ABSTRACT

Background: Zinc (Zn) is an essential nutrient that is required in humans and animals for the growth, development, and maintenance of healthy bones. The aim of this study is to investigate the effects of zinc-deficient nutrition on the dental, mandibular, maxillary, and cranial dimensions of rats.

Materials and Methods: This experimental study was carried out on 14 male Wistar rats. The rats were randomly divided into two groups. Group I rats were fed with a Zn-deficient (ZD) diet, and Group II rats with a Zn-containing (ZC) diet. All the rats on the experimental diet were killed at the end of the fourth week and their blood samples were taken. The serum Zn levels were measured by an atomic absorption spectrophotometer. Radiographic assessment of the jaw bone density was done at the end of the study. Subsequently, the final measurements were made on the dry skulls, the mandibles, and teeth in both the groups. Statistical evaluation was performed by the student's t-test and repeated measures analysis. The difference between the groups was considered statistically significant if P < 0.05.

Results: The ZD group showed a significantly lower value in body weight (P < 0.05), serum level of zinc (P < 0.0001), and radiographic bone density of the mandible (P = 0.02). With regard to the craniofacial parameters, a significant difference was observed only in the length of the clinical crowns of the teeth (L13), which were longer in group II as compared to group I (P = 0.03).

Conclusion: This study confirmed that changes in zinc intake could not affect the growth of craniofacial structures. Also, it might change the radiographic bone density of the mandible.

Key Words: Craniofacial bones, rats, zinc deficiency

INTRODUCTION

Zinc (Zn) is an essential trace element required for the growth, development, and maintenance of healthy bones.[1-4] Zn is present at a concentration of up to 300 mg/g, and it has been considered an important factor in bone metabolism.[5] Zn stimulates reproduction and differentiation in osteoblastic cells and inhibits osteoclastic activity in the bone tissue. It also helps protein synthesis in osteoblastic cells and plays a role in the preservation of bone mass.[6,7] Evidence indicates that Zn may play an essential role in the regulation of bone metabolism.[1,8,9] Zn-deficient rats consume less food, and hence, have significantly reduced growth, which has been associated with abnormalities in bone growth, formation, and mineralization.[1,10-12] The effects of Zn deficiency are similar in most animal species and include dermatitis, alopecia, ocular lesions, testicular atrophy, growth retardation, and anorexia.[1,8,11,13-15] The previously reported skeletal changes, including delayed maturation, reduced alkaline phosphate activity, reduced premenopausal bone mass, and postmenopausal osteoporosis, have been associated with Zn deficiency.[16-19] To our knowledge, the specific effects of Zn deficiency on the craniofacial structures have been demonstrated.

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Address for correspondence:
Dr. Maryam Seyedmajidi,
Dental Materials Research Center, School of Dentistry, Babol University of Medical Sciences, Babol, Iran.
E-mail: ms_majidi79@yahoo.com

Access this article online
Website: http://drj.mui.ac.ir
in Kara et al.’s study. Due to lack of adequate literature in this field of investigation, in the present experiment, we try to evaluate the effects of a zinc-deficient nutrition program on the dental, mandibular, maxillary, and cranial dimensions of rats.

MATERIALS AND METHODS

Study setting
This investigation was carried out at the Pharmacology Department of the Babol University of Medical Sciences (Babol, Iran). The experiment protocol was reviewed and approved by the Animal Ethics Committee of the Babol University of Medical Sciences. The study setting was similar to the study of Kara et al. In this study, 14 male Wistar rats were used after cessation of lactation, on the twenty-fourth day of birth. The rats were randomly divided into two equal groups: The Group I rats were fed with a zinc-deficient diet (ZD), and the group II rats (controls) with a zinc-containing diet (ZC). The formulated ZD and ZC diets were identical except for the zinc content. The zinc-deficient diet was stored at 4°C in plastic containers and handled with plastic gloves and appropriate tools to avoid contamination. The rats were kept individually in stainless steel cages and maintained at 22-25°C with a 12-hour light/dark cycle. They were allowed free access to distilled water. Features of zinc deficiency, including oral lesions, loss of appetite, reduced weight gain, hair loss, and diarrhea, were observed in all ZD rats.

Atomic absorption spectrophotometry
Changes in the oral tissue of the study groups were recorded at the end of the fourth week on the experimental diets. Then, all the rats were sacrificed after anesthesia with chloroform. Blood samples were taken from the auxiliary vessels and centrifuged at 3000 rpm for five minutes. The blood samples were stored at a temperature of -20°C. Later, the serum zinc level was measured by an atomic absorption spectrophotometer (Flame-type UNICAM 929; ATI-Unicam, Cambridge, UK).

Calibration of the radiographic method
For evaluation of the radiographic pattern of the rat skull, a mature rat was anesthetized with chloroform. The head of the rat was irradiated at 50 kVp and 8 mA for 0.25 seconds. The lateral skull projection was provided by a digital occlusal sensor and photostimulable phosphor (PSP) digital imaging sensors (Digora Optime, Soredex, Tuusula, Finland). After image processing in a computer, the densities of five points of the mandible and maxilla were randomly measured for ten days using the digora software. No significant difference was observed in the mean bone density of the measured points in the maxilla and mandible within ten days.

Radiographic assessment of the jaw bone density
Lateral skull radiographs were taken at the beginning and end of the study. The radiographs were exposed at 50 kVp and 8 mA. The distance between the x-ray tube and the head of the rat was 50 cm. Lateral skull projections from the skull of the rats were provided by using photostimulable phosphor (PSP) digital imaging sensors (Digora Optime, Soredex, Tuusula, Finland). After image processing in a computer, the radiographic densities of the maxilla and mandible were assessed using the Digora software and the mean radiographic density of each jaw was measured and recorded.

Measurement of the craniofacial parameters
The heads of the rats were carefully macerated and fixed in 10% formalin for one week. These specimens were immersed in 2.5% NaOCl for 24 hours to remove the organic tissue [Figure 1]. The skulls and mandibles were divided into two halves midsagittally using a sharp blade. Then, the following 13 parameters were evaluated on the skulls, mandibles, and teeth using a caliper with an accuracy of 0.01 mm, (identical to the study of Kara et al.).

L1: Anterior edge of the glenoid fossa — extreme anterior extension of the maxillary bone between the incisors.
L2: Anterior edge of the glenoid fossa — junction of the mesial surface of the first molar with an alveolar bone.
L3: Maximum skull length: The intersection of the frontoparietal suture and the interparietal suture.
RESULTS

This investigation was carried out on 14 male rats divided into two groups with seven rats in each. Group I rats were fed with a ZD diet, and Group II rats with a ZC diet. The first observation of appetite reduction, loss of hair, diarrhea, and ulcerations of the skin and mucosa, in ZD rats occurred on the fifth day of the study and continued until the end of the experiment.

Body weight

The rats’ weight was approximately equal at the beginning of the investigation and there was no statistical difference between them (P > 0.05). The weights were measured on days 5, 10, 15, 20, 25 and 28 of the study, in both groups. The weight of the rats in Group II was more than in Group I throughout the study. A significant difference was seen from day 15 until the end of the study (P < 0.05) [Table 1]. By comparing weight changes using the repeated measures test, there was a significant difference in both the inter-group and intra-group (P < 0.001) [Figure 2].

Serum level of Zinc

The serum zinc level of the ZD rats (Group I) was lower than that of the controls (Group II) (P < 0.0001) [Table 2].

Statistical analysis

Statistical evaluation was performed via the student’s t-test and repeated measures analysis. The difference between groups was considered statistically significant at P < 0.05.

Table 1: Comparison of the weight (g) of rats in Group I and Group II at the beginning and during the study

<table>
<thead>
<tr>
<th>Weight groups</th>
<th>At the beginning</th>
<th>Day 5</th>
<th>Day 10</th>
<th>Day 15</th>
<th>Day 20</th>
<th>Day 25</th>
<th>Day 28</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>42.71±5.62</td>
<td>48.71±5.53</td>
<td>55.57±5.38</td>
<td>63.43±6.05</td>
<td>72.00±5.77</td>
<td>79.29±5.41</td>
<td>84.00±5.66</td>
<td>NS*</td>
</tr>
<tr>
<td>Group 2</td>
<td>42.14±5.43</td>
<td>51.86±4.49</td>
<td>59.71±4.68</td>
<td>71.00±4.69</td>
<td>79.71±5.62</td>
<td>89.43±5.44</td>
<td>95.14±5.30</td>
<td>0.023</td>
</tr>
<tr>
<td>P-value</td>
<td>NS*</td>
<td>NS</td>
<td>NS</td>
<td>0.026</td>
<td>0.004</td>
<td>0.003</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

NS*: Non-significant

Figure 2: Weight changes on a daily basis in the study groups. Significant difference can be observed from day 15 of the study.
Bone density of the jaws
At the beginning of the study, the bone density of the maxilla and mandible did not show significant difference between the two groups. At the end of the study, although the bone density of the mandible in Group II was more than in Group I \((P = 0.02)\), the bone density of the maxilla was not statistically different between the two groups [Table 3].

Measurement of the craniofacial parameters
A statistically significant difference was not found in the entire total craniofacial parameters measured between the groups except the length of the clinical crowns of the teeth (L13), which were longer in Group II as compared to group I \((P = 0.03)\), [Table 4].

DISCUSSION
The goal of this study was to assess the effects of dietary Zn-deficiency on the craniofacial parameters during growth, in rats. Zn with its extensive and crucial roles in the mammalian system, is regarded as a key trace element for the growth of humans and many animal species.\(^{[21,22]}\) Furthermore, it has been reported that it is essential for bone metabolism, as a cofactor for specific enzymes.\(^{[23]}\) It is a widely accepted view that the most obvious indicator of Zn deficiency is inadequate food intake, in other words loss of appetite and a decrease in body weight.\(^{[24]}\) As expected, the ZD diet caused a reduction in both body weight and craniofacial bone growth. Zn inadequacy caused this effect and it was not the consequence of a general reduction of food intake.

Rats, which are more susceptible than other animals to Zn-deficiency, are useful models for the study of the effects of dietary Zn-deficiency on their craniofacial structures, during growth. In fact, growth impairment is significantly greater in ZD rats than in control rats. Zn plays multiple roles related to bone metabolism.\(^{[4,11,16,21,23]}\) Zn stimulates bone formation and bone protein synthesis by increasing the activity of critical enzymes, such as alkaline phosphatase.\(^{[12,25,26]}\) Previous investigations have examined the effect of Zn deficiency on bones in young rats\(^{[8,11,27]}\). The growth decrease in Zn-deficient rats and growth acceleration in Zn-added rats in our study have confirmed that Zn is a necessary element for growth. This was in agreement with Kara et al.’s study, in which changes in Zn intake exerted an effect on the growth of the craniofacial structures.\(^{[20]}\) Orbak et al., in their study, also found that oral health was better in control rats (those fed with a ZC diet) than in ZD rats and showed that Zn-deficiency was a potential risk factor for oral and periodontal diseases.\(^{[28]}\) The diagnosis of Zn deficiency can be confirmed by both clinical features and laboratory findings.\(^{[21,29]}\) Previous studies evaluated Zn concentrations in the serum by using atomic absorption spectrophotometry.\(^{[28-30]}\) We also used this method in our study and identified that the serum Zn level of ZD rats was lower than that of the rats given a ZC diet.

Zn-deficiency causes skeletal growth retardation and reduction in bone mass.\(^{[17,19]}\) In the previous studies, the morphometric abnormality of the growth plates, likely related to the role of Zn in cell division, differentiation, and apoptosis, explain the skeletal longitudinal growth retardation and the greater deformability of the long bones in Zn-deficient rats than in the control rats.\(^{[5]}\)

### Table 2: Comparison of serum zinc levels (ppm) of Group I and Group II

<table>
<thead>
<tr>
<th></th>
<th>Group 1 (ZD)</th>
<th>Group 2 (ZC)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>7</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>0.036 ± 0.02</td>
<td>0.33 ± 0.097</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

### Table 3: Mean ± SD of bone density of the maxilla and mandible in the study groups before and after the experiment

<table>
<thead>
<tr>
<th>Bone density</th>
<th>Group I</th>
<th>Group II</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maxilla at the start</td>
<td>183.4 ± 6.3</td>
<td>147.9 ± 9.9</td>
<td></td>
</tr>
<tr>
<td>Maxilla at the end</td>
<td>191.3 ± 8.2</td>
<td>147.4 ± 8.0</td>
<td></td>
</tr>
<tr>
<td>Mandible at the start</td>
<td>162.7 ± 9.2</td>
<td>162.3 ± 7.5</td>
<td></td>
</tr>
<tr>
<td>Mandible at the end</td>
<td>166.1 ± 9.1</td>
<td>166.5 ± 10.1</td>
<td></td>
</tr>
</tbody>
</table>

NS*: Non-significant

### Table 4: Measurements (distances, L in mm) made on the teeth, skulls, and mandibular halves of 14 rats

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group I</th>
<th>Group II</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>L1</td>
<td>24.36 ± 0.63</td>
<td>24.24 ± 0.30</td>
<td>NS*</td>
</tr>
<tr>
<td>L2</td>
<td>12.31 ± 0.29</td>
<td>11.99 ± 0.41</td>
<td>NS</td>
</tr>
<tr>
<td>L3</td>
<td>14.47 ± 0.46</td>
<td>14.48 ± 0.37</td>
<td>NS</td>
</tr>
<tr>
<td>L4</td>
<td>14.88 ± 0.29</td>
<td>14.93 ± 0.32</td>
<td>NS</td>
</tr>
<tr>
<td>L5</td>
<td>21.41 ± 0.68</td>
<td>21.89 ± 0.44</td>
<td>NS</td>
</tr>
<tr>
<td>L6</td>
<td>15.60 ± 0.38</td>
<td>15.66 ± 0.44</td>
<td>NS</td>
</tr>
<tr>
<td>L7</td>
<td>9.64 ± 0.34</td>
<td>9.30 ± 0.40</td>
<td>NS</td>
</tr>
<tr>
<td>L8</td>
<td>37.04 ± 0.70</td>
<td>37.62 ± 1.04</td>
<td>NS</td>
</tr>
<tr>
<td>L9</td>
<td>12.92 ± 0.52</td>
<td>13.44 ± 0.38</td>
<td>NS</td>
</tr>
<tr>
<td>L10</td>
<td>18.16 ± 0.53</td>
<td>18.61 ± 0.34</td>
<td>NS</td>
</tr>
<tr>
<td>L11</td>
<td>15.05 ± 0.39</td>
<td>15.17 ± 0.23</td>
<td>NS</td>
</tr>
<tr>
<td>L12</td>
<td>2.84 ± 0.16</td>
<td>2.74 ± 0.24</td>
<td>NS</td>
</tr>
<tr>
<td>L13</td>
<td>5.32 ± 0.29</td>
<td>5.72 ± 0.33</td>
<td>0.03</td>
</tr>
</tbody>
</table>

NS*: Non-significant
In this study, we especially examined the effects of low levels of Zn intake on the teeth, mandible, and maxilla of the rats, during growth. In the study by Maki et al., on the effects of low levels of Zn intake on bone density in rat mandibles during the growth stage, with peripheral quantitative computed tomography, the low-Zn group showed significantly lower trabecular bone density compared to the control group. This was similar to our study, in which the density of the mandible in Group II was more than in Group I, however, the bone density of the maxilla was not statistically different between the two groups. In the present study, a statistically significant difference was not found in the entire total craniofacial parameters measured between the groups except the length of the clinical crowns of the teeth (L13), which was longer in Group II as compared to Group I. This was in contrast to Kara et al.’s study, in which the means of all the lengths of the rats in the Zn-deficient group were significantly shorter than those in the control group. In addition to the results of measuring the lengths, a low growth of the maxilla was seen in the ZD group as compared to the control group.

Zn seems to play several roles related to bone metabolism. There are few known reports demonstrating the effect of deficient Zn intake on the osseous tissue during the growth stage. Weissman and Hoyer reported that Zn deficiency reduced the sensitivity of the receptors to the growth hormone. Golub et al. reported that a low-Zn diet during adolescence might slow down the bone growth, enhancing the risk of osteoporosis in later years.

**CONCLUSION**

This study confirmed the fact that Zn played an important role in gaining body weight and stimulated bone mineralization. Also, it confirmed that the changes in Zn intake did not affect the growth of craniofacial structures, and it could change the radiographic bone density of mandible.

**REFERENCES**


