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## Vasoprotective Effect of Foods as Treatments: Chicken Collagen Hydrolysate

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### 1. Introduction

Collagen is a major protein in living organisms and accounts for about one-third of all protein in mammalian bodies, including the human body. Recently, collagen peptides have been used as foods that take advantage of their tertiary functions. We have been focusing on the vasoprotective effect of collagen peptides.

Chicken collagen hydrolysate (CCH) is obtained by treating chicken feet with enzymes to produce an angiotensin-converting enzyme (ACE) inhibitory peptide. Administration of this CCH for 12 weeks reduces blood pressure in humans. We therefore investigated the mechanism of the vasoprotective effect of CCH. We tested whether prolonged CCH treatment of rats or mice would restore endothelial cell function and improve proinflammatory cytokine levels. We found that CCH treatment improved the vasorelaxation of rat aorta damaged with L-NG-nitroarginine methyl ester, an NO synthesis inhibitor. CCH treatment also reduced the serum levels of IL-6, sICAM-1, and TNF- $\alpha$  in an atherosclerotic mouse model, C57BL/6.KOR-ApoE<sup>sh1</sup>.

These findings indicate the usefulness of collagen peptides as foods promoting anti-atherogenesis via a vasoprotective effect.

Years have passed since functional foods and their tertiary function first attracted attention. The primary function of foods is to supply the nutrients required to sustain life, and the secondary function is to satisfy taste preferences. The tertiary function of foods is to exert biological regulatory effects, such as biophylaxis, homeostatic maintenance, and disease prevention, which are activated upon food intake. Purified food ingredients that have tertiary functions are widely consumed as supplements. Multitudes of supplements are available on today's market: besides common vitamins, minerals, and amino acids, there are catechins, which are antioxidant constituents of tea (Katiyar, 2003), soy isoflavones, which have female hormone-like actions (Weijer, 2002), and docosahexaenoic acids and eicosapentaenoic acids, which decrease triglyceride levels (Tamai, 2004). Collagen is being used widely, not only in supplements but also as an ingredient of common food products such as beverages, yogurts, and breads.

Collagen is a major protein in living organisms and accounts for about one-third of all protein in mammalian bodies, including the human body. It forms an extracellular matrix that plays a role in the formation of connective tissues and acts as a scaffold for cells, but its accumulation declines with age. The majority of the collagen in the body exhibits a triple

helix structure; with heating, this structure is lost and the collagen becomes gelatin like. Moreover, as a result of enzymatic degradation that eliminates its gelation ability, the gelatin increases in solubility and becomes collagen peptides (Fig. 1), which are frequently consumed by women, in particular. Collagen peptides are consumed as a food product to supply the collagen lost from the body with age, and a substantial number of reports have shown that treatment with collagen peptides increases well-being.

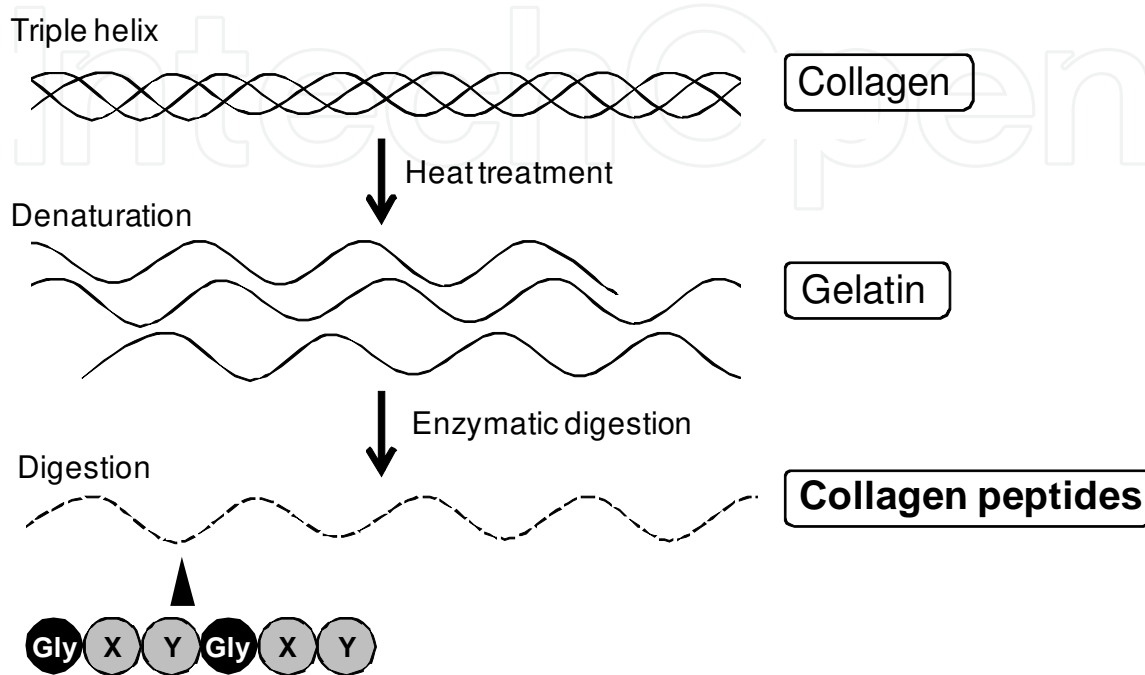


Fig. 1. Collagen in the body exhibits a triple helix structure but is denatured and becomes gelatin-like if heated. Enzyme treatment of denatured collagen produces collagen peptides, which are composed of atypical repetitions of -Gly-X-Y-Gly-X-Y- and are consumed as functional foods.

### 1.1 The tertiary function of collagen peptides

In recent years, vigorous research has been conducted to elucidate both the mechanism by which collagen peptides are absorbed from food into the body and the tertiary functions of this protein. Orally administered collagen peptides are transferred to the blood in the form of dipeptides or tripeptides, without being completely degraded to amino acids (Iwai, 2009 and Shigemura, 2009). A double-blind placebo-controlled trial has confirmed that collagen peptide treatment increases the skin's moisture content (Ohara, 2009). The primary structure of collagen consists of atypical repetitions of -Gly-X-Y- and characteristically includes hydroxyproline, which is produced by posttranslational modification. Many studies have suggested that this particular sequence enables collagen to exert multiple bioactivities, not only in skin and bones, but also in blood vessels, which contain large amounts of collagen (Arborelius, 1999). Accordingly, collagen peptides are expected to have tertiary functions additional to those already known.

### 1.2 Targeting blood vessels

The blood vessels are referred to as the largest organ in the body, because the vascular endothelial cells, which line the vessel lumens, cover an area as large as six tennis courts and

weigh 1.5 kg; they are therefore as heavy as the liver. Although blood vessels were once seen as simply the “pipes” that circulate blood, it has become increasingly clear that the vascular endothelial cells receive signals from organs and control blood supply and the secretion of various cytokines on demand (Kato, 2004).

There are many diseases caused by vascular abnormalities, especially in Japan. According to the cause-specific death rates reported by the Ministry of Health, Labor, and Welfare of Japan in 2006, death rates due to circulatory system diseases are extremely high: after malignant neoplasms (30.4%), cardiovascular diseases account for 15.9% of all deaths and cerebrovascular diseases account for 11.8%. From this perspective, protecting the blood vessels from disease should increase the quality of life of many people. We therefore took advantage of the absorbability of collagen peptides and aimed to develop ones targeting the protection of blood vessels.

### **1.3 Development of a low-molecular-weight chicken collagen hydrolysate**

Collagen peptides are generally extracted from pig skin or fish scales. However, here we used chicken legs as sources of the new collagen peptides. This was because, although gelatin is known to be allergenic, our previous study showed that the allergenicity of chicken-derived gelatin is the lowest among a number of types (Taguchi, 2002).

Chicken legs were solubilized by acid treatment and the extracted collagen was processed by proteases. The resulting low-molecular-weight collagen peptides were then dried and powdered for subsequent use as low-molecular-weight chicken collagen hydrolysate (CCH) (Saiga, 2008) (Fig. 2). Our preliminary *in vitro* experiments showed that CCH strongly inhibits angiotensin-converting enzyme (ACE). Production of angiotensin II, a vasopressor, is suppressed by the inhibition of ACE in the blood and organs, thereby resulting in a hypotensive effect (Gupta, 2010). Because hypertension is closely related to arteriosclerosis, the inhibition of blood pressure elevation is expected to have a protective effect on the blood vessels. In addition, ACE serves as a kininase II (Sharma, 2009). Because kininase II degrades bradykinin, a vasodilator, inhibition of ACE (or kininase II) by CCH causes bradykinin accumulation in the body. Bradykinin activates endothelial nitric oxide synthase (eNOS) and increases the production of nitric oxide (NO), a vasodilator. In this manner, CCH was expected to have a vasoprotective function—a novel tertiary function of foods—through its ACE inhibitory activity.

## **2. Hypotensive effects of chicken collagen hydrolysate in subjects with hypertension**

Arteriosclerosis and hypertension are closely associated with each other. If strong pressure is applied continuously to an artery because of hypertension, the arterial walls are damaged and blood cholesterol infiltrate the walls through the damaged areas and cause arteriosclerosis. In addition, advanced arteriosclerosis narrows the blood vessels and causes blood flow to deteriorate. The heartbeat is then enhanced to improve blood flow, and this causes the blood pressure to increase. In this manner, hypertension accelerates arteriosclerosis and produces a vicious cycle. If we could alleviate hypertension, we would thus also be able to ameliorate arteriosclerosis. We therefore initially conducted a clinical trial to verify the hypotensive effect of CCH in humans (Kouguchi, 2008).

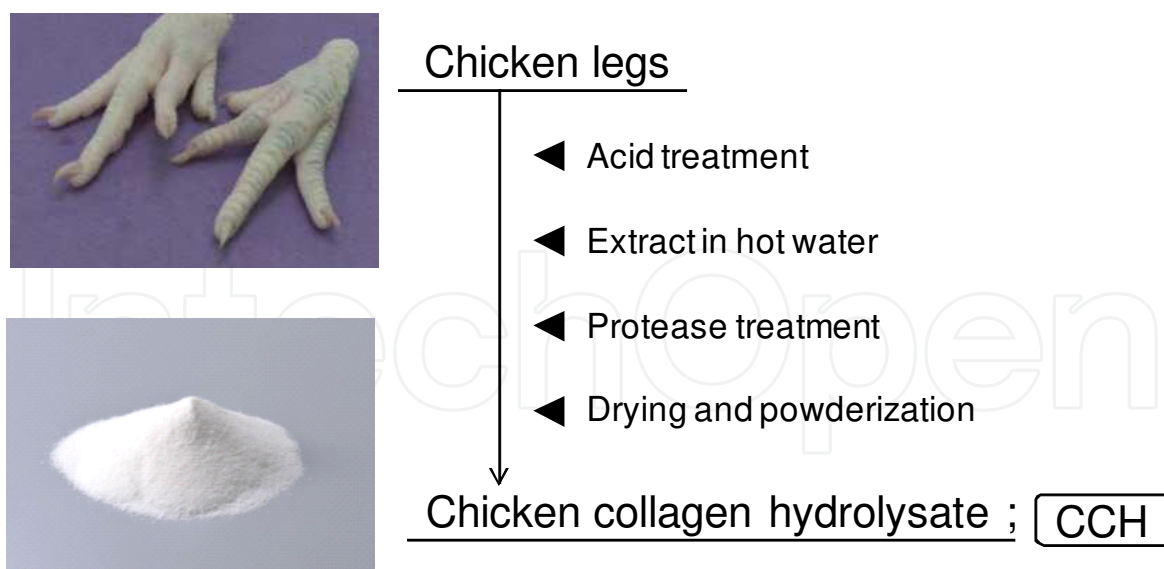


Fig. 2. Process of production of chicken collagen hydrolysate (CCH). Chicken legs are used as the basic ingredient and are treated with acid and then hot water to extract collagen, which was then processed with proteases. The resulting low-molecular-weight collagen peptides are then dried and powered for subsequent use as CCH.

## 2.1 Subjects

Subjects for the test were 120 healthy, antihypertensive drug-free, adult males and females with mild hypertension or high-normal blood pressure. The subjects (males, 59; females, 61) were randomly assigned to two groups. No significant differences in subject characteristics, including sex, age, height, body weight, body mass index, systolic blood pressure, diastolic blood pressure, and pulse rate, were observed between the two groups ( $P > 0.2$ ).

The study was approved by the institutional review board and was performed under the close supervision of the study investigators. The subjects were well informed about the test contents and methods by the study investigators, and they provided written informed consent to protect their rights in accordance with the spirit of the Declaration of Helsinki.

## 2.2 Experimental diets

A drink containing CCH (hereafter, referred to as the test food) or its counterpart without CCH (hereafter, referred to as the placebo) was used in the experiment. The test food contained 2.9 g of CCH; for the placebo, the raw material composition was the same as that of the test food, but without the CCH.

## 2.3 Trial design

The trial was designed as a placebo-controlled, double-blind, parallel-group comparison study. The study ran for a total of 18 weeks: 2 observational weeks before the treatment (pre-treatment observation period), a 12-week treatment period, and 4 weeks for post-treatment observation (post-treatment observation period). All subjects were given a bottle of drink daily during the treatment period. All subjects were directed not to change their daily diets and exercise regimens (Fig.3). They were advised strongly not to overeat, over-drink, or over-exercise.

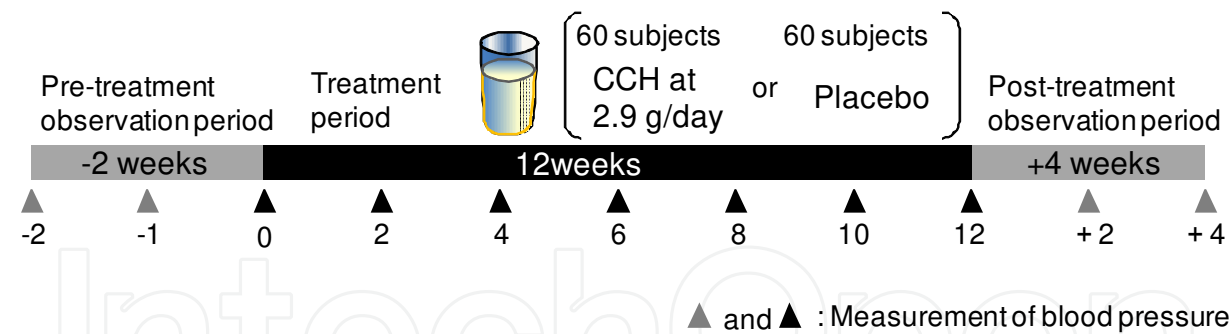


Fig. 3. Clinical trial schedule for CCH administration. The 120 subjects were assigned to two groups and given the experimental or placebo diet for 12 weeks. Blood pressure was measured a total of 11 times in the course of the experiment: twice in the pre-treatment observation period, 7 times in the treatment period, and twice in the post-treatment observation period.

2.4 Measurement of blood pressure

Blood pressure was measured a total of 11 times in the course of the experiment: twice in the pre-treatment observation period, 7 times in the treatment period, and twice in the post-treatment observation period. The subjects were kept at rest for at least 10 min before the measurement. Blood pressure in the left cubital fossa was measured while the subjects were seated. Blood pressure was measured more than once with a mercury manometer. The average value of 2 stable measurements (i.e. when the difference of the values was less than 5 mmHg) was recorded as the value recorded. Pulse rate was measured once at each visit. The subjects' condition was also interviewed by a doctor at the time of measurement of blood pressure.

2.5 Results

Systolic blood pressures in the test food group were non-significantly lower ( $P < 0.1$ ) than those of the placebo group after 2 weeks of treatment and were significantly lower ( $P < 0.05$ ) than in the placebo group after 12 weeks of treatment (Fig. 4). In the test food group, in comparison with the mean pre-treatment blood pressure (139.7 mm Hg), the blood pressure was significantly lower after 2 weeks (133.9 mm Hg;  $P < 0.001$ ), 4 weeks (135.7 mm Hg;  $P < 0.01$ ), 6 weeks (134.6 mm Hg;  $P < 0.001$ ), 8 weeks (134.4 mm Hg;  $P < 0.01$ ), 10 weeks (134.6 mm Hg;  $P < 0.001$ ), and 12 weeks (133.5 mm Hg;  $P < 0.001$ ). After 2 weeks of treatment, the blood pressure in the test food group was 135.5 mm Hg; this was non-significantly lower than the pre-treatment blood pressure ( $P < 0.1$ ). In the placebo group, blood pressure after 6 weeks of treatment (135.9 mm Hg) was significantly lower than the pre-treatment blood pressure (139.8 mm Hg) ( $P < 0.05$ ).

2.6 Discussion

Blood pressures in the test food group decreased continuously during the treatment period. Because the compositional difference between the test food and placebo in this experiment was only the presence or the absence of CCH, the observed antihypertensive effect was considered to be due to CCH treatment. We had previously confirmed that CCH exhibits ACE inhibitory activity and antihypertensive effects in rats (Saiga, 2008). The results of this study indicated that CCH had a similar antihypertensive effect in humans. Moreover, on

medical examination some subjects reported a dry cough. Dry cough is typically observed with ACE inhibitor administration and is attributed to bradykinin accumulation in the body. This raises the possibility that the CCH inhibited kininase II and thus caused accumulation of bradykinin, a vasoprotector, which then induced NO production via the stimulation of eNOS. The results suggest that CCH exerts vasoprotective effects by ameliorating blood pressure in humans.

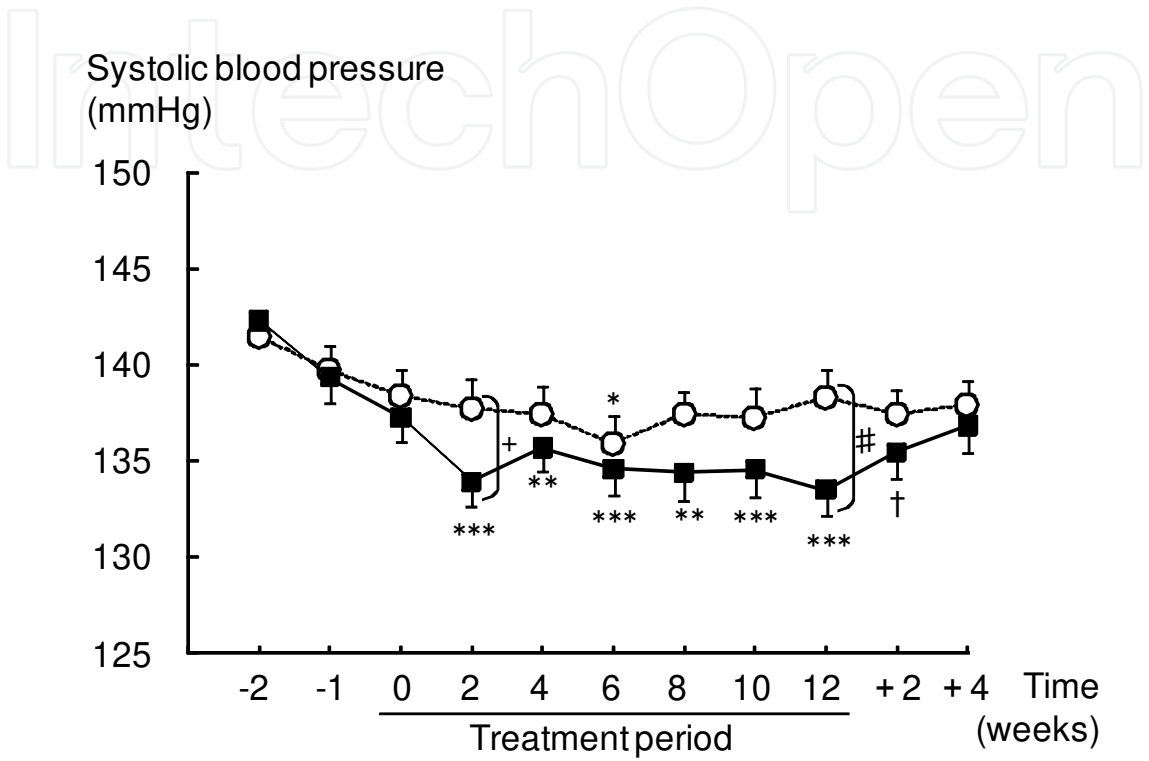


Fig. 4. Time-course of changes in systolic blood pressure in the subjects. Systolic blood pressures in the test food group were non-significantly lower than those in the placebo group after 2 weeks of treatment and were significantly lower than in the placebo group after 12 weeks of treatment. In comparison with the pre-treatment blood pressure (mean of the values at -2, -1, and 0 weeks), the blood pressure in the test food group was consistently and significantly lower throughout the treatment period. Data are mean  $\pm$  SE values. +  $P < 0.1$ , #  $P < 0.05$  versus placebo group. †  $P < 0.1$ , \*  $P < 0.05$ , \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$  versus pre-treatment blood pressure.

In our previous *in vitro* studies, we found that CCH treatment of human umbilical vein endothelial cells directly increased eNOS activation (data not shown). When eNOS expressed in vascular endothelial cells is activated, the cells produce NO. The NO functions as a signal to relax adjacent vascular smooth muscle cells; consequently, this dilates arteries and increases blood flow. Other than NO, vascular endothelial cells excrete vasoactive substances such as endothelin, a vasopressor, and maintain the balance of constriction and dilation of blood vessels. NO production via eNOS is particularly important in maintaining the homeostasis of blood vessels. Taken together, these findings indicate that oral administration of CCH improves blood pressure by inhibiting ACE and protects blood vessels by inducing NO production, thereby inhibiting the development of arteriosclerosis.

### 3. CCH treatment improves vascular endothelial function in rats and thus exerts protective effects on organs

The clinical trial described in the preceding section suggested that CCH protects the blood vessels by inducing NO production. Therefore, we next directly investigated the vasodilatory effect of CCH *ex vivo* by using rat blood vessels. We administered L-NG-nitroarginine methyl ester (L-NAME), an NO synthesis inhibitor, to rats to trigger vascular endothelial dysfunction. We then tested whether prolonged CCH treatment of the rats would restore their endothelial function (Zhang, 2010).

#### 3.1 Experimental animals

Thirty-six male WKY rats (10 weeks old) were randomly allocated to three groups. The first group (control group) received untreated chow and drinking water. The second group (L-NAME group) received L-NAME in their drinking water (0.5 g/L) for 8 weeks. The third group (L-NAME+CCH group) received L-NAME in their drinking water and CCH (2.0 g/kg daily) via a metal oral Zonde needle. All animal procedures were performed in accordance with the Animal Experimentation Guidelines of the Japanese Association for Laboratory Animal Science and were approved by the Animal Use and Care Committee of Nippon Meat Packers, Inc.

#### 3.2 Vasorelaxation assay

A vasorelaxation assay was performed on the tissue of eight or nine rats from each group after 8 weeks of treatment. The rats were anesthetized with diethyl ether and the thoracic aorta was removed. The surrounding connective tissue and fat were carefully removed from the thoracic aorta, which was then cut into 2- to 3-mm-wide rings. Segments of thoracic aorta were mounted between two steel hooks in isolated tissue chambers containing Krebs-Henseleit solution at 37 °C. The isometric tension was recorded with an isometric force-displacement transducer. After an equilibration period, L-norepinephrine bitartrate was added to cause contraction. This was followed by the addition of cumulative doses of acetylcholine chloride to the bath solution to produce relaxation. Vascular relaxation was expressed as a percentage of tension development (Fig.5).

#### 3.3 Results

After 8 weeks of treatment, the survival rate of the L-NAME group rats, which had received L-NAME in their drinking water, was 66.7% of that of the control rats. However, rats that had ingested CCH (L-NAME+CCH group) had a significantly better survival rate (91.7% of that of the control group) than the L-NAME rats ( $P < 0.05$ ) (Fig.6). During all of the experiments, monitoring revealed that the rats drank 17 to 30 mL of water and ate 16 to 30 g of chow every day, confirming that their drinking and eating patterns were unaffected by the treatment protocols. Body weight gains did not differ among groups (data not shown). We measured the vasorelaxant effects of CCH treatment after 8 weeks of treatment (Fig. 7). Treatment with acetylcholine chloride caused concentration-dependent relaxation of the thoracic aorta preparations from all groups after the preparations had been caused to contract by the addition of L-norepinephrine bitartrate. The acetylcholine chloride induced a relaxation response in the thoracic aortas from the L-NAME group (12.7% vasorelaxation); this was significantly less than that in preparations from the control group (69.5%).

Compared with that of the L-NAME group, vasorelaxation of the thoracic aortas from the L-NAME + CCH group (36.0%) was significantly improved by long-term administration of CCH ( $P < 0.05$ ).

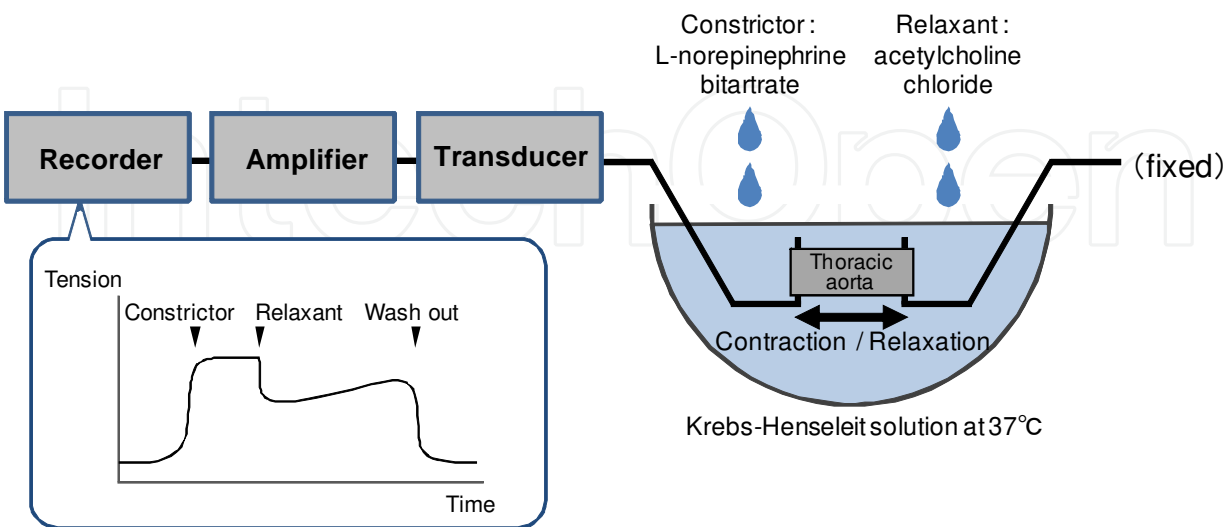


Fig. 5. Schematic of the Magnus apparatus. The excised rat thoracic aorta was cut into 2- to 3-mm-wide rings and the segments were mounted between two steel hooks in isolated tissue chambers containing Krebs-Henseleit solution at 37°C. Drops of L-norepinephrine bitartrate were then added to the tissue chamber to cause the aorta to contract. This was followed by the addition of various doses of acetylcholine chloride to trigger aortic relaxation. The electrical signals for this contraction-relaxation reaction were amplified via a transducer and recorded.

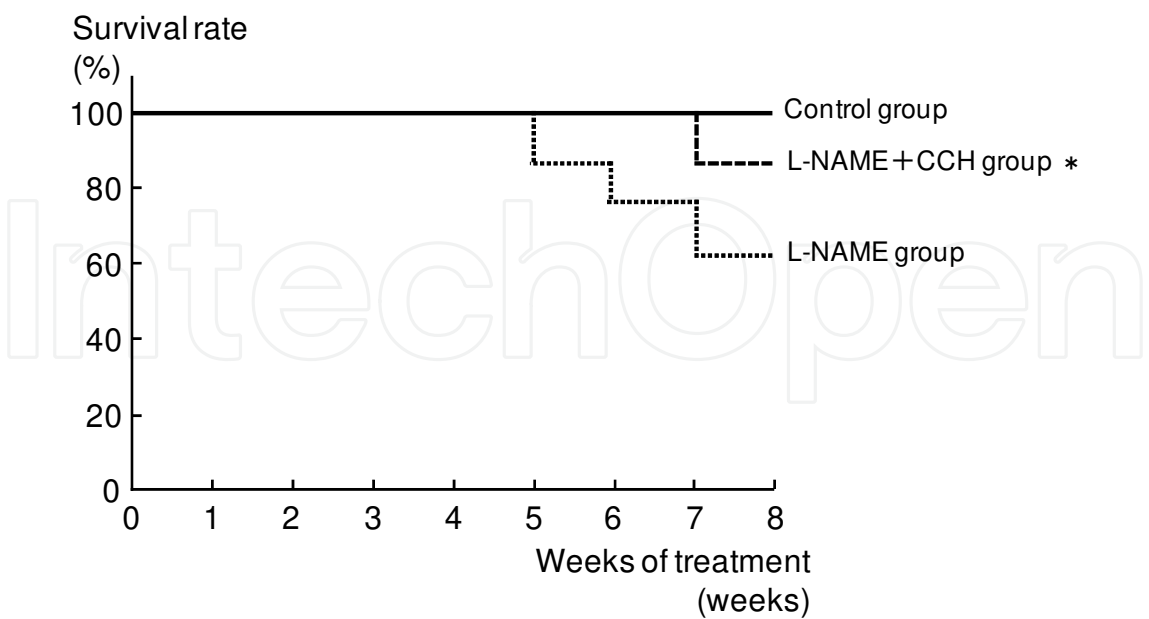


Fig. 6. Survival rates of rats during the test period. Eight weeks into the test period, the survival rate of the L-NAME + CCH group was significantly higher than that of the L-NAME group. Data are mean  $\pm$  SE values ( $n=12$  rats). \*  $P < 0.05$  versus L-NAME group.

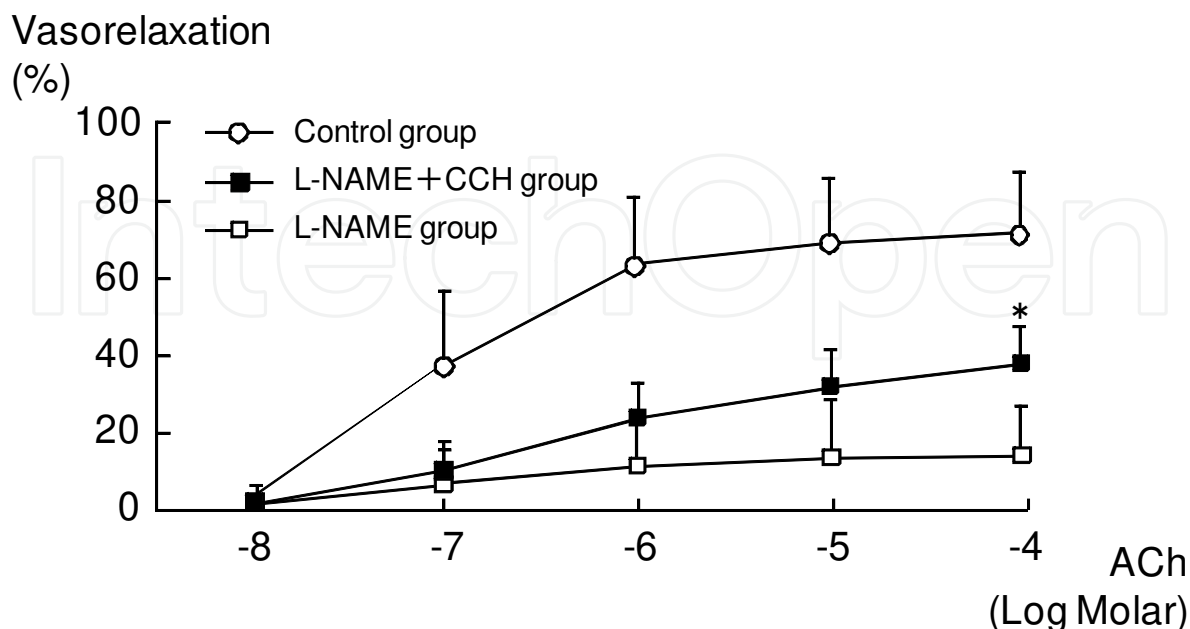


Fig. 7. Vasorelaxation of rat thoracic aortas over the 8 weeks of the test period. Treatment with acetylcholine chloride caused concentration-dependent relaxation of the thoracic aorta preparations from all groups. Especially at high acetylcholine concentrations, vasorelaxation was significantly higher in the L-NAME + CCH group than in the L-NAME group. Data are mean  $\pm$  SE values ( $n=8-9$  rats). \*  $P < 0.05$  versus L-NAME group.

### 3.4 Discussion

We found that CCH treatment improved vascular endothelial function. Acetylcholine activates eNOS expressed in vascular endothelial cells and induces NO production, thereby dilating blood vessels. In L-NAME-treated rats, the vasodilation response associated with NO production induced by an acetylcholine stimulus was inhibited; however, CCH treatment improved this response. As stated earlier, our previous studies have confirmed that CCH activates eNOS in vascular endothelial cells *in vitro*; this result was again supported by our study. In essence, therefore, CCH treatment strongly activated eNOS, promoted NO production, and thus triggered a vasodilatory response.

Moreover, the survival curves showed that the survival rate of L-NAME-treated rats was significantly enhanced by CCH administration. This may have been because CCH treatment alleviated the various organ failures caused by L-NAME-induced vascular disorders. We previously conducted the same experiment by using a higher concentration of L-NAME (1 g/L) and prepared tissue sections for observation. We identified substantial tissue damage associated with L-NAME treatment in the blood vessels, kidney, and heart; this damage was alleviated by CCH treatment (Fig. 8). The substantial fibrosis observed, especially in the heart and liver, was relieved by CCH treatment. Although further investigations of this attenuation effect of CCH treatment on tissue damage are required, we consider that it results from tissue protection via the vasoprotective effects of CCH.

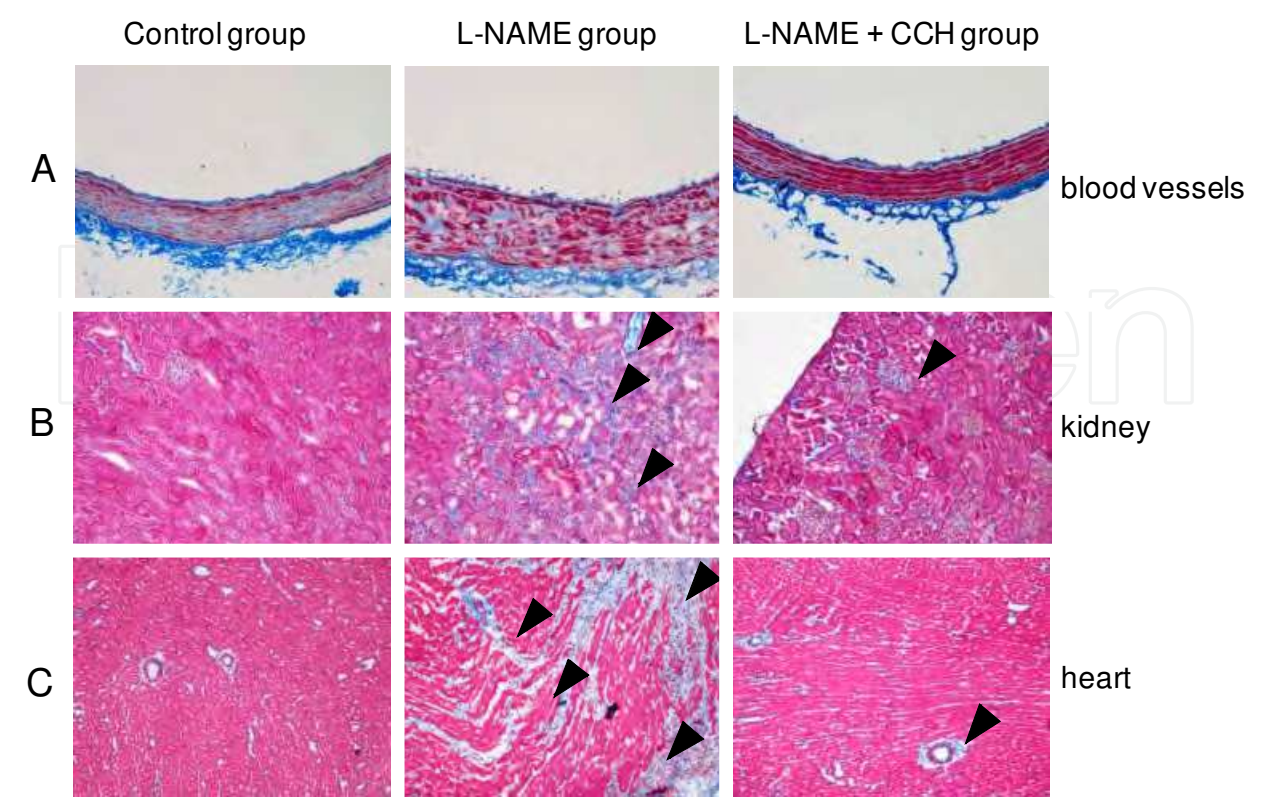


Fig. 8. Tissue sections of blood vessels (A,  $\times 160$ ), kidney (B,  $\times 80$ ) and heart (C,  $\times 80$ ) after treatment with L-NAME at a high concentration (1 g/L). Tissues were stained with Masson trichrome. These sections are from a similarly designed previous experiment of ours. Significant tissue damage caused by L-NAME was observed in the blood vessels, kidneys, and heart tissues, whereas CCH treatment alleviated these damages. Arrows indicate signs of fibrosis.

**4. CCH treatment inhibits proinflammatory cytokine expression in a mouse model of arteriosclerosis**

Previous studies have indicated that CCH exerts vasoprotective effects and thus organ protective effects. We therefore investigated the effects of CCH in an atherosclerosis mouse model, C57BL/6.KOR-ApoE<sup>sh1</sup>. This mouse is spontaneously hyperlipidemic and characteristically has high total cholesterol (TC) levels and arteriosclerotic lesions. Using this mouse model, we examined the changes in blood cholesterol levels and proinflammatory cytokine expression in response to prolonged CCH treatment (Zhang, 2010).

**4.1 Experimental animals**

Eighteen male C57BL/6.KOR-ApoE<sup>sh1</sup> mice (7 weeks old) were randomly allocated to two groups (n = 9) and fed on a normal diet or a diet supplemented with 10% CCH for 12 weeks. At the end of the 12-week experiment, the mice were sacrificed, blood was obtained from their veins, and tissues were collected for further analysis. All animal procedures were performed in accordance with the Animal Experimentation Guidelines of the Japanese Association for Laboratory Animal Science and were approved by the Animal Use and Care Committee of Nippon Meat Packers, Inc.

4.2 Measurement of plasma and hepatic lipids

Levels of TC, triglycerides (TG), low-density lipoprotein cholesterol (LDL-C), and high-density lipoprotein cholesterol (HDL-C) in the plasma and liver were determined. Total lipids extracted from the liver were also analyzed. In addition, plasma levels of interleukin-6 (IL-6), soluble intercellular adhesion molecule-1 (sICAM-1), and tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) were measured by ELISA.

4.3 Observation of tissue sections

At the end of the 12-week test period, the thoracic aorta and liver were excised from the dissected rats and were fixed in formalin, paraffin-embedded, and sliced with a microtome to prepare thin sections, which were then stained with Oil Red O or hematoxylin-eosin for histological observation.

4.4 Results

The mice were treated with CCH for 12 weeks and then sacrificed for analysis. Compared with those in the controls, the amounts of plasma TC and hepatic lipid and TG in the CCH group were reduced by 14.4%, 24.7%, and 42.8%, respectively (Table 1). However, CCH administration had no obvious influence on the concentrations of TG, LDL-C, and HDL-C in the plasma or of TC in the liver.

	Plasma (mg/100ml)				Liver (mg/g)		
	TC	TG	LDL-C	HDL-C	Lipid	TC	TG
Control	1208 $\pm$ 93	308 $\pm$ 72	678 $\pm$ 68	10.2 $\pm$ 1.5	75.3 $\pm$ 6.6	0.31 $\pm$ 0.11	4.9 $\pm$ 2.4
10% CCH	880 $\pm$ 73 *	306 $\pm$ 64	550 $\pm$ 80	11.3 $\pm$ 0.9	56.7 $\pm$ 4.8 *	0.27 $\pm$ 0.07	2.8 $\pm$ 1.5 *

Table 1. Effect of CCH treatment on plasma concentrations of TC, TG, LDL-C, and HDL-C and on hepatic total lipid, TC, and TG in C57BL/6.KOR-ApoE<sup>sh1</sup> mice at the end of the 12-week test period. Plasma TC, hepatic total lipid, and hepatic TG concentrations were significantly lower in the 10% CCH group than in the control group. Data are mean  $\pm$  SE values (n=9 mice). \*  $P < 0.05$  versus L-NAME group.

We also investigated the effects of CCH treatment on plasma proinflammatory cytokine levels in C57BL/6.KOR-ApoE<sup>sh1</sup> mice. Administration of CCH resulted in decreases in plasma levels of IL-6 (by 43.4%,  $P < 0.01$ ), sICAM-1 (by 17.9%,  $P < 0.05$ ), and TNF- $\alpha$  (by 24.1%,  $P < 0.01$ ) (Fig.9). To investigate whether CCH had a preventive and therapeutic effect on arteriosclerosis, atherosclerotic lesions in the aorta were observed by microscopy with Oil Red O staining (Fig.10). There were no obvious differences in the aortas of the CCH and control groups. We then tested whether CCH treatment had alleviated liver damage in the C57BL/6.KOR-ApoE<sup>sh1</sup> mouse model. Sections of paraffin-embedded liver were stained with hematoxylin-eosin or Oil Red O. Treatment with 10% CCH for 12 weeks decreased the abundance of diffuse lipid droplets and fat vacuoles compared with that in the control group (Fig.10).

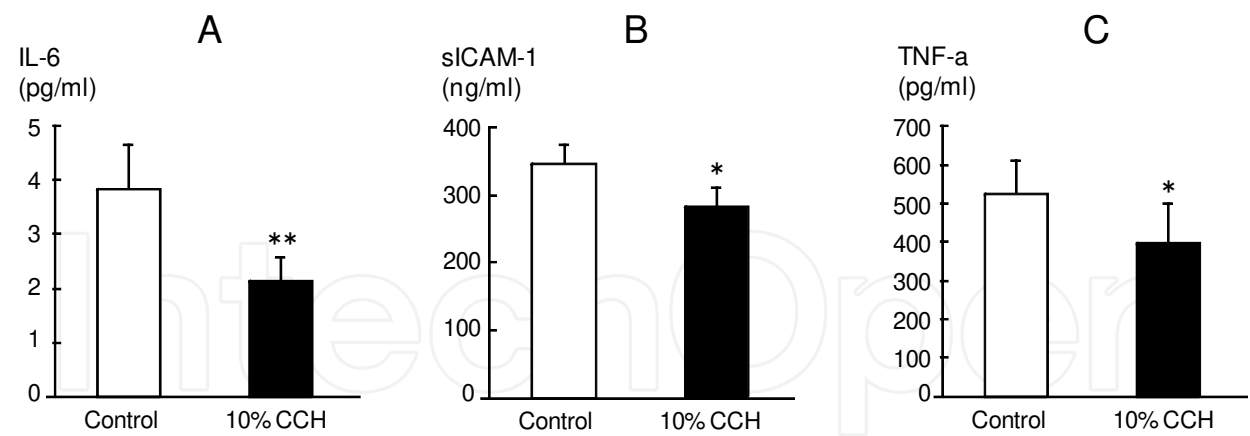


Fig. 9. Effect of CCH treatment on plasma proinflammatory cytokine levels in C57BL/6.KOR-ApoE<sup>sh1</sup> mice at the end of the 12-week test period. Interleukin-6 (IL-6) (A), soluble intercellular adhesion molecule-1 (sICAM-1) (B), tumor necrosis factor alpha (TNF-α) (C). The levels of all plasma proinflammatory cytokines were significantly lower in the 10% CCH group than in the control group. Data are mean ± SE values (n=9 mice). \*  $P < 0.05$ , \* \*  $P < 0.01$  versus control group.

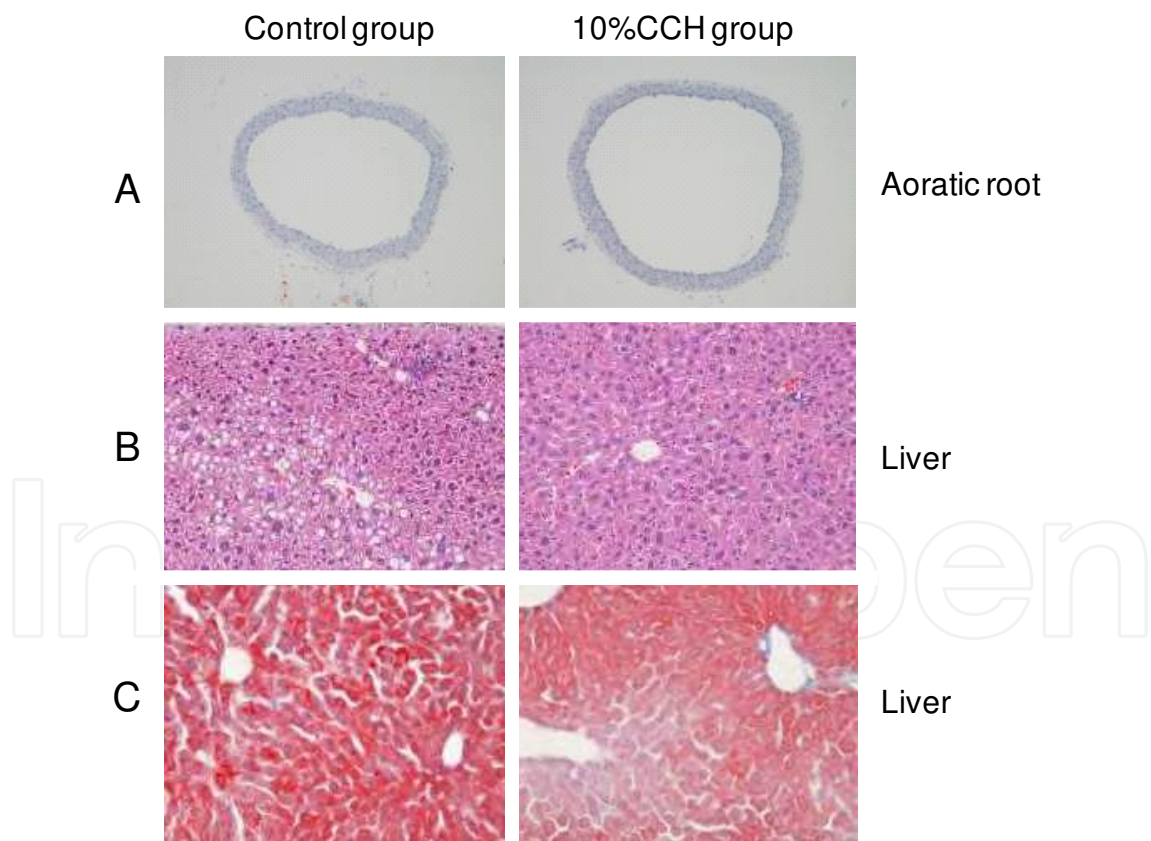


Fig. 10. Tissue sections of aortic root (A) and liver (B and C) at the end of the 12-week test period. Tissues were stained with Oil Red O (A, ×80; C, ×140) or hematoxylin-eosin (B, ×140). No obvious change was observed in the aortic root of the 10% CCH group; however, diffuse lipid droplets and fat vacuoles in the livers of the treatment group were less abundant than in those of the control group.

#### 4.5 Discussion

Our results suggested that, as well as lowering plasma TC, CCH had a lipid-lowering effect through regulation of hepatic lipid biosynthesis to suppress TG levels. In humans, collagen-specific peptides are absorbed into the blood as a result of CCH treatment (Iwai, 2009). Once absorbed into the body, the CCH peptides function as regulatory factors to influence cholesterol homeostasis. This effect may have contributed to the decrease in the abundance of lipid droplets and fat vacuoles observed in the liver tissues.

Because inflammation plays an important role in the development of arteriosclerosis, inflammatory markers were also examined to investigate the anti-inflammatory function of dietary intervention. IL-6, sICAM-1, and TNF- $\alpha$  are the major proinflammatory cytokines secreted by adipocytes. At the same time, NO inhibits the expression of these proinflammatory cytokines in the vascular endothelium. Our previous studies indicate that orally ingested CCH induces NO production in the body. Hence, the results imply that CCH treatment downregulates several proinflammatory cytokines via NO production, thereby having beneficial effects on the fat tissues. Further detailed investigations are, however, necessary to elucidate more of the direct effects of CCH on fat cells.

Unfortunately, no direct therapeutic effect of CCH on arteriosclerotic plaques was observed in this study. Nevertheless, the data demonstrated that CCH treatment substantially reduced both the total lipid content in the liver and the production of proinflammatory cytokines such as IL-6, TNF- $\alpha$ , and sICAM-1 in a mouse model highly susceptible to arteriosclerosis. High levels of expression of these factors lead to the progression of arteriosclerosis. From this perspective, long-term CCH treatment may be effective as a simple dietary, rather than drug, treatment for preventing arteriosclerosis.

#### 5. Conclusion: The availability of collagen peptides as a food providing anti-atherogenesis via a vasoprotective effect

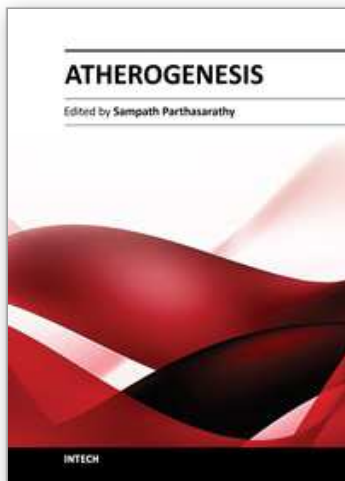
It has been frequently reported that externally applied collagen peptides help to increase water retention owing to their high water retentivity. On the other hand, the functionality of orally ingested collagen is not fully understood. However, much of the evidence reported in recent years, including the results of this study, supports the specific physiological activities of collagen absorbed by the body.

In this study, we examined the impacts of collagen peptides on blood vessels from various perspectives. We demonstrated that collagen peptides exhibit vasoprotective functions via NO production and effectively protect against atherogenesis.

Functional foods will not replace pharmaceuticals. However, what humans continue to do regularly for survival is to eat. Whereas a balanced diet obviously supports healthy life, elucidation of the tertiary function of food ingredients by precisely following their mechanisms is a long-term mission for food researchers. We focused on collagen and analyzed the whole process from development of, to research into, novel chicken-derived collagen peptides. We clarified the efficacy of vasoprotection, which is a novel tertiary function of collagen peptides. We intend to continue our efforts to demonstrate the beneficial functionalities of collagen in the hope of improving the global quality of life through the consumption of this food product.

## 6. References

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## **Atherogenesis**

Edited by Prof. Sampath Parthasarathy

ISBN 978-953-307-992-9

Hard cover, 570 pages

**Publisher** InTech

**Published online** 11, January, 2012

**Published in print edition** January, 2012

This monograph will bring out the state-of-the-art advances in the dynamics of cholesterol transport and will address several important issues that pertain to oxidative stress and inflammation. The book is divided into three major sections. The book will offer insights into the roles of specific cytokines, inflammation, and oxidative stress in atherosclerosis and is intended for new researchers who are curious about atherosclerosis as well as for established senior researchers and clinicians who would be interested in novel findings that may link various aspects of the disease.

### **How to reference**

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Tomomi Kouguchi, Youzuo Zhang, Mikako Sato, Yoshihisa Takahata and Fumiki Morimatsu (2012). Vasoprotective Effect of Foods as Treatments: Chicken Collagen Hydrolysate, Atherogenesis, Prof. Sampath Parthasarathy (Ed.), ISBN: 978-953-307-992-9, InTech, Available from:  
<http://www.intechopen.com/books/atherogenesis/vasoprotective-effect-of-foods-as-treatments-chicken-collagen-hydrolysate>

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