prepared chicken-meat and pork extracts showed that the former, but not the latter, attenuated cardiac hypertrophy in experimentally-induced cardiac hypertrophic rat [11]. Certain data suggest that Pork CP has less ACE-inhibitory activity. It is important to note that the serum NOx, a metabolite of NO, value was significantly higher at 12 weeks than at week 0 in the chicken CP study [9]. Zhang et al. [12] demonstrated that chicken CP administration increased serum NO concentration in rats.

Ohara et al. [13] showed that the structure and amount of food-derived peptides in human blood differs according to the collagen type and collagen source. The biological activity of orally administered collagen peptide is thought to depend on the collagen type or collagen source.

This study has a limitation that warrant attention. As the sample size was small and made inferences on baPWV, this study was not powered to differentiate pork CP ingestion in terms of CVD risk reduction. A longitudinal study using any method of measuring atherosclerosis is therefore required to clarify this problem.

We conclude our findings indicate that ingestion of pork CP may become an important component of preventive medicine.

Author contributions

M.I., K.K., K.I., N.I., H.M., and Y.O. designed research, M.I., and Y.O. collected clinical samples, M.O., and T.K. performed the experiments, M.I., and K.I. analyzed data, and M.I., and K.I. wrote the manuscript. All authors have read and approved the final manuscript.

Disclosure statement

No potential conflict of interest was reported by the authors.

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