Effects of the chitosan and purified salt supplements on the blood pressure, ACE, and electrolyte concentrations in middle-aged adults

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Abstract   This study evaluated the effects of the chitosan salt and purified salt on adults’ clinical
physiology by observing the changes of the systolic blood pressure (SBP), diastolic blood pressure (DBP),
serum angiotensin converting enzymes (ACE) activity, and the electrolyte concentrations after taking the
two kinds of salts. The chitosan salt was produced by mixing korean solar salt and 3% of chitosan. The
purified salt was produced using an ion exchange membrane method. The SBP and DBP for the acute
supplement of the purified and chitosan salt with the 0.2g dosage showed no difference by the time course.
This pointed out the inefficiency of the acute intake of salts on the SBP or DBP. The activity of the serum
ACE in the male and female adults which was measured 60 minutes after the chitosan intake significantly
decreased (p<0.01 and p<0.05, respectively), compared to the before supplement. On the other hand, the
ACE significantly increased after the intake of purified salt (p<0.001 and p<0.05, respectively). These
results indicated that the intake of chitosan salt presented more suppressive effect on the ACE than that of
the purified salt for both male and female adults. Moreover, the plasma sodium (Na⁺) concentration
exhibited a significantly low level after 60 minutes from the chitosan salt intake for both male and female
adults (p<0.05 and p<0.01, respectively). In the case of the intake of purified salt, while male adults
presented no significant differences in plasma sodium concentration between before and after the
supplement, female adults presented a significantly increased level (p<0.001). The plasma chloride (Cl⁻)
concentration presented no differences after the intake of the chitosan salt for both males and females.
Both male and female adults showed an increase of the plasma chloride concentration after the intake of
the purified salt (ns and p<0.01, respectively). In summary, there was no significant effect of the
supplements on the SBP and DBP but a significantly suppressive effect of the chitosan salt on the ACE
and the Na⁺ concentration for both male and female subjects.

Key Word : chitosan & purified salt, blood pressure, serum ACE, plasma Na⁺, Cl⁻
Introduction

Solar salt is an essential nutritious element in human beings because it provides essential minerals which help maintain physiological functions of the human body. Salt controls the electrolyte balance in the cell membrane, neural excitement, and muscle contraction, maintains the osmotic pressure of the body fluid, and transfers nutrients. It is also essential for better tastes of food and as one of the most effective food preservatives (1).

However, an excessive intake of salt is not recommended because it is known that an excessive salt intake is associated with hypertension and cardiovascular diseases (2, 3). It is well established that high salt intake is associated with the occurrence of hypertension (4). Moreover, much interest has focused on the association between insulin resistance and hypertension in high salt diet (5, 6, 7). The major reason for the salt-related diseases is not due to salt itself, but to the harmful substances, which are included in salt. This perhaps results from the contamination of the sea, where industrial and domestic wastes are discharged without any filtering. An existing law prohibits direct production of solar salt and encourages the refining process, which extracts only sodium chloride, using an ion exchange membrane method. However, there is an upsurge of interest in salt as functional food that considers the safety and health, due to the issue that minerals, which are beneficial components for the human body, are removed in the process of salt refining.

Hypertension, which accounts for 15-20% of adult diseases in Korea, can lead to complications, such as cerebral hemorrhage, heart disease, and kidney disease. In particular, chronic hypertension damages blood vessels and becomes a major cause of cerebral apoplexy. Essential hypertension, which refers to the hypertension with no identifiable cause and accounts for 75-90% of the hypertension cases, is assumed its cause as an excessive intake of salt. Therefore, people diagnosed with hypertension is restricted an excessive intake of salt (8). However, continuous research on this issue is required because some studies reported that salt doesn’t have a relationship with hypertension, or with its related diseases (9, 10).
Table salt (NaCl) plays an important role as an essential mineral for the extracellular fluid maintaining the amount of body fluid. The Cl ion in salt activates digestive enzymes, such as pepsin and alpha-amylase, and Na ion facilitates intestinal absorption of the digestive materials. Table salt increases the appetite, and is an essential mineral for digestion and absorption. In addition, salt affects human growth and development according to the amount of intake.

There still exists a controversy about the relationship between table salt and hypertension, and the question about which ion (Na ? or Cl ?) is a major factor for hypertension. During the initial stage of the studies, Ambard & Bearjard (11) reported that the Cl ion of the table salt was a major component of hypertension. However, Dahl (12) found that the Na ion presented a valid evidence as a cause of hypertension in his experiment using salt-sensitive rats.

In an experiment about Na ion ; 1) When the amount of table salt decreased in meals, the blood pressure decreased. When the amount of table salt increased, the blood pressure increased. 2) The decreased blood pressure, which was caused by a decrease in table salt intake, did not change by intaking chloride substances such as ammonium chloride, which did not contain sodium. 3) The excessive intake of table salt led to the increase in the blood pressure. However, the limitation of the Na ion, that is, substituting table salt with potassium chloride and ammonium chloride, didn’t lead to the high blood pressure.

In recent years, some researchers suggested that an excessive intake of table salt leads to sodium disorder in kidney, which again leads to the increase in the blood pressure, the increase in the amount of body fluid, and the extracellular fluid. The results provide the basis of the sodium-hypertension theory. However, the basis of this theory presents weak theoretical backgrounds which are contracted by the stronger theory, the chloride-hypertension theory.

Hiromichi (13) found that seven healthy male adults whose ages ranged from 20 to 55 increased their blood pressure after 1 hour from the meals with highly salted food (table salt of 13g/ 1100kcal/meal), but their increased blood pressure decreased when the highly salted meals were supplemented with 4 g of
chitosan in an experiment conducted a week after. Sodium and potassium, which are the major positive 
(+) ions in blood, showed little change by the highly salted food, but HCO₃ in arterial blood decreased, 
which was caused by the increase of negative (-) ion, chlorine. After the intake of chitosan, the negative 
ion did not increase and the ACE activity, which had increased after 1 hour from the intake of highly 
salted food, was suppressed by the chitosan. As proved in the animal test, it was explained that chitosan 
supplement decreased the blood pressure by lowering the activity of ACE that was caused by the 
decrease of the Cl concentration in blood.

The relationship between table salt and hypertension is still in controversy. Even though 
limiting the table salt intake is an available way to prevent and cure hypertension, more studies are 
demanded to uncover which ion is the major factor for the disease.

This study evaluates the effects of the chitosan salt and purified salt on adults’ clinical 
physiology by observing the changes of the systolic blood pressure (SBP), diastolic blood pressure (DBP), 
serum angiotensin converting enzymes (ACE) activity, and electrolyte concentrations in male and female 
adults by the acute supplement of the chitosan and purified salt (0.2g/kg.bw/day).

Material and Methods

Subjects. The subjects participating in this study consisted of 10 males and 10 females. Subjects were 
performed repeatedly testing by 1 week interval according to the test conditions. The subcategories are as 
follows: 1) male chitosan supplement group, MCGS; 2) female chitosan supplement group, FCSG; 3) 
males purified salt supplement group, MPSG; 4) female purified salt supplement group, FPSG. The 
medical and physical conditions of the subjects were examined before the experiment. Table 1 presents 
the physical characteristics of all subjects.
### Table 1. Physical characteristics of the subjects

<table>
<thead>
<tr>
<th>Item</th>
<th>Group</th>
<th>Age [yrs]</th>
<th>Weight [kg]</th>
<th>Height [cm]</th>
<th>%fat</th>
<th>Suppl. Amount [g]</th>
<th>BP [mmHg]</th>
<th>SBP</th>
<th>DBP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MCSG (n=10)</td>
<td>41.30 ± 4.11</td>
<td>72.58 ± 8.45</td>
<td>72.92 ± 6.83</td>
<td>23.94 ± 3.75</td>
<td>14.52 ± 1.69</td>
<td>121.50 ± 6.69</td>
<td>81.00 ± 7.38</td>
<td></td>
</tr>
<tr>
<td></td>
<td>FCSG (n=10)</td>
<td>36.50 ± 2.68</td>
<td>55.54 ± 6.98</td>
<td>157.30 ± 4.28</td>
<td>27.96 ± 5.88</td>
<td>11.11 ± 1.41</td>
<td>104.00 ± 7.38</td>
<td>64.00 ± 7.38</td>
<td></td>
</tr>
<tr>
<td>t-value</td>
<td></td>
<td>3.094**</td>
<td>4.914***</td>
<td>6.126***</td>
<td>1.823</td>
<td>4.914***</td>
<td>5.557**</td>
<td>5.152***</td>
<td></td>
</tr>
</tbody>
</table>

Values are mean and standard deviation (Mean±SD)

** abbr. > **, p<0.01; ***, p<0.001 significant difference between MCSG and FCSG

MCSG, Male Chitosan supplement group; FCSG, Female Chitosan supplement group;

%fat, fat percent; BP, Blood pressure; SBP, Systolic blood pressure; DBP, Diastolic blood pressure

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**Materials** The materials used in this study were chitosan salt and purified salt. The chitosan salt was produced by the addition of 3% of chitosan to the solar salt produced in Docho in Shinsan, South Korea. Purified salt was produced using an ion exchange membrane from the ocean water.

**Supplement method of Chitosan and Purified salt** The acute supplement amount of the chitosan and purified salt was given as part of their meal with the amount of 0.2g/kg/day per person. The amount of acute supplement was referred to a previous study (14) which supplemented bamboo salt with the amount of 14g/70kg/day (0.2g/kg/day). The supplement was provided twice a day if necessary. All subjects were advised to avoid excessive exercise 12 hours before the test and to avoid any beverage, which contained caffeine.
The tests were conducted twice at 1-week interval; the first for chitosan salt and then purified salt after one week. A counter balanced design was utilized in order to prevent a contamination effect from repeated tests.

**Measurement of blood pressure** The blood pressure of all subjects was measured after taking an enough rest in the laboratory. Then, the chitosan salt was administrated twice during a day. The SBP and DBP were measured immediately after the supplement and every 30 minutes after for 2 hours. One week after the test, the blood pressure was repeatedly measured for the supplement of purified salt using the same method used in the test of chitosan salt. The blood pressure monitor used in this study was a sphygmomanometer (Japan) of mercury type, and the blood pressure classification was referred to using the standard of National Institute of Health (NIH), USA (15).

**Blood collection and analysis** The blood sampling was conducted after 12 hours of fasting and 60 minutes after the supplement. In order to separate the serum and plasma, 10 ml of blood was collected from the antecubital vein of the subjects using a disposable syringe and a vacutainer tube (Becton Dickinson Vacutainer), which was treated by the ethylenediaminetetraacetic acid (EDTA). The collected blood was divided into 5 ml, respectively. The collected blood samples were centrifuged using a centrifuge (Hanil 5000, Korea) at 3,000 rpm for 15 minutes. The separated supernatant was moved into an eppendorf tube and stored in a freezer, which was maintained at -80°C until the analysis.

An automatic blood chemistry analyzer (Vitro, DT 60 II Chemistry System, Johnson & Johnson, USA) was used to analyze the Na⁺ and Cl⁻ concentrations of the blood plasma of the samples. A spectrophotometer was used to analyze the serum ACE by measuring the absorbance at 228 nm. Data were obtained from duplicate experiments.
Statistics  The data obtained from the study was processed using the SPSS statistical package (V. 11.01). In addition, the mean and standard deviation (Mean and SD) for the variables were produced and the one-way ANOVA were conducted to examine the differences between chitosan and purified salt according to the time course of the male and the female subjects. Independent samples t-tests were run to examine the differences between the groups, and paired samples t-tests were utilized to compare the differences between before and after the supplement. In addition, post-hoc tests (Newmann-keuls) were conducted with the variables that presented a significant difference from the ANOVA. Values of p<0.05 were considered statistical significant.

Results and Discussion

This study analyzes the changes in the SBP, DBP, serum ACE activity, and electrolyte concentrations in the blood plasma according to the time course after supplementing the purified salt and chitosan salt.

Table 2 showed the changes of the SBP according to the intake of chitosan salt and purified salt for middle-aged male and female subjects. The SBP of the MCSG presented a small increase 30 minutes after the supplement compared to the pre-supplement. However, this increase was not significant, and the values decreased gradually between 60 minutes and 120 minutes, where the decrease was also not significant. These tendencies were similar to that of the MPSG. From these results, it was concluded that an acute intake of salt didn’t significantly increase the SBP of male subjects. There were no significant difference between the MCSG and the MPSG according to the time course. Similarly, there was no significant difference in the SBP according to the time course for the case of the FCSG and FPSG. However, the SBP presented a significant difference between the male and the female subjects, in which the male subjects showed higher values than female subjects for all periods.
Table 2. The changes of the systolic blood pressure following the time course for the chitosan salt and purified salt supplement in both males and females

<table>
<thead>
<tr>
<th>Time Group</th>
<th>pre suppl.</th>
<th>-30min.</th>
<th>-60min.</th>
<th>-90min.</th>
<th>-120min.</th>
<th>F-value</th>
<th>post-hoc</th>
</tr>
</thead>
<tbody>
<tr>
<td>MCSG</td>
<td>121.50</td>
<td>128.00</td>
<td>124.50</td>
<td>119.50</td>
<td>118.50</td>
<td>1.553</td>
<td>ns</td>
</tr>
<tr>
<td></td>
<td>±6.69</td>
<td>±10.33</td>
<td>±11.17</td>
<td>±10.12</td>
<td>±10.29</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MPSG</td>
<td>124.50</td>
<td>131.50</td>
<td>127.50</td>
<td>126.50</td>
<td>125.00</td>
<td>0.885</td>
<td>ns</td>
</tr>
<tr>
<td></td>
<td>±8.96</td>
<td>±10.55</td>
<td>±8.58</td>
<td>±9.14</td>
<td>±9.43</td>
<td></td>
<td></td>
</tr>
<tr>
<td>t-value</td>
<td>0.849</td>
<td>0.750</td>
<td>0.674</td>
<td>1.623</td>
<td>1.473</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>P</td>
<td>0.408</td>
<td>0.463</td>
<td>0.510</td>
<td>0.122</td>
<td>0.158</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>FCSG</td>
<td>104.00***</td>
<td>113.00**</td>
<td>105.00***</td>
<td>105.50**</td>
<td>108.50'</td>
<td>1.726</td>
<td>ns</td>
</tr>
<tr>
<td></td>
<td>±7.38</td>
<td>±11.59</td>
<td>±8.49</td>
<td>±7.98</td>
<td>±7.84</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FPSG</td>
<td>108.50***</td>
<td>116.30**</td>
<td>109.50**</td>
<td>108.00'</td>
<td>110.00'</td>
<td>1.477</td>
<td>ns</td>
</tr>
<tr>
<td></td>
<td>±6.69</td>
<td>±9.44</td>
<td>±8.32</td>
<td>±9.19</td>
<td>±9.72</td>
<td></td>
<td></td>
</tr>
<tr>
<td>t-value</td>
<td>1.429</td>
<td>0.698</td>
<td>1.197</td>
<td>0.650</td>
<td>0.380</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>P</td>
<td>0.170</td>
<td>0.495</td>
<td>0.247</td>
<td>0.524</td>
<td>0.709</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Values are mean and standard deviation

Abbr> ns; not significant

*, p<0.05; **, p<0.01; ***, p<0.001 Significant difference between male and female

MCSG; Male Chitosan salt supplement group, MPSG; Male Purified salt supplement group,

FCSG; Female Chitosan salt supplement group, FPSG; Female Purified salt supplement group

Table 3 showed the changes of the DBP of the male and female subjects according to the supplement of chitosan and purified salt. The DBP values didn’t present a significant difference in either male or female subjects according the time course for the acute supplement of chitosan and purified salt.
The acute intake of salt didn’t affect the DBP of the subjects. However, the DBP values of the male subjects were higher than those of the female subjects similar to the SBP.

Table 3. The changes of the diastolic blood pressure following the time course for the chitosan salt and purified salt supplement in both males and females

<table>
<thead>
<tr>
<th>Time Group</th>
<th>pre suppl.</th>
<th>-30min.</th>
<th>-60min.</th>
<th>-90min.</th>
<th>-120min.</th>
<th>F-value</th>
<th>post-hoc</th>
</tr>
</thead>
<tbody>
<tr>
<td>MCSG</td>
<td>81.00 ± 7.38</td>
<td>89.00 ± 14.49</td>
<td>83.50 ± 12.03</td>
<td>80.00 ± 11.30</td>
<td>79.00 ± 10.22</td>
<td>1.247 ns</td>
<td></td>
</tr>
<tr>
<td>MPSG</td>
<td>88.80 ± 14.22</td>
<td>91.50 ± 14.35</td>
<td>84.00 ± 9.66</td>
<td>85.00 ± 9.72</td>
<td>84.00 ± 10.22</td>
<td>0.802 ns</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>t-value</th>
<th>P</th>
<th>Values are mean and standard deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.540</td>
<td>0.141</td>
<td>**, p&lt;.01; ***, p&lt;.001 Significant difference between male and female</td>
</tr>
<tr>
<td>0.388</td>
<td>0.703</td>
<td>@, p&lt;.05 significant difference between FCSG and FPCG</td>
</tr>
<tr>
<td>0.102</td>
<td>0.920</td>
<td>No significant difference between chitosan and purified salt supplement, ns; no significant</td>
</tr>
<tr>
<td>1.061</td>
<td>0.303</td>
<td></td>
</tr>
<tr>
<td>1.094</td>
<td>0.288</td>
<td></td>
</tr>
<tr>
<td>0.746</td>
<td>0.015</td>
<td></td>
</tr>
<tr>
<td>0.501</td>
<td>0.623</td>
<td></td>
</tr>
<tr>
<td>0.172</td>
<td>0.866</td>
<td></td>
</tr>
<tr>
<td>0.990</td>
<td>0.335</td>
<td></td>
</tr>
<tr>
<td>0.250</td>
<td>0.806</td>
<td></td>
</tr>
<tr>
<td>0.159</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.015</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Fig. 1 and Fig. 2 showed the changes in the activity of angiotensin converting enzyme (ACE) before the supplement and 60 minutes after the supplement of chitosan and purified salt, respectively, for male (Fig. 1) and female (Fig. 2) adults. In the case of the male subjects, the acute intake of chitosan salt presented a significantly decreased level ($p<0.01$) of the activity of ACE 60 minutes after the supplement compared to the before supplement. On the contrary, the acute intake of purified salt presented a significantly increased level ($p<0.001$). This indicated that the intake of chitosan salt suppressed the ACE, compared to the purified salt. In the case of the female subjects, the results didn’t show a significant difference 60 minutes after the supplement of chitosan salt even though they showed a decreased level like the male case. However, the ACE presented a significant increase ($p<0.05$) 60 minutes after the purified salt supplement. This indicated that the intake of chitosan salt suppressed the ACE in both male ($p<0.01$) and female (ns) subjects. However, there was no significant difference between the male and the female subjects for the serum ACE activity.

Fig. 1. The alteration in the ACE activities following the acute chitosan salt and purified salt supplement in males

*Abbr* > **, $p<0.01$; ***, $p<0.001$ significant difference between before suppl. and after 60min.

*ns*; not significant
Fig. 2. The alteration in the ACE activities following the acute chitosan salt and purified salt supplement in females

* abbr: * p<0.05; significant difference between before suppl. and after 60min.

ns: not significant

Fig. 3 and Fig. 4 showed changes in the plasma Na\(^+\) concentration according to the supplement of purified and chitosan salt in the male and female adults. The plasma sodium (Na\(^+\)) concentration of both male and female subjects for the supplement of chitosan salt presented a significantly decreased level (p<0.05 and p<0.01, respectively) 60 minutes after the supplement compared to the before supplement. Contrary to the chitosan salt, the plasma sodium concentration of the male and female subjects for the supplement of purified salt presented an increased level 60 minutes after the supplement compared to the before supplement (ns and p<0.001, respectively). The concentration values presented significant differences 60 minutes after the supplement between chitosan and purified salt for both the male and the female subjects (p<0.01 for the males and p<0.001 for the females). This result presented
different effects on the plasma Na\textsuperscript{+} concentration according to the two different kinds of salts. However, there was not a significant difference between the male and female subjects for all the treatments.

![Graph showing Na\textsuperscript{+} concentration following chitosan salt and purified salt supplement in males.](image)

**Fig. 3.** The Na\textsuperscript{+} (mmol/l) concentration following the chitosan salt and purified salt supplement in males

* **abbr**: * p<0.05; significant difference between before suppl. and after 60 min. p<0.01; significant difference between before suppl. and after 60 min of intake.

**ns**: not significant
Fig. 4. The Na⁺ (mmol/l) concentration following the chitosan salt and purified salt supplement in females

*abr* **, *p*<0.01; significant difference between before suppl. and after 60min.

*ns*: not significant

Fig. 5 and Fig. 6 showed changes in the plasma Cl⁻ ion concentration. The plasma chlorine (Cl⁻) concentration presented no significant difference between the before and after the supplement for both chitosan and purified salt, in the case of the male subjects. In addition, there was no significant difference between the chitosan and purified salt. In the case of the female subjects, while the plasma chlorine concentration presented a decreased level for the supplement of the chitosan salt (ns), the concentration presented a significantly increased level (*p*<0.01) 60 minutes after the supplement of the purified salt. There was a significant difference (*p*<0.001) in the chlorine concentration between the supplement of chitosan and purified salt 60 minutes after the supplement.
Fig. 5. The Cl⁻ (mmol/l) concentration following the chitosan salt and purified salt supplement in male

ns: not significant
Fig. 6. The Cl\textsuperscript{-} (mmol/l) concentration following the chitosan salt and purified salt supplement in females

abbr\textsuperscript{> **, p<0.01; significant difference between before and after 60min.}

ns: not significant

Chitosan is a kind of polysaccharide, which exists in nature, and has recently drawn interests as a new functional substance. Invertebrate animals (shell fish, such as shrimp and crab, insects, and various other animals and fish) contain chitosan. Chitin, the polysaccharide polymer from which chitosan is derived, is a kind of mucopolysaccharide, which is produced by the bond of N-acetyl-glucosamine with beta-1-4glycoside. It has a chemical structure that was replaced the oxalic acid from the C-2, a glucose residue of cellulose, with acetylamino radical. Chitosan has a chemical structure removed of the acetyl radical that exists in chitin (16). That is, chitosan is derived from chitin by deacetylation using highly concentrated alkali. Chitin is obtained by removing calcium using HCl after removing protein from shellfish such as shrimp and crap using NaOH (16). Kas (17) reported that chitosan has biodegradability
and safety as a source of polymer. Researchers suggested that the derivatives from chitin and chitosan presented various bioactivities, such as antitumor action improving the immune function and regeneration, antivirus, antimold activation, cholesterol control, and hypertension suppression (18, 19, 20).

Based on the prior studies, this study evaluated the effects of the chitosan salt comparing it to the purified salt on clinical physiology by observing the changes of the systolic blood pressure (SBP), diastolic blood pressure (DBP), serum angiotensin converting enzymes (ACE) activity, and the electrolyte concentrations in male and female adults by the intake of the chitosan and purified salt.

Many studies found that an excessive acute intake of salt increased the blood pressure. The increased blood pressure is due to the fact that the sodium ion, which exists in salt, absorbs excessive water into the blood vessel. Hypertension, a disease that 15-20% of Korean adults are exposed to, is a major cause of cerebral apoplexy because it damages blood vessels (21). Therefore, an excessive intake of salt by the patients with hypertension is not recommended. However, further research on this issue is required because some studies reported that salt doesn’t have a significant effect on hypertension and its related diseases (11, 12).

In the current study, the SBP in the four testing of the two groups (MCSG, MPSG, FCSG, and FPSG) presented relatively increased levels 30 minutes after the supplement of chitosan salt, compared to the before supplement (ns). The changes decreased gradually after 60 minutes until 120 minutes after the supplement (ns). The results showed that an excessive acute intake of salt presented no significant increase in the level of the SBP for both purified and chitosan salt. Similarly, the DBP values didn’t present a difference by the time course in either male or female subjects according to the acute supplement of chitosan and purified salt. From these results, the acute intake of salt didn’t affect the SBP and DBP changes. The DBP of the male subjects presented a higher level than that of the female subjects, similarly to the SBP.

The results from the study were a little different that of the results from previous studies. Kato & Hiromichi (22) reported that there was an increase in the blood pressure 1 hour after the intake of
highly salted food in their clinical study of chitosan where highly salted food (table salt, 13gram/1,100kcal/meal) was served to seven male adults. One week after the test, however, they found that there was no increase in the blood pressure when they supplemented the highly salted food with 4g of chitosan. As a result, the intake of chitosan presented a suppressive effect on the blood pressure. Another study (23) was conducted with laboratory animals for 8 weeks, which investigated the changes of the blood pressure at 1 week intervals according to the different test conditions: supplement of lowered salted food, chitosan salt supplemented food, and regular endurance exercises. They found that a 0% lowered salted food supplement group presented no significant decrease in the blood pressure through the test. On the contrary, a group, which was provided with 3% chitosan salt supplemented food, presented 2 mmHg decrease in the blood pressure (from 134.97±7.87 mmHg to 132.90±7.62 mmHg) during the test. In addition, a group, which provided with 3% chitosan salt supplemented food along with the endurance exercises during the test period, showed 3 mmHg of the blood pressure decrease (from 134.83±7.05 mmHg to 131.82±6.33 mmHg). There was no significant difference in the blood pressure between the group with the intake of 3% table salt supplemented food and the group with the intake a 3% table salt supplemented food along with endurance exercises (23). These results indicate that while the supplement of the table salt resulted in an increase in the blood pressure, chitosan salt supplement decreased the blood pressure.

The activity of the serum ACE for the male adults after the intake of chitosan salt significantly (p<0.01) decreased after 60 minutes from the supplement compared to the before supplement. However, the intake of the purified salt increased the ACE level at a significant level (p<0.001). As a result, the intake of chitosan salt presented a more suppressive effect for the ACE than that of the purified salt for males. In the case of female adults, the activity of the serum ACE for the intake of chitosan salt showed a decreased level 60 minutes after the supplement (ns), but the purified salt showed an increased level (p<0.05). These results indicate that the intake of chitosan salt suppresses the ACE in both the male and
the female subjects. In addition, there was no significant difference in the ACE according to the sex of the subjects.

In a previous study, Kato (24) reported that the SBP of the salt-susceptible rats increased by the supplement of high sodium chloride, but that the SBP didn’t increase when sodium bicarbonate and sodium amino acid were administered instead of the table salt. This result indicates that a negative ion was related to the outbreak of hypertension. He also found that there was no increase in the blood pressure by administering alginic acid to the normal rats and hypertensive rats that were cultivated with highly salted food with some vegetable fibers for several weeks, even though the excrement with sodium increased. However, the chlorine concentration in the blood decreased according to the supplement of chitosan with chlorine-increased excrement (24). The suppressive effect of chitosan on the blood chlorine concentration was similar to the results of this study, in which chitosan was applied to human beings. As a result, we conclude that the intake of chitosan suppressed the activity of ACE by reducing the chlorine concentration in the blood, which lead to the blood pressure suppression. In this study, the plasma \( \text{Na}^+ \) and \( \text{Cl}^- \) ion concentration presented a decreased level after 60 minutes of the chitosan salt intake in both male and female subjects. However, the intake of purified salt increased the \( \text{Na}^+ \) and \( \text{Cl}^- \) ion concentration. This result suggests that the supplement of chitosan salt was a major factor for the suppression of blood pressure.

As demonstrated in this study, the supplement of chitosan salt presented a more effective means to suppress the ACE activity in order to control the increase in the blood pressure than that of purified salt. In addition, this study propose that the supplement of chitosan salt is healthy and safe for human body because chitosan exhibited positive effects on the blood pressure suppression as exhibited in both the \( \text{Na}^+ \) and \( \text{Cl}^- \) ion concentration.
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