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Long Term Low Salt Feeding Led to the Changes in Food Intake, Body Weight and Depressive-Like Behavior in Mice

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ABSTRACT

Low salt intake is associated with depression, but the experimental evidence is not clear. Sixty adult mice were randomly divided into four groups (mild, moderate and severe salt deficiency groups and control) during the seasonal splitting from winter to spring. The mice in the control group were fed with standard ordinary diet (salt content 0.26%), while in the mild, moderate and severe groups, the mice were fed with 10%, 30% and 50% salt deficient feedstuff, lasting for approximately 3 months. The results showed that the salt content of feed was negatively correlated with the food intake and body weight of mice. The sucrose preference test found that only the mild salt deficiency group had no difference between the beginning and the end of the experiment, and the other three groups including the control, showed a significant decrease. These results suggest that dietary salt content has an impact on the food intake and body weight of mice and is associated with the emergence of depressive-like behavior. Furthermore, the seasonal splitting from winter to spring may also have a differential synergistic effect on the change of depression-like behavior associated with low salt intake in mice.

1. INTRODUCTION

Depression is a serious mental illness and the main cause of mental disability among adolescents and young people all over the world. In recent years, more and more studies have begun to explore the relationship between dietary nutrition intake and depression [1], and salt is widely used as a seasoning in daily diet. Therefore, the relationship between salt intake and depression has attracted attention [2, 3]. In a co-

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hort study involving 23,510 people, it was found that there was a U-shaped association between dietary salt intake and depression, that is, too much or too little salt intake in the daily diet would increase the risk of depression [4]. In animal experiments, rats were injected with deoxycorticosterone acetate for a long time to cause their strong sodium appetite without providing saline [5]. Sodium deficiency-induced depression-like phenotype occurred in animals, which was manifested in the reduction of sugar water intake and the enhancement of self-stimulation brain current. After giving normal saline, the depressive-like symptoms of rats were significantly relieved [5-8]. In addition, in animal models, sodium deficiency and excess can lead to withdrawal social activities [9]. These findings suggest that a low salt diet is associated with depressive-like behavior. In addition, seasonal changes are highly correlated with depressive symptoms, which is particularly obvious in seasonal affective disorder (SAD) [10-12]. But so far, the effect of long-term low salt diet on depressive-like behavior and its relationship with seasonal changes are not clear. Therefore, the purpose of this study is to explore the effects of long term low salt feeding on depression-like behaviors related to seasonal changes.

2. MATERIAL AND METHODS

2.1. Experimental Animals and Feeding

Adult male mice (C57BL/6) aged 8 - 9 weeks were purchased from Hubei Provincial Laboratory Animal Public Service Center (Wuhan, China) and housed under standard conditions (12-h light/dark cycle, room temperature 18˚C - 25˚C, 40% - 50% humidity) with access to food and water ad libitum. The protocols were approved by the committee on the Ethics of Animal Experiments of South-Central Minzu University.

2.2. Experimental Implementation Season and Scheme

Since depressed patients, especially patients with double-phase emotional disorders, there is a certain seasonal law [13]. This experiment was conducted in the alternate season of winter and spring, from October 27, 2019 to February 1, 2020, for a total of 96 days. Sixty mice were randomly divided into four groups (control group, mild, moderate and severe salt deficiency groups) with 15 mice in each group. Each mouse from the treated and control groups was raised in a separate cage. The mice in control group were fed with standard diet (salt content 0.26%) purchased from Commercial animal feed company and the salt content of foodstuff was decreased by 10% (salt content 0.234%), 30% (salt content 0.182%) and 50% (salt content 0.13%), respectively, in the mild, moderate and severe salt deficiency groups compared with the control group. Before the beginning of the experiment, all mice were tested for the sucrose preference, and the data were used as the benchmark value of depressive-like behavior state of mice. After the beginning of the experiment, the food intake of mice within 24 hours was measured and recorded every four days. An appropriate amount of foodstuff was weighed and put on the feed rack of cage, and after 24 hours, the remaining foodstuff was weighed and the data were recorded for a total of 20 times during the experiment. The mice were weighed with an electronic scale at an interval of about 10 days and the data were recorded for a total of 9 times during the experiment. Before the end of the experiment, the mice were tested for sucrose preference again.

2.3. Sucrose Preference Test

Sucrose preference test (SPT) was performed to observe anhedonia-like behavior. 1% sucrose solution was prepared and filtrated with 0.22 μ membrane filter. All mice were habituated to 1% sucrose solution and water for 24 hours. On the testing day, mice were deprived of water for 24 hours and then presented with two bottles, one containing 1% sucrose and the other with drinking water. Twenty-four hours later, the bottles were removed and weighed. The sucrose preference was expressed as mean sucrose consumption relative to total fluid consumption [14].

2.4. Data Analysis

Data were analyzed using Graphpad prism v 8.0. For multiple group comparisons and to determine

treatment effects, one-way ANOVA post hoc Tukey's multiple comparison tests were used to determine statistical significance. For comparisons between two groups, either unpaired or paired Student's t test (two tailed) was performed to determine statistical significance. $P < 0.05$ was considered as statistically significant.

3. RESULTS

3.1. The Changes in Food Intake

During this experiment, the observation on 15 mice in each group was completed except that 2 and 3 mice died in the mild and severe salt deficiency group, respectively. Overall, the food intake of mice in each group fluctuated up and down to a certain extent in 20 tests (**Figure 1(a)**). After the analysis of variance on the food intake data of mice in each group, it was found that there was no significant difference between each group before the day 17. The food intake of mice in the four groups had similar change trends since the day 21, and was significantly higher in the moderate and severe salt deficiency groups than that of the control group. Such differences disappeared on the day 29, but reappeared in the next two tests. Interestingly, the differences disappeared again on day 45, and then the differences between the moderate and severe salt deficiency group and the control group reached the maximum from the day 49 to day 63. After that, the food intake of mice showed a little or no difference between the groups until the end of experiment with the exception of that it was significantly higher in the severe salt deficiency group than that of the control group ($P < 0.001$) on the day 96 (**Figure 1(b)**). It should be noted that there was no significant difference in food intake between the mild salt deficiency group and the control group from the beginning to the end of the experiment. Based on these results, it could be concluded that the food intake of mice in each salt deficiency group has three stages including an adaptation period (13 - 45 days), the large intake period (49 - 67 days) and the maintenance period (71 - 96 days). In addition, by analyzing the data in each group on day 96, it was found that the food intake of mice was negatively correlated with the salt content of feed (**Table 1**), that is, the lower the salt content, the higher the food intake.

3.2. The Changes in Body Weight

Figure 2 shows the change trend of the average body weight of mice in each group in 9 tests, and overall, the body weight of mice in each group presents an upward trend. However, according to the statistical analysis, the significant differences in the body weight of the mild and severe salt deficiency group were only found on day 54, 65 or 73 as compared to the control group ($P < 0.05$). In the last test on day 96, the body weight of mice in the mild salt deficiency group was significantly lower than that of the other three groups, but there were no significant differences between the moderate and severe salt deficiency group, and the moderate salt deficiency group and the control (**Figure 2(b)**). It was also found that the salt content of feed was negatively correlated with the body weight of mice (**Table 1**). Interestingly, the body weight of mice in the mild salt deficiency group began to deviate from that in the severe salt deficiency group from the second test and remained until the end of the experiment, suggesting that the long-term intake of mild salt deficiency diet has a greater impact on body weight as compared with the severe salt deficiency diet.

Table 1. Correlation analysis between salt content, food intake and body weight at day 96.

	Salt content (group)	Average food intake (24 h) (g)	Average body weight (g)
	0.26% (Con)	2.96	27.59
	0.234% (I)	2.80	25.25
	0.182% (II)	3.35	27.14
	0.13% (III)	3.78	28.99
r		−0.9439 (P = 0.0561)	−0.6266 (P = 0.3734)
R^2		0.8909	0.3927

Con: control group; I, II and III: mild, moderate and severe salt deficiency groups.

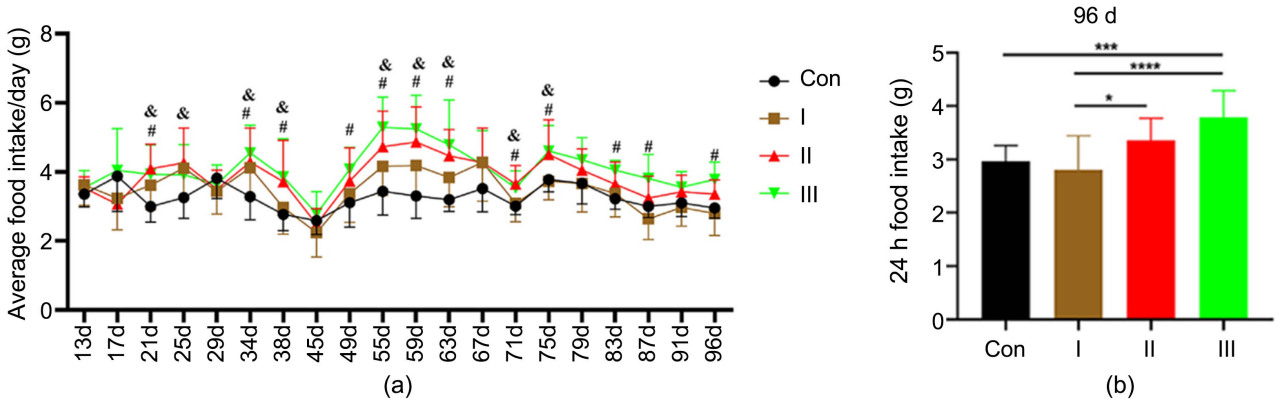

Figure 1. The changes in 24 h food intake of mice at different time points. (a) Black, brown, red and green markers represent the control (con, n = 15), the mild (I, n = 13), moderate (II, n = 15) and severe (III, n = 12) salt deficiency group, respectively. Data are expressed as the mean ± s. e. m. &: Con vs II group ($P < 0.05$); #: Con vs III group ($P < 0.05$); (b) The food intake of each group on day 96 was statistically compared (* $P < 0.05$, *** $P < 0.001$, **** $P < 0.0001$).

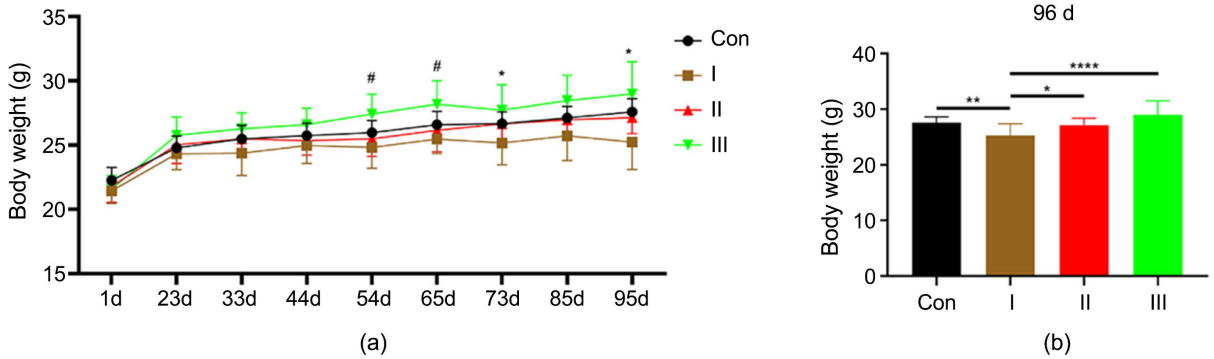

Figure 2. The changes in body weight of mice at different time points. (a) Black, brown, red and green makers represent the control (con, n = 15), the mild (I, n = 13), moderate (II, n = 15) and severe (III, n = 12) salt deficiency group, respectively. Data are expressed as the mean ± s. e. m. * Con vs I group ($P < 0.05$); # Con vs III group ($P < 0.05$); (b) The body weight of each group on day 96 was statistically compared (* $P < 0.05$, ** $P < 0.01$, **** $P < 0.0001$).

3.3. The Changes in Sucrose Preference Test

The sucrose preference values were decreased in the low, medium and severe salt deficiency group, but the significant differences were found in the medium and severe group, and unexpectedly, there was also significant difference in the control group (Figure 3).

4. DISCUSSION

In this study, we found that mice had significant changes in food intake and body weight after one month of low salt diet. Compared with the control group, the food intake of mice in three salt deficiency groups increased significantly, and such differences still existed by the end of the experiment. The body weight gain in the severe salt deficiency group was the most obvious and significantly higher than that in other groups. Interestingly, the food intake of mice in the mild salt deficiency group was significant greater than that of mice in the control group, but the trend of body weight change was reduced. This finding suggests that low salt diet may promote the eating desire of mice.

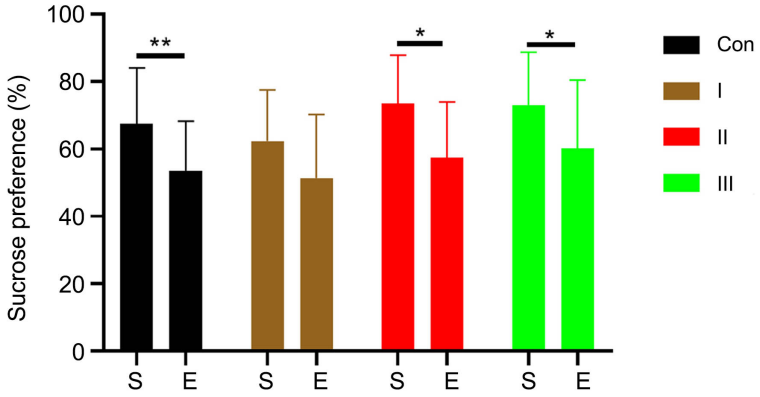

Figure 3. The comparison of sucrose preference in two tests at the start (S) and the end (E) of the experiment. Black, brown, red and green markers represent the control (con, n = 15), the mild (I, n = 13), moderate (II, n = 15) and severe (III, n = 12) salt deficiency group, respectively. Data are expressed as the mean ± s. e. m. * P < 0.05, ** P < 0.01.

The previous study found that 8-week-old male rats were fed with very low salt content (0.01%) and normal salt content (0.9%), respectively, when the rats reached the age of 16 weeks, the body weight in the low salt group was significantly higher than that in the normal group [15], which was similar to the results in the moderate and severe salt deficiency groups in the present study. Our findings suggested that a slight decrease in salt content in diet may cause body weight loss, but the lower salt content could increase body weight, however, the reason for this phenomenon is not clear. We suspect that the possible reason is that severe low salt feeding caused mice to crave salt and compensate by eating more. This hypothesis was supported by increased food intake in the severely salt-deficient group. Increased overall food intake led to increased intake of nutrients other than salt, and severe salt deficiency groups weight gain. However, due to insensitivity to salt deficiency, there was no difference in food intake between the mild group and the control group. In the case of reduced salt intake, body weight also decreased, which was consistent with the report [16].

In this study, sucrose preference test was used to evaluate the possible change in depressive-like behavior in mice fed with different degrees of salt deficiency foodstuff [17, 18]. The results showed that all groups including control group showed obvious changes in depression-like behavior during the experimental period for 96 days. It was found that the sucrose preference values were decreased in the low, medium and severe salt deficiency groups, but the significant differences were only found in the medium and severe salt deficiency group, and unexpectedly, there was also significant difference in the control group. In the present study, the finding that the obvious change in sucrose preference test was also noticed in the control group during the seasonal splitting from winter to spring suggested that seasonal change may have an impact on the depressive-like behavior of animals, which may also explain the characteristic changes of depressive-like behavior in three salt deficient groups. It would be more interesting to observe long-term seasonal changes in mice that could be maintained throughout the year.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest regarding the publication of this paper.

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