Letter to the Editor

Potential Effects of Desalinated Seawater on Arteriosclerosis in Rats

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To evaluate the potential risk of arteriosclerosis caused by desalinated seawater, Wistar rats were provided desalinated seawater over a 1-year period, and blood samples were collected at 0, 90, 180, and 360 days. Blood calcium, magnesium, and arteriosclerosis-related indicators were investigated. Female rats treated with desalinated seawater for 180 days showed lower magnesium levels than the control rats (P < 0.05). The calcium and magnesium levels in female rats and the magnesium level in male rats were lower than the levels in the controls, following treatment with desalinated seawater for 360 days (P < 0.05). Blood levels of arteriosclerosis-related lipid peroxidation indicators and C-reactive protein (CRP) in the treatment group did not differ from those in the controls. The levels of lipid peroxidation indicators and CRP in rats were not significantly affected by drinking desalinated seawater, and no increase in risk of arteriosclerosis was observed.

Key words: Desalinated seawater; Lipid peroxidation; CRP; Arteriosclerosis

In many regions, particularly those with rising populations and declining rainfall, fresh water demand exceeds the available supply. To help meet the demands for potable water, facilities for desalinating seawater have been constructed in many countries. Owing to the use of reverse osmosis technology, desalinated water contains little or no calcium (Ca) or magnesium (Mg)⁴. Research has shown that reduced Mg and Ca intake due to drinking desalinated water could potentially increase the risk of cardiac abnormalities and elevated C-reactive protein (CRP) levels¹. A 2009 meta-analysis of case-control and cohort studies examining the possible relationship between water hardness and cardiovascular mortality concluded that the concentration of Mg in drinking water is inversely related to cardiovascular mortality (pooled odds ratio of 0.75, P < 0.001)⁵.

Previous studies have shown that low Mg levels and lipid peroxidation are directly correlated⁴, and this correlation is an important mechanism underlying arteriosclerosis. In addition, Mg levels indirectly affect Ca influx, potentially via potassium channels. Under normal circumstances, an imbalance between Ca and Mg levels may be an important risk factor for arteriosclerosis⁵.

To evaluate whether long-term consumption of desalinated seawater with low Ca and Mg levels would lead to arteriosclerosis, we conducted a 1-year study using experimental rats to explore the effects of drinking desalinated seawater on arteriosclerosis markers. In this study, we mainly analyzed the potential effect of desalinated seawater on some important factors for arteriosclerosis. In addition to blood Ca and Mg levels, the levels of representative antioxidant enzymes [superoxide dismutase (SOD), glutathione peroxidase (GSH), and malondialdehyde (MDA)] and oxidized low-density lipoprotein (ox-LDL, an important risk factor of the formation and development of arteriosclerosis) were measured to determine the effects of desalinated seawater on lipid peroxidation in rats. CRP is an inflammatory marker intrinsically linked to arteriosclerosis, and researchers have suggested that CRP is the most useful predictive marker of future cardiovascular risks⁶. Therefore, in our study, CRP was also considered an important marker for the effects of desalinated seawater on arteriosclerosis.

Animal Grouping and Exposure  One hundred

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and sixty Wistar rats of clean grade (80 males and 80 females) weighing 200 ± 10 g were used in this study. They were supplied by the Institute for Laboratory Animal Resources of the China Institutes for Food and Drug Control [license number: SCXK (Beijing) 2014-0013] and kept in the clean animal room of the China Center for Disease Control and Prevention [license number: SYXK (Beijing) 2014-0043]. The rats were randomly divided into two groups based on their body weight, with males and females constituting each half of each group respectively. The treatment group was provided desalinated seawater, while the control group was provided municipal water. The rats were also fed normal rat chow and allowed to drink water freely (desalinated seawater or municipal water). The rats were kept at room temperature (25 °C), and the room temperature and under a 12:12 light: dark cycle.

**Blood Sample Collection and Testing**

Samples were collected one day before the start of the experiment (0 d), and 90 (90 d), 180 (180 d), and 360 (360 d) days after the start of the experiment. At each time-point, the rats were fasted, but were allowed to drink water for one day prior to sample collection. For each group, 20 rats (males and females constituted each half of the group, respectively) were selected and blood was collected via the cardiac puncture method. At least 5 mL whole blood was collected, of which 1 mL was placed in a blood collection tube containing anticoagulant for Ca and Mg determination. The rest of the blood (4 mL or more) was placed in a blood collection tube without anticoagulant. The coagulated blood samples were centrifuged to obtain serum, which was further aliquoted for use in different marker tests.

**Determination of Blood Ca and Mg Levels in Rats**

Whole blood (0.04 mL) was added to the whole blood diluent (specifically used for the MBS element analyzer, Beijing Persee General Instrument Co., Ltd.) and mixed well. The Ca and Mg levels in blood were analyzed using a MBS element analyzer, and the data are expressed in Figure 1.

**Determination of Levels of Lipid Peroxidation Indicators and CRP**

The kits used for superoxide dismutase (SOD), glutathione (GSH), and malondialdehyde (MDA) detection were obtained from the Nanjing Jiancheng Bioengineering Research Institute. The levels of these enzymes were analyzed using a Spectral Scanning Multimode Reader (Thermo Scientific Varioskan Flash). The levels of oxidized low-density lipoprotein (ox-LDL) and CRP in rats were determined using ELISA and an automated microplate reader (BIO-RAD Model 550). The reagents for ELISA kit were supplied by Adlitteram Diagnostic Laboratories (ADL, USA), and the data are expressed in Figure 2.

**Statistical Analysis**

Results were expressed as the $x \pm s$. Statistical analyses were performed by a one-way analysis of variance (ANOVA) and means of the groups were compared by Fischer’s least significant difference (LSD) test. $P < 0.05$ was considered statistically significant.

**Detection and Evaluation of Water Sample**

The desalinating seawater sample was detected according to the ‘Standard examination method of drinking water’ (GB/T 5750-2006), and the results are shown in the Supplemental Table 1 (available in, www.besjournal.com). The pH of desalinating seawater was 6.94 (faintly acid), the total dissolved solid concentration in water was 186 mg/L, and the total hardness was 13 mg/L. These values are in compliance with ‘Standard of living drinking water’ (5,749-2,006). Of the 73 toxicological parameters of desalinating seawater detected, the Boron content was 1.3 mg/L, which was higher than the limit (limit is ≤ 0.5 mg/L), and the Fluoride content was 0.02 mg/L, which was lower than the limit (limit is ≤ 1 mg/L). The other test results were lower than the detection limit or not detected.

**Effects of Drinking Desalinated Seawater on the Blood Levels of Ca and Mg in Rats**

The blood levels of Ca and Mg in rats were measured at all four time-points. As shown in Figure 1, female rats given desalinated seawater for 360 d showed lower blood Ca levels than female rats in the control group ($P < 0.05$). However, male rats given desalinated seawater for the same time-period did not exhibit statistically significant differences compared to those in the control group. Female rats given desalinated seawater for 180 d and 360 d, as well as male rats given desalinated seawater for 360 d showed significantly lower blood Mg levels than their corresponding controls ($P < 0.05$).

**Effects of Drinking Desalinated Seawater on the Blood Levels of Lipid Peroxidation Markers in Rats**

The levels of the two important antioxidant enzymes SOD and GSH, the lipid peroxidation product MDA, and ox-LDL are shown in Figure 2. Female and male rats given desalinated seawater showed no significant statistically differences in the levels of SOD, GSH, MDA, and ox-LDL at any time-point when compared to their corresponding controls. Thus, it was concluded that administering desalinated water
in rats for 360 days would not have a significant effect on the levels of blood lipid peroxidation.

**Effects of Drinking Desalinated Seawater on the Blood CRP Level in Rats** As shown in Figure 3, female and male rats given desalinated seawater at any time-point showed no statistically significant differences in their CRP levels compared to their corresponding controls. Hence, it was concluded that administering desalinated seawater in rats for 360 days would not affect their blood CRP levels.

Owing to the widespread utilization of desalinated seawater as residential drinking water, assessment of the effects of desalinated seawater on health has garnered attention. Various studies have suggested that desalinated seawater could increase the risk of cardiovascular diseases, because it is a type of ‘soft water’ containing low levels of Ca and Mg\(^7\)\(^8\). Arteriosclerosis is a common cardiovascular

**Figure 1.** Effects of drinking desalinated seawater on the blood levels of Ca and Mg in rats (n = 10). *Significant compared to Municipal water group at P < 0.05.

**Figure 2.** Effects of drinking desalinated seawater on the blood SOD (A), GSH (B), MDA (C), and ox-LDL (D) levels in rats (n = 10).
Disease with a high incidence rate. Dyslipidemia, lipid peroxidation, endothelial dysfunction, and inflammatory cytokines can directly or indirectly lead to the formation and development of arteriosclerosis. In this study, we found that the lipid peroxidation indicators (SOD, GSH, MDA, and ox-LDL) and CRP levels of rats were not significantly affected after drinking desalinated seawater for 1 year. Some lipid parameters, which are common clinical indicators of atherosclerosis, were detected in our previous study, and we concluded that long-term consumption of desalinated water had some influence on blood lipid levels in female rat. However, these changes were not enough to induce arteriosclerosis. Early studies have shown that drinking water containing low levels of Mg might increase MDA levels in experimental animals, as well as CRP levels. These results are inconsistent with the findings of the present study. We surmise that this inconsistency might arise from the different levels of Mg in the seawater samples. Thus, Mg levels in our seawater were not sufficient to increase the levels of MDA and CRP.

In conclusion, drinking desalinated seawater did not cause arteriosclerosis in rats as determined through the analysis of changes in levels of blood lipid peroxidation markers and inflammatory cytokines at various time-points over a 360-day period. Because of the differences between humans and experimental animals, as well as the limitation of short experimental period (one year; no life), related uncertainty factors should be considered in the process of adopting the results for risk assessment, and a comprehensive analysis combining people data should be conducted.

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Figure 3. Effects of drinking desalinated seawater on the blood CRP level in rats (n = 10).
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### Attached List. The general chemical index result of the desalination seawater sample

<table>
<thead>
<tr>
<th>Parameter</th>
<th>turbidity degree (NTU)</th>
<th>pH</th>
<th>Fe (mg/L)</th>
<th>Dissolved Solid Concentration (mg/L)</th>
<th>Total Hardness (mg/L)</th>
<th>Oxygen Consumption (mg/L)</th>
<th>Manganese (mg/L)</th>
<th>Chloride (mg/L)</th>
<th>Sulfate (mg/L)</th>
<th>Na (mg/L)</th>
<th>Anion Synthetic Detergent (mg/L)</th>
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</thead>
<tbody>
<tr>
<td>Desalinating Seawater</td>
<td>0.18</td>
<td>6.49</td>
<td>&lt;0.01</td>
<td>186</td>
<td>13</td>
<td>0.83</td>
<td>&lt;0.005</td>
<td>115</td>
<td>2.15</td>
<td>69</td>
<td>&lt;0.10</td>
</tr>
<tr>
<td>Limitation</td>
<td>≤1</td>
<td>6.5~8.5</td>
<td>≤0.3</td>
<td>≤1000</td>
<td>≤450</td>
<td>≤3</td>
<td>≤0.1</td>
<td>≤250</td>
<td>≤250</td>
<td>≤200</td>
<td>≤0.3</td>
</tr>
</tbody>
</table>
