

Occlusal reduction of unilateral molars influences change of stress-related hormones in rats

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Summary

In order to investigate the change of stress-related hormones by dental occlusal reduction, we ground molars in Sprague-Dawley (SD) rats and evaluated the effect on hormone levels. Thirteen and 18 weeks after occlusal reduction, cortisol concentration was increased 2.75 and 2.17 fold respectively, whereas corticosterone concentration was slightly elevated by 31.2% and 13.5%, respectively. Body weight was slightly decreased, but feed and water intake, and blood chemistry were the same in the experimental group as in the control group. Our results suggest that unilateral molar occlusal reduction may influence cortisol and corticosterone levels and the endocrine system, leading to hormone imbalance through the body.

Introduction

Occlusion is crucial for chewing, pronunciation and swallowing in prothodontics, orthodontics and dentistry (*Budtz-Jørgensen, 1981; Hashimoto, 1998*). However, the physiological significance of occlusion remains unclear. Recent clinical reports have shown that dental occlusion is related to many chronic diseases, such as headache, rhinitis, backache and memory loss (*Kato et al., 1997; Onozuka et al., 2000; Ueda, 1991*). However, to date there have been no long-term studies investigating whether dental occlusion has any effect on homeostasis. Sumioka reported that the trigeminal nuclear complex in dogs not only controls sensory perception in the face and chewing, but is also probably involved in posture control and the autonomic nervous system (*Sumioka, 1991*). This suggests that dental occlusion plays an important part in the normal functioning of the trigeminal nerve system. Azuma et al (1999) found that occlusal reduction affects head position, postural control and cardiac function. They put forward the suggestion that

occlusal abnormalities could exert physical effects on systemic health.

Therefore, we carried out the experiments to investigate whether reduction of dental occlusion influences stress-related hormone levels in Sprague-Dawley (SD) rats.

Materials and Methods

Animals

All the procedures were performed in compliance with the Guiding Principles in the Care and Use of Animals (National Research Council, 1996) and the Animal Welfare Committee of Korea Research Institute of Bioscience & Biotechnology (KRIBB). SD rats were obtained from Daehan Biolink Co. (Umsung, Korea) and housed in specific pathogen-free (SPF) barrier facilities of KRIBB, a sub-center of International Council for Lab Animal Science (ICLAS; Code No. Kist). The animal received tap water and rat chow (Rodent Diet 2918C, Harlan, Madison, WI) *ad libitum* and were housed singly, maintained in a room under standard laboratory conditions (23°C ± 1°C and 50% ± 5% humidity) with 12 hr of dark and 12 hr of light. We strictly kept the rules for animal experiments including

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ethical care under the guidance of the Committee. The number of each experimental group was 7.

Occlusal reduction

Occlusal reduction was carried out as described previously (Azuma *et al.*, 1999). Eight-week-old SD male rats were anaesthetised by intraperitoneal injection of pentobarbital (25 mg/kg body weight). The occlusal surface of right-hand, upper and lower molars was reduced with a rotary diamond bur (Diatech, No. 830L, 1 x reduction) at a low speed and by cooling the bur to avoid any damage such as heat-induced inflammation. Each tooth was ground down by ~0.3 mm, taking care not to expose the pulp. Control rats were injected with pentobarbital without being subjected to occlusal reduction. In a preliminary experiment, hormonal changes were examined in rats with reduced anterior teeth. How-

ever, after 2 weeks, anterior teeth had grown by up to 70%. Ground molars did not show signs of growth after up to 18 weeks; therefore, we decided to use ground molars in our experiment instead of anterior teeth (Fig. 1). Food and water intake, body weight, blood chemistry and hormonal changes were observed for up to 18 weeks. Each week, 1.2 ml of blood was obtained from the inferior vena cava of control and experimental rats.

Hormone determinations

Serum was extracted from the remaining 0.7 ml blood and stored in a freezer (-70°C), then the concentration of cortisol, corticosterone, and other hormones was measured by radioimmunoassay (Brand, 1999; Prado *et al.*, 2001) as described in the manufacturer's manual. In brief, the concentration of standard cortisol, and corticosterone was 0-50

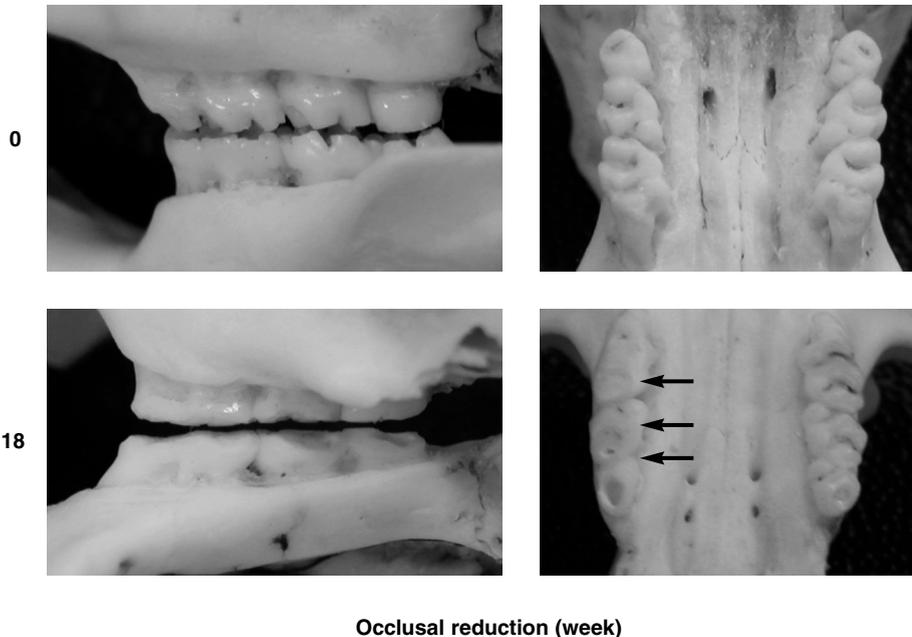


Figure 1. Occlusal reduction of unilateral molars of SD rats. Zero and 18 weeks after occlusal reduction, the rats were killed and their teeth examined. The method of occlusal reduction is described in Materials and Methods.

mg/dl, 0-200 ng/ml, respectively. Each volume (25 μ l, 50 μ l in order, respectively) of serum was added to 1 ml of iodine 125-labelled cortisol (Diagnostic Products Corporation, Los Angeles, CA), or corticosterone (Diagnostic Products Corporation, Los Angeles, CA) antibody. After discarding the free antibody, the bound complex was determined by the radioactivity using a γ -counter (Wallac, Turku, Finland). All the data were analyzed by *Student's t-test*.

Results and Discussion

We studied the effect of molar occlusal reduction on cortisol and corticosterone levels in SD rats (Figs. 2A and B). After the first 2 weeks, there was not much change in cortisol concentration and similarly after 7 weeks (data not shown). However, after 13 and 18 weeks, serum cortisol concentration increased 2.75 and 2.17-fold respectively (Fig. 2A) and serum corticosterone increased by up to 31.2

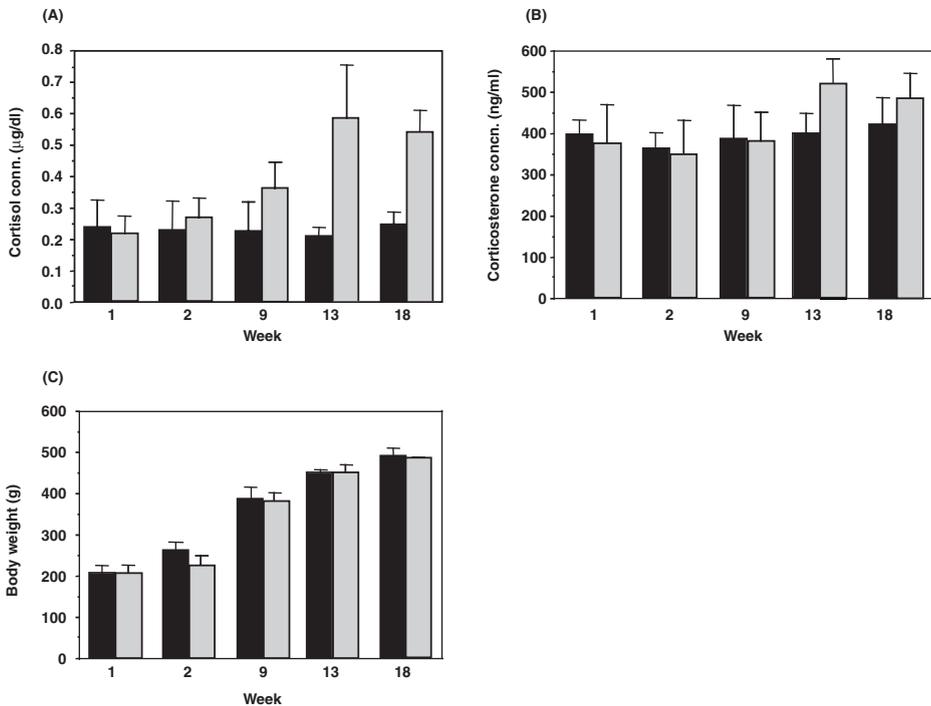


Figure 2. Change of level of stress hormone concentration in serum by occlusal reduction of unilateral molars. (A), Cortisol concentration. Standard cortisol concentration was 0-50 mg/dl. 25 μ l of serum were added to 1 ml of iodine 125-labelled cortisol antibody. Black columns, controls; white columns, occlusal reduction. The data represent mean \pm S.D. of two independent experiments (n=7). (B), Change of corticosterone concentration. The procedure of corticosterone determination is similar to that of cortisol. The iodine 125-labelled corticosterone antibody was purchased from Diagnostic Products Corporation. Black columns, controls; white columns, occlusal reduction. (C), Change of body weight. The body weight was weighed every day. The data represent mean \pm S.D. of two independent experiments (n=7). Black columns, controls; white columns, occlusal reduction. Significance was determined using *Student's t-test*.

and 13.5% after 13 and 18 weeks respectively (Fig. 2B). Serum insulin level, body weight, food and water intake, and blood chemistry were not affected in the experimental group (Fig. 2C and data not shown). The occlusal influence between experimental and control groups can be ruled out by careful histological analysis of pulp tissue. There was also no change in plasma epinephrine or norepinephrine (data not shown).

This is the first finding that molar occlusal reduction induces changes in levels of cortisol and corticosterone in SD male rats. The reduction of molars influences the body homeostasis, and we have found strong evidence that the act of reducing the molar teeth can cause the hormonal content, especially cortisol, to change. Cortisol, a stress-related hormone originating in the adrenal cortex, is well known to stimulate transfer of amino acids from muscle, fatty acid, and adipose cells to liver. Its target tissues are liver, adipose tissue and muscle (Brand *et al.*, 1995).

Reducing molar occlusion has been shown to have several effects on the body. In Guinea pigs it has been shown to reduce life span (Azuma *et al.*, 1999). Onozuka *et al.* (2000) reported that the decrease of biting ability caused by molar occlusal reduction may be involved in the development of Alzheimer's disease. In senescence-accelerated mice with molar reduction, learning ability and memory were drastically reduced. It has been observed that molar reduction leads to impaired immunological responses in hippocampal cells and a decrease in c-fos expression (Hiroshima *et al.*, 2001). Additionally, in rats, orally fed with powder, learning ability and memory were decreased compared with rats fed a solid meal (Kato *et al.*, 1997). Recently, it is reported that occlusal disharmony increased corticosterone and norepinephrine in with a circadian fashion, suggesting that the treatment causes chronic stress in rat (Yoshihara *et al.*, 2001). Corticosterone is also known as a stress hormone induced by some environmental stimuli, which is associated with cortisol (Rose, 2000). Similarly, we found that 13 and 18 weeks after the unilateral

molars were reduced by occlusion (Fig. 2B), the corticosterone concentration was elevated by up to 31.2% and 13.5%, respectively. Additionally, body weight, insulin concentration in serum, feed and water intake, and blood chemistry did not change in the experimental group compared with the control group. We could not also find a change of epinephrine or norepinephrine in plasma (data not shown).

According to our results, unilateral reduction of molars in SD rats increases serum cortisol and corticosterone concentration (Figs. 2A and B). We suggest that changes in molar occlusion increase stress and affect the endocrine system, leading to hormone imbalance through the body. Further studies on molar occlusal reduction using dogs or apes may give us consistent information about stress hormonal change caused by the treatment.

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