Effects of a Chicken Collagen Hydrolysate on the Circulation System in Subjects with Mild Hypertension or High-Normal Blood Pressure

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Received September 12, 2012; Accepted December 14, 2012; Online Publication, April 7, 2013 [doi:10.1271/bbb.120718]

Key words: collage peptides; circulation system; angiotensin converting enzyme (ACE)-inhibitory activity; brachial-ankle pulse wave velocity (baPWV); arterial stiffness

We investigated the effects of a chicken collagen hydrolysate (CCH) on the circulation system in humans. A total of 58 subjects with either mild hypertension (systolic blood pressure (SBP) of 140–159 mmHg or diastolic blood pressure (DBP) 90–99 mmHg) or high-normal blood pressure (SBP 130–139 mmHg or DBP 85–89 mmHg) were assigned to two groups, one involving a placebo and the other, the test food (including CCH of 2.9 g/d). The parameters related to each subject’s circulation system were monitored over the study period of 18 weeks. The brachial-ankle pulse wave velocity (baPWV), an indicator of arterial stiffness and marker of vascular damage, was significantly lower in the test food group than in the placebo group during the treatment period. The blood pressure in the test food group was also significantly lower than that in the placebo group, while the serum nitrogen oxide was higher in the test food group after the treatment. These results suggest that CCH exerted modulatory effects on the human circulation system.

Materials and Methods

Subjects. The subjects (30 males and 28 females) were randomly selected from among 120 people with mild hypertension (a systolic di- or tri-peptides, without complete degradation to amino acids. Furthermore, these di- or tri-peptides, which are present in human peripheral blood after ingesting collagen peptides, had physiological functions.

The growing body of evidence on the physiological functions of ingested collagen peptides prompted us to investigate their effects on the circulation system. An intake of 2.9 g/d of a chicken collagen hydrolysate (CCH), which had been extracted from chicken legs, over 12 weeks had an antihypertensive effect when compared with that on a placebo group. We also found that the administration of CCH (5.2 g/d) for 4 weeks significantly decreased SBP by 11.8 mmHg in mildly hypertensive subjects. In addition, the activation of endothelial progenitor cells in the peripheral blood was confirmed in subjects who had ingested 5.2 g of CCH daily for 4 weeks. The number of endothelial progenitor cells has been reported to be negatively correlated with the presence of coronary risk factors, and to be positively correlated with the vascular endothelial function. A rat model of cardiovascular damage has confirmed that the intake of CCH for 8 weeks improved vascular relaxation. These results suggest that a wide variety of protective effects on the circulation system, as well as antihypertensive activity, can be expected from the intake of CCH.

However, the dosage of CCH (5.2 g/d) used in our previous study was high. We found, by comparing three doses (1.4, 2.9, and 4.4 g/d) of CCH in mildly hypertensive subjects, that the most appropriate dose of CCH was 2.9 g/d. That study was also conducted without setting a placebo group. Therefore, in this present study, we performed a double-blind placebo-controlled evaluation by using a CCH dose of 2.9 g/d to test for further protective effects of CCH on the circulation system.

Materials and Methods

Subjects. The subjects (30 males and 28 females) were randomly selected from among 120 people with mild hypertension (a systolic...
blood pressure (SBP) of 140–159 mmHg or diastolic blood pressure (DBP) of 90–99 mmHg or high-normal blood pressure (SBP of 130–139 mmHg or DBP of 85–89 mmHg) who had not been treated with any antihypertensive agents.

The 58 subjects were randomly assigned to two groups on the basis of measurements determined during the observation period before administering the experimental diets. No significant differences in subject characteristics such as sex, age, body height, body weight, body mass index (BMI), SBP, DBP, and pulse rate existed between the two groups (Table 1).

The study was conducted after acceptance by the institutional review board (IRB) for clinical trial services at Soiken Inc. (Osaka, Japan) and was performed under inspection by the study investigators. The subjects were informed on the test contents and methods by the study investigators and provided their written informed consent, thus protecting their rights in accordance with the spirit of the Declaration of Helsinki.

**Experimental diets.** A lactic acid bacterial beverage containing CCH (test food) and its counterpart without CCH (placebo) were used as the experimental diets. CCH was obtained by using a procedure basically on the method of Saiga et al.\(^1\) First, gelatin was extracted from chicken legs and purified by filtration. The extracted gelatin was then degraded with proteases, sterilized, and dried. Each experimental diet was prepared in the form of a 120-mL lactic acid drink. The test food contained 2.9 g of CCH. The placebo lactic acid drink was identical to the test food but without CCH. IRB confirmed there to be no difference in taste or texture between these supplementary drinks. Table 2 shows the compositions of CCH and the experimental diets.

**Trial design.** The trial was designed as a placebo-controlled, double-blind, parallel-group comparison study. The 18-week study period comprised 2 weeks for pre-treatment observation, 12 weeks for treatment, and 4 weeks for post-treatment observation (Fig. 1). All the subjects were given a bottle of lactic acid drink daily during the treatment period. The subjects in the test-food group were given the test food containing CCH at 2.9 g/d, while the subjects in the placebo group were given the same lactic acid drink, but without CCH. All the subjects in the two groups were directed not to change their daily diets or exercise regimens. Eleven study visits were scheduled: −2 and −1 wk (the pre-treatment observation period); 0, 2, 4, 6, 8, 10, and 12 wk (the treatment period); and +2 and +4 wk (the post-treatment observation period). The subjects attended each of these visits after fasting for more than 10 h following supper on the day before the visit, and were examined and interviewed by a doctor, or by a nurse under the supervision of a doctor.

**Body height and body weight.** Body height was determined by a preliminary physical examination. Body weight was measured a total of six times: once during the pre-treatment observation period (−2 wk), 4 times during the treatment period (0, 4, 8, and 12 wk), and once during the post-treatment observation period (+4 wk). The measured values were used to calculate BMI on each day of the study visit.

**Blood pressure and pulse rate.** Blood pressure and pulse rate were determined at all visits. Blood pressure was measured more than once by using a mercury manometer. An average value of 2 stable readings was used to calculate BMI on each day of the study visit.

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### Table 1. Clinical Characteristics of the Two Subject Groups

<table>
<thead>
<tr>
<th></th>
<th>Test food group</th>
<th>Placebo group</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male/Female</td>
<td>15/14</td>
<td>15/14</td>
<td>30/28</td>
</tr>
<tr>
<td>Age (y.o.)</td>
<td>54.3 ± 9.2</td>
<td>51.2 ± 7.8</td>
<td>52.8 ± 8.6</td>
</tr>
<tr>
<td>Body height (cm)</td>
<td>162.6 ± 7.7</td>
<td>163.6 ± 9.3</td>
<td>163.1 ± 8.5</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>65.2 ± 11.3</td>
<td>66.5 ± 10.2</td>
<td>65.9 ± 10.7</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>24.6 ± 2.9</td>
<td>24.8 ± 3.0</td>
<td>24.7 ± 2.9</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>141.1 ± 8.1</td>
<td>140.6 ± 9.3</td>
<td>140.8 ± 8.6</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>86.2 ± 6.1</td>
<td>88.2 ± 7.3</td>
<td>87.2 ± 6.8</td>
</tr>
<tr>
<td>Pulse rate (beats/min)</td>
<td>66.1 ± 7.6</td>
<td>67.4 ± 8.1</td>
<td>66.7 ± 7.8</td>
</tr>
</tbody>
</table>

Values are expressed as the mean ± standard deviation.
BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure.

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### Table 2. Composition of the CCH and Experimental Diets

#### A

<table>
<thead>
<tr>
<th>Components</th>
<th>CCH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein (g/100 g)</td>
<td>91.2</td>
</tr>
<tr>
<td>Carbohydrate (g/100 g)</td>
<td>3.1</td>
</tr>
<tr>
<td>Fat (g/100 g)</td>
<td>0.0</td>
</tr>
<tr>
<td>Ash (g/100 g)</td>
<td>0.1</td>
</tr>
<tr>
<td>Water (g/100 g)</td>
<td>5.6</td>
</tr>
</tbody>
</table>

#### B

<table>
<thead>
<tr>
<th>Components</th>
<th>Test food</th>
<th>Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein (g/120 mL)</td>
<td>4.4</td>
<td>1.3</td>
</tr>
<tr>
<td>Carbohydrate (g/120 mL)</td>
<td>10.1</td>
<td>9.3</td>
</tr>
<tr>
<td>Fat (g/120 mL)</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Ash (g/120 mL)</td>
<td>0.4</td>
<td>0.3</td>
</tr>
</tbody>
</table>

CCH, chicken collagen hydrolysate

**Blood pressure and pulse rate.** Blood pressure and pulse rate were determined at all visits. Blood pressure was measured more than once by using a mercury manometer. An average value of 2 stable readings was used to calculate BMI on each day of the study visit.

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**Fig. 1.** Clinical Trial Schedule for CCH Administration.

The 58 subjects were assigned to two groups and given the test food or a placebo for 12 weeks. Eleven study visits were scheduled: −2 and −1 wk (the pre-treatment observation period); 0, 2, 4, 6, 8, 10, and 12 wk (the treatment period); and +2 and +4 wk (the post-treatment observation period). Arrowheads show the time for clinical and laboratory examinations. CCH, chicken collagen hydrolysate; baPWV, brachial-ankle pulse wave velocity.
Blood and urine examinations

All hematological and urine examinations remained within the standard ranges (data not shown). It is important to note that the serum NOx value after 12 wk was higher ($p < 0.1$) than at 0 wk in the test food group, but not in the placebo group (Fig. 4).

Consultations and interviews

There were no adverse events associated with the intake of either experimental diet.
Table 3. Changes in Blood Pressure and Pulse Rate of Subjects during the Pre- to Post-Treatment Period with the Test Food or Placebo

<table>
<thead>
<tr>
<th>Group</th>
<th>SBP (mmHg)</th>
<th>DBP (mmHg)</th>
<th>Pulse rate (beats/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test food</td>
<td>143±7.3</td>
<td>87±5.0</td>
<td>69±6.3</td>
</tr>
<tr>
<td>Placebo</td>
<td>143±7.3</td>
<td>87±5.0</td>
<td>69±6.3</td>
</tr>
</tbody>
</table>

Values are expressed as the mean ± standard deviation.

SBP: systolic blood pressure; DBP: diastolic blood pressure

Discussion

Angiotensin converting enzyme (ACE) inhibitors can reduce the vasopressive effect of angiotensin II by inhibiting the ACE-mediated biosynthesis of angiotensin II. They can also simultaneously inhibit kininase II (the bradykinin catabolic enzyme), resulting in an increase in the bradykinin level. Bradykinin promotes NO production by activating endothelial nitric oxide synthase (eNOS). NO increases the blood flow by relaxing the vascular smooth muscles and thus expanding the arteries (i.e., by the vasodilating effect); it also inhibits platelet adhesion to the vascular endothelium and platelet aggregation. In addition, angiotensin II directly promotes the proliferation of vascular smooth muscle cells. ACE inhibitors can thus be expected to have both an antihypertensive effect and vasoprotective activity that act against arteriosclerosis.

CCH activates endothelial progenitor cells and promotes vascular relaxation. Therefore we measured baPWV in 58 subjects with mild hypertension or high-normal blood pressure. baPWV is used by medical institutions as an indicator of the arterial stiffness value and as a marker of vascular damage. Those subjects with a flexible aorta show a low baPWV value, because the value reflects the aorta flexibility. baPWV is associated with aging and sex: aging is positively correlated with baPWV, and males tend to show a higher baPWV value than females. A cut-off value of 1400 cm/s has been proposed as an index of cardiovascular disease in Japan.

All baPWV values in the placebo group (right, left, and average) significantly increased with time (Table 4). baPWV is also known to be higher in the winter than in the summer. We speculate that the time-dependent increase in baPWV observed in the placebo group was due to seasonal variation, since the study was conducted from July to December. On the other hand, the baPWV values for the test food group did not significantly change (Table 4). These data suggest that the intake of CCH improved arterial flexibility more effectively than that of the placebo.

A trend toward an increase in the serum level of NOx, a metabolite of NO, was found in the test food group after 12 wk when compared with that at 0 wk (Fig. 4). This might be supported by our previous data indicating that a CCH administration increased the serum NO concentration in rats. Since NO inhibits the proliferation of vascular smooth muscle cells, CCH might facilitate the NO-induced inhibition of arteriosclerosis in humans. However, further studies are required to fully elucidate this increase in NOx level.

Vascular protection is important to improve and maintain the quality of life. The mortality rates in countries such as the USA, England, Germany, and France from cardiovascular diseases, including myocardial infarction and cerebral infarction, are greater than those from malignant neoplasms. There have been some reports on foods with vasoprotective activity: peptides derived from sardine, sesame, and milk casein had ACE-inhibitory activity, and a pine bark extract increased the blood flow and exerted a vasorelaxant effect. However, there are few reports of a diet-attributable improvement in baPWV. CCH also showed both a
Effects of Ingesting a Chicken Collagen Hydrolysate

Table 4. Changes in baPWV of Subjects during the Treatment Period with the Test Food or Placebo

<table>
<thead>
<tr>
<th>Group</th>
<th>0 wk</th>
<th>4 wk</th>
<th>8 wk</th>
<th>12 wk</th>
<th>Two-way-layout ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>baPWV (right) Test food group</td>
<td>1540.7</td>
<td>1512.1</td>
<td>1536.3</td>
<td>1543.9</td>
<td>p &lt; 0.05</td>
</tr>
<tr>
<td>(cm/s) Placebo group</td>
<td>1489.4</td>
<td>1543.5</td>
<td>1614.1</td>
<td>1567.8</td>
<td></td>
</tr>
<tr>
<td>baPWV (left) Test food group</td>
<td>1514.1</td>
<td>1514.9</td>
<td>1538.7</td>
<td>1539.4</td>
<td>n.s.</td>
</tr>
<tr>
<td>(cm/s) Placebo group</td>
<td>1490.2</td>
<td>1557.1</td>
<td>1571.5</td>
<td>1565.5</td>
<td></td>
</tr>
<tr>
<td>baPWV (average) Test food group</td>
<td>1527.6</td>
<td>1513.7</td>
<td>1537.7</td>
<td>1541.9</td>
<td>p &lt; 0.1</td>
</tr>
<tr>
<td>(cm/s) Placebo group</td>
<td>1490.0</td>
<td>1550.5</td>
<td>1566.8</td>
<td>1566.9</td>
<td></td>
</tr>
</tbody>
</table>

Values are expressed as the mean ± standard deviation. 
**p < 0.01, *p < 0.05: Significantly different from 0 wk (Bonferroni’s multiple comparison test)
†p < 0.1: Tendency for difference from 0 wk (Bonferroni’s multiple-comparison test)
n.s., not significant
baPWV, brachial-ankle pulse wave velocity

Fig. 3. Changes in ΔbaPWV of the Subjects against the Measurements at 0 wk during the Treatment Period with the Test Food and Placebo.

Fig. 4. Changes in the Serum Nitrogen Oxide (NOx) Level of the Subjects during the Treatment Period with the Test Food or Placebo.

Acknowledgments

This study was supported by the Promotion of Research Activities in the Private Sector Project of the Institute of Agricultural Machinery, part of the National Agriculture and Food Research Organization of Japan.

References

3) Shigemura Y, Iwai K, Morimatsu F, Iwamoto T, Mori T, Oda C,