Short Communication

Korean solar salts reduce obesity and alter its related markers in diet-induced obese mice

Jaehyun Ju¹, Jia-Le Song², Eui-Seong Park³, Myoung-Sool Do³ and Kun-Young Park¹,4§

¹Department of Food Science and Biotechnology, Cha University, 335 Pan-gyo-ro, Bundang-gu, Seongnam, Gyeonggi 13488, Korea
²Department of Food Science and Nutrition, Pusan National University, Busan 46241, Korea
³School of Life Science, Handong Global University, Pohang, Gyeongbuk 37554, Korea
⁴Chongqing Collaborative Innovation Center for Functional Food, Chongqing University of Education, Chongqing 400067, People's Republic of China

INTRODUCTION

Table salt is an essential condiment and is available as solar salt, purified salt, and processed salts such as bamboo salt, all of which have unique mineral content and processing method [1]. Solar salt, defined as “a crystalline material obtained from sea water by natural evaporation in salt fields,” contains 92.4-94.4% of sodium chloride, and other minerals including potassium, magnesium, calcium, while purified salt, which is manufactured by evaporation of sea water after dialytic condensation, is 99.9% sodium chloride [1-3]. The mineral compositions of solar salts depend on the salt field [4], the duration of aging and manufacturing process [5]. Fleur de Sel, solar salt from Guerande, France, is abundant in minerals like calcium, magnesium, and potassium. However, solar salt produced in Korea contains more minerals than European salt [6]. Solar salt possesses anti-cancer properties in sarcoma-180-transplanted mice, lower mutagenic effect in comparison with purified salt and anti-hypertensive effect in Dahl salt-sensitive rats [7-9]. Obesity is characterized as an abnormal increase in body fat due to dysregulated energy homeostasis; untreated obesity is associated with type 2 diabetes, hyperlipidemia, atherosclerosis, hypertension and even osteoarthritis [10-12]. Bamboo salt, possibly owing to higher concentration of minerals (potassium, calcium and sulfur) than other commercially-available salt, has an anti-obesity effect [13], while differences in suppression of obesity by the different origin of Korean solar salts have not been researched. Thus, we have selected representative solar salts from different salt fields in Korea, along with Guerande solar salt from France and purified salt, which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.
to be tested and compared for anti-obesity effects and related markers in the serum and tissues in mice with diet-induced obesity (DIO).

MATERIALS AND METHODS

Samples, preparation of the experimental diet, and mineral analyses

Solar salt was obtained from different salt fields as follows; Y Corporation (Tae-an-gun, Chungcheongnam-do, South Korea; SS-Y), T Corporation (Shin-an-gun, Jeollanam-do, South Korea; SS-T), S Corporation (Shinan-gun, Jeollanamdo, South Korea; SS-S), and Sas Bourdic Co. (Guerande salt; Batz-sur-Mer, France; SS-G). Purified salt was provided from H Corporation (Ulsan, South Korea; PS).

Each salt sample was mixed into a high-fat diet (45% calories from fat; TD.06415. Harlan Laboratories, Madison, WI, USA) at 0.47% (wt/wt). The daily dosage administered was equivalent to 5 grams for a 60-kilogram human [14]. AIN-76A, which was administered to Normal group, was purchased from Samtaco (Osan, Gyeonggido, Korea).

Salts samples, heat-dried at 60°C for 12 hrs, were analyzed for mineral composition by ICP-OES (inductively coupled plasma optical emission spectrometry) using an Optima 8300 unit (Perkin-Elmer, Waltham, MA, USA), in accordance with EPA method 6010 [15].

Animal study

Male C57BL/6J mice (6 weeks old, 16-18 g) were purchased from Samtaco Bio Korea (Gyeonggi-do, South Korea). After obtaining study approval from the Institutional Animal Care and Use Committee (PNU-IACUC; PNU-2015-0889) of Pusan National University in Busan (Korea), animals were housed under a 12-h light/dark cycle at room temperature with ad libitum access to food and water. Animals were randomly divided into eight groups of ten mice per group as follows; a chow-diet-fed group (Normal), an HFD-fed group (Control), and HFD + PS, HFD + SS-T, HFD + SS-Y, HFD + SS-S, and HFD + SS-G groups. DIO was induced in all groups except for a Normal group feeding with a high fat diet (45% calories from fat and 0.47% (wt/wt) of salt). The daily dosage administered to Normal group, was purchased from Samtaco (Osan, Gyeonggido, South Korea; PS).

At the end of the 16-week experimental period, mice were sacrificed without fasting [17], and tissues were harvested, weighed, snap-frozen in liquid nitrogen, and stored at -80°C until required. Plasma was obtained from blood collected from individual aortas by centrifugation at 3,000 × g for 10 min and stored at -80°C until analyzed.

Serum levels of triglyceride, total cholesterol, leptin, and insulin

Serum levels of triglyceride (TG), and total cholesterol (TC) were determined using commercial assay kits purchased from Asan Pharmaceutical (Seoul, South Korea). Serum insulin and leptin levels were also determined using commercial ELISA kits (R&D Systems, Minneapolis, MN, USA).

RESULTS

Analyses of serum lipids and hormones

Serum levels of triglyceride, total cholesterol, leptin, and insulin were determined. Triglyceride and total cholesterol levels were higher in the HFD group compared to the Normal group. Leptin levels were also significantly higher in the HFD group compared to the Normal group.

Molecular analysis of gene expression

RT-PCR was performed to analyze gene expression levels. The expression levels of PPARα, CPT-1, FAS, and PPARγ were significantly lower in the HFD group compared to the Normal group. The expression levels of PPARα, CPT-1, FAS, and PPARγ were significantly higher in the HFD + SS-G group compared to the HFD group.

Statistical analysis

Results are presented as means ± SDs. The significance of differences between mean values were assessed using one-way ANOVA with Duncan's multiple range test. Null hypotheses of no difference were rejected if P-values were less than 0.05. The analysis was performed using SAS v 9.1 software (SAS Institute Inc., Cary, NC, USA).

CONCLUSION

Solar salt from different regions was administered to mice to evaluate its anti-obesity effects. The results showed that solar salt from different regions had different effects on anti-obesity. The HFD + SS-G group showed the most significant anti-obesity effects among the groups tested.
Table 1. Changes in body weight, food efficiency ratio, and EAT weight after treatment with solar salts

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>Body weight</th>
<th>Food efficiency ratio (FER)</th>
<th>EAT weight (grams)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial (grams)</td>
<td>Week 8 (grams)</td>
<td>Week 16 (grams)</td>
</tr>
<tr>
<td>Normal¹</td>
<td>238 ± 0.6b</td>
<td>29.1 ± 2.2a</td>
<td>346 ± 1.8a</td>
</tr>
<tr>
<td>Control²</td>
<td>238 ± 0.7</td>
<td>40.9 ± 2.0a</td>
<td>487 ± 1.7a</td>
</tr>
<tr>
<td>HFD + PS²</td>
<td>238 ± 0.8</td>
<td>42.3 ± 2.9a</td>
<td>483.3 ± 3.4a</td>
</tr>
<tr>
<td>HFD + SS-G²</td>
<td>240.0 ± 0.5</td>
<td>41.7 ± 4.2a</td>
<td>493 ± 4.2a</td>
</tr>
<tr>
<td>HFD + SS-Y²</td>
<td>239.6 ± 0.6</td>
<td>42.8 ± 1.6a</td>
<td>49.1 ± 2.1b</td>
</tr>
<tr>
<td>HFD + SS-T²</td>
<td>243 ± 0.6</td>
<td>42.5 ± 2.3b</td>
<td>46.3 ± 2.7b</td>
</tr>
<tr>
<td>HFD + SS-S²</td>
<td>240 ± 0.9</td>
<td>42.1 ± 1.8a</td>
<td>46.2 ± 1.3a</td>
</tr>
</tbody>
</table>

¹ Values are presented as means ± SD (n = 10).
² Not significant.
³ Normal: chow diet; Control: high-fat diet (HFD; 45% calories from fat); HFD + PS: HFD supplemented with 0.47% of purified salt; HFD + SS-G: HFD supplemented with 0.47% of solar salt from G Co.; HFD + SS-Y: HFD supplemented with 0.47% of solar salt from Y Co.; HFD + SS-T: HFD supplemented with 0.47% of solar salt from T Co.; HFD + SS-S: HFD supplemented with 0.47% of solar salt from S Co.

Table 2. Changes in triacylglycerol (TG) and total cholesterol (TC) induced by the 8-week administration of salt in an HFD

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>TG (mg/dl)</th>
<th>TC (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal¹</td>
<td>154.5 ± 2.2c</td>
<td>100.5 ± 1.7f</td>
</tr>
<tr>
<td>Control²</td>
<td>164.2 ± 2.2a</td>
<td>189.5 ± 3.2c</td>
</tr>
<tr>
<td>HFD + PS²</td>
<td>164.2 ± 2.2a</td>
<td>189.5 ± 3.2c</td>
</tr>
<tr>
<td>HFD + SS-G²</td>
<td>143.5 ± 0.7d</td>
<td>205.3 ± 1.0a</td>
</tr>
<tr>
<td>HFD + SS-Y²</td>
<td>142.6 ± 3.6e</td>
<td>196.3 ± 2.1a</td>
</tr>
<tr>
<td>HFD + SS-T²</td>
<td>134.2 ± 2.2d</td>
<td>163.1 ± 1.1a</td>
</tr>
<tr>
<td>HFD + SS-S²</td>
<td>120.2 ± 1.4a</td>
<td>165.1 ± 1.1a</td>
</tr>
</tbody>
</table>

¹ Values are presented as means ± SD (n = 10).
² Not significant.

Serum analysis; triacylglycerol, total cholesterol, leptin and insulin

Among the salt-administered groups, the HFD + SS-S group had significantly lower TG levels (164.5 ± 2.2 mg/dl) than Control (160.5 ± 1.7 mg/dl) (Table 2).

The lowest TC concentration among the salt-administered groups occurred in the HFD + SS-T group (163.1 ± 1.1 mg/dl), followed by the HFD + SS-G group (165.1 ± 1.1 mg/dl) and HFD + PS groups (189.5 ± 3.2 mg/dl). Conversely, the HFD + PS group showed a higher TG level (164.5 ± 2.2 mg/dl) than Control (160.5 ± 1.7 mg/dl) (Table 2).

The leptin concentrations in salt-administered groups were significantly lower than in the Control (4.8 ± 0.3 ng/mL). The HFD + SS-T (3.1 ± 0.3 ng/mL) and the HFD + SS-S (3.3 ± 0.1 ng/mL) group had the lowest leptin level, followed by HFD + PS, HFD + SS-Y and HFD + SS-G (Fig. 1A).

Administration of HFD significantly increased the serum concentration of insulin (17.2 ± 0.1 ng/mL; P < 0.05) versus the Normal group (6.9 ± 0.7 ng/mL). The lowest insulin concentration among the salt-administered groups was observed in the HFD + SS-G group (13.0 ± 0.4 ng/mL; P < 0.05). On the other hand, HFD + SS-Y group had the highest insulin among the salt-administered groups (17.2 ± 0.1 ng/mL) (Fig. 1B).

Table 3. Mineral proportions of salt samples determined by ICP-OES

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>Na (% dry weight)</th>
<th>Mg (% dry weight)</th>
<th>K (% dry weight)</th>
<th>Ca (% dry weight)</th>
<th>S (% dry weight)</th>
<th>Zn (% dry weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PS¹</td>
<td>38.90 ± 0.56a</td>
<td>37.87 ± 0.39a</td>
<td>57.97 ± 1.21a</td>
<td>30.30 ± 0.59a</td>
<td>34.61 ± 0.72a</td>
<td></td>
</tr>
<tr>
<td>SS-G¹</td>
<td>38.90 ± 0.56a</td>
<td>37.87 ± 0.39a</td>
<td>57.97 ± 1.21a</td>
<td>30.30 ± 0.59a</td>
<td>34.61 ± 0.72a</td>
<td></td>
</tr>
<tr>
<td>SS-Y¹</td>
<td>38.90 ± 0.56a</td>
<td>37.87 ± 0.39a</td>
<td>57.97 ± 1.21a</td>
<td>30.30 ± 0.59a</td>
<td>34.61 ± 0.72a</td>
<td></td>
</tr>
<tr>
<td>SS-T¹</td>
<td>38.90 ± 0.56a</td>
<td>37.87 ± 0.39a</td>
<td>57.97 ± 1.21a</td>
<td>30.30 ± 0.59a</td>
<td>34.61 ± 0.72a</td>
<td></td>
</tr>
<tr>
<td>SS-S¹</td>
<td>38.90 ± 0.56a</td>
<td>37.87 ± 0.39a</td>
<td>57.97 ± 1.21a</td>
<td>30.30 ± 0.59a</td>
<td>34.61 ± 0.72a</td>
<td></td>
</tr>
</tbody>
</table>

¹ Values are presented as means ± SD (n = 3).
² Means with the different letters in each row are significantly different (P<0.05) by Duncan’s multiple range test.
³ Not significant.
⁴ PS: purified salt; SS-G: Guerande solar salt; SS-S: solar salt from Y Co.; SS-T: solar salt from T Co.; SS-G: Guerande solar salt;

mRNA expression of adipogenic-/lipogenic- and β-oxidation-related factors in the liver and EAT

The hepatic expression of peroxisome proliferator-activated receptor gamma (PPARγ) in the Control was 4.04 fold higher than the Normal group, but its expression was suppressed in the HFD + SS-S (0.00 fold) and HFD + SS-T (0.53 fold) versus the Control. HFD + SS-Y adversely boosted its mRNA expression by 1.95 fold higher than the Control. The expression of fatty acid synthase (FAS), in comparison with the Control, was suppressed in the HFD + SS-T (0.33 fold), HFD + SS-S group (0.63 fold), while HFD + SS-Y and HFD + PS showed higher expression levels of 1.41 fold and 1.35 fold of Control, respectively. The strongest expression level in HFD + PS group was the lowest among the salt-administered groups (0.68 fold) (Fig. 2A).
Fig. 1. Changes in serum concentrations of (A) leptin and (B) insulin by administration of salt samples to diet-induced obese mice. *Results are presented as means ± SDs (n=3). Means with the different letters on the bars are significantly different (P < 0.05) by Duncan's multiple range test.

Fig. 2. Changes in mRNA expression of obesity-related factors in (A) liver and (B) EAT by administration of salt samples to diet-induced obese mice. *Results are presented as means ± SDs (n=3). Means with the different letters on the bars are significantly different (P < 0.05) by Duncan's multiple range test.
The strong suppression in expression of C/EBPα of EAT, in comparison with the Control (47.5 folds of Normal), occurred in the HFD + SS-T group (0.05 folds) and HFD + SS-S (0.37 fold). Conversely, HFD + SS-Y (1.23 fold) and HFD + PS group (2.19 folds) had higher expression levels than the Control. The lowest expression level of PPARγ, in comparison with the Control (16.5 folds of Normal), occurred in HFD + SS-T group (0.03 fold) and HFD + SS-S (0.29 fold). Conversely, HFD + PS (1.16 fold) and HFD + SS-Y (1.25 fold) groups had higher levels than Control (P < 0.05).

In addition, the expression level of PPARα in Control was 0.84 fold that in Normal group. However, expression levels of HFD + SS-S and HFD + SS-T groups were higher than HFD + PS, HFD + SS-G and HFD + SS-Y (Fig. 2B).

**DISCUSSION**

Administration of solar salt samples from different salt fields resulted in different changes in body weight gain, FER, and weight of EAT. SS-S and SS-T suppressed those factors more strongly than SS-Y, PS and even SS-G. Weight gain suppression was stronger in the SS-T and SS-S groups than other salt groups, even surpassing Normal.

The food efficiency ratio (FER) can be used as a scale of obesity, and a low FER is a powerful predictor of reduction of obesity without possible anorectic activity [16]. It may thus be inferred that administration of Korean solar salt in a DIO model, especially HFD + SS-T and HFD + SS-S, suppresses weight gain and reduces serum lipid without changing dietary intake.

HFD-administered mice generally showed higher TG and TC concentration. Mice in HFD + SS-S and HFD-SS-T group, however, had significantly lower serum TG and TC concentration than Control. The present study shows that Korean solar salts, especially SS-S and SS-T, significantly reduced leptin and insulin concentrations, which suggests they may be able to suppress TG accumulation in adipose tissue by their unique chemical features. Clinically, high sodium intake is associated with a high serum leptin concentration, high serum insulin concentration, and insulin resistance [19,20]. Owing to low sodium content and Na/K ratios, Korean solar salts may suppress the progress of obesity and eventually contribute to reductions in serum insulin concentrations. PPARγ is expressed in liver and adipose tissues, either alone or in cooperation with C/EBPα via a positive feedback mechanism, and induces the transcription of adipogenic genes like FAS [21,22]. Therefore, reduction in expression of these factors is of high importance; fortunately, SS-T and SS-S produced that reduction. On the other hand, PPARα induces the transcription of β-oxidation-related factors such as CPT-1 [23]. Significant liver weight reduction was achieved in HFD + SS-T (2.1 ± 0.2 g; P < 0.05) and HFD-SS-S (2.2 ± 0.1 g; P < 0.05) in comparison with HFD-fed group (2.7 ± 0.1 g) (data not shown).

Based on these findings, Korean solar salt, especially SS-S and SS-T, suppresses the expression of adipogenic- and lipogenic factors (PPARγ, C/EBPα, and FAS), promotes the expression of β-oxidative factors (PPARα and CPT-1), and reduces fat accumulation in the liver and EATs.

Rats on a high sodium diet (3.12% Na+) have more adipose tissue mass than those fed a normal sodium diet (0.5% Na+) [24]. A strong association exists between obesity and urinary Na/K ratio, and a high intake of sodium and a low intake of potassium are positively associated with a high total body fat percentage [25]. Solar salts in Korea, especially SS-S and SS-T, have a lower Na/K ratio than other solar salts, so these specific features of Korean solar salts might have partially contributed to anti-obesity action. The only difference made to the HFD was addition of salt at a concentration of 0.47%, which corresponds to the human dose of 5 grams per day. Oral administration of bamboo salt, which has more minerals than other salt samples, suppresses progression of obesity in vivo, reflected in reduced weight gain, lowered FER, suppressed expression of adipogenic factors, etc [13]. Conversely, it was reported that supplement of dietary sodium with a high-fat diet at a concentration higher than 1% suppresses weight gain by reduction in digestive efficiency, potentially due to suppression of rennin-angiotensin system [26]. Our in vitro research using 3T3-L1 adipocytes revealed that solar salt prepared with new concentrated salt water, instead of using mixture of new and recycled concentrated salt water, significantly suppressed gene expression of adipogenesis (C/EBPα, SREBP-1c) / lipogenesis (LPL, FAS) and promoted lipolysis (PPARα, CPT-1), even in the same salt field (data not shown).

Our future research will examine the differences among salts as well as the total mineral compositions and ratios, and processing methods of salt. Differences in salt fields seem to occur due to different manufacture methods, environmental factors surrounding salt fields, and chemical compositions including minerals and organic phytochemicals, etc. Also, we assume that turbidity of concentrated salt water in Hae-Ju (temporary storage of concentrated salt water in the salt field) seems to change the patterns of crystallization, mineral composition, taste and functionality of solar salt. Thus, detailed investigation on mechanisms of functionality, as well as manufacturing methods and the active compounds of solar salt are needed in the near future.

**CONFLICT OF INTEREST**

The authors declare no potential conflicts of interests.

**REFERENCES**

2. Lee HM, Lee WK, Jin JH, Kim IC. Physicochemical properties and microbial analysis of Korean solar salt and flower of salt. J Korean...
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