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Effects of Changes in Vertical Occlusal Dimension on Heart Rate Fluctuations in Guinea Pigs

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Abstract. *Background. We have previously reported that the decrease of the vertical occlusal dimension (VOD) led to heart failure and abnormalities in creatine phosphokinase (CPK) in guinea pigs. In the present study, we investigated the autonomic activity and the origin of the abnormality in CPK under different occlusal conditions. Materials and Methods: Guinea pigs were separated into the following five groups: untreated control, reduced VOD, slit, restored VOD and increased VOD groups and compared for their electrocardiogram and heart rate fluctuations for two weeks using Fluclet, computer software. Results: The control group revealed no changes in heart rate fluctuations or posture. The reduced VOD group exhibited a two-phase wave of heart rate fluctuations, with the first peak 0-2 days after teeth grinding, and the second peak starting from 4 days after teeth grinding until sudden death (usually 12th day), accompanied by head drop. The slit and the restored VOD groups exhibited only the first peak. The increased VOD group, with approximately 3 mm-thick acrylic pellets bonded to the posterior teeth, showed no heart rate fluctuations. Body weight loss was most prominent in the reduced VOD group, and became much milder in the order of increased VOD, restored and slit groups. The reduced VOD group exhibited transient elevation of skeletal muscle type of CPK isozyme activity within two days after treatment. Conclusion: The present study suggests that the first peak of heart rate fluctuations is caused by pulpal stimulation, and the second peak by excessive contraction (excessive excitation of the motor output system and the autonomic nervous system) of the masticatory muscles. On the other hand, increased VOD did not influence either the motor or the autonomic nervous system.*

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There have been many clinical reports of various systemic symptoms such as headache, shoulder stiffness and malposture due to changes in the occlusal position (1-4). On the other hand, there have been reports describing the effects of artificial occlusal abnormalities attributed to teeth extraction, bite raising and tooth grinding on the whole body in experimental animals (5-11). These studies suggest that the trigeminal responses to occlusal changes induce various systemic symptoms by as yet unidentified mechanisms. Out of these symptoms, head drop (Figure 1) and the drooping of the submandibular mental area to the ground were observed approximately one week after grinding the posterior maxillary teeth of guinea pigs to the cervical area. Abnormal waveforms, including T-wave inversion, were also observed on the electrocardiogram (ECG) (11). In order to elucidate the mechanism of heart failure caused by the reduced vertical occlusal dimension (VOD), we investigated how the changes of several occlusal positions affect the autonomic and motor responses of guinea pigs.

Materials and Methods

Experimental animals. Male Hartley guinea pigs aged 7 weeks and weighing about 500 g (Japan SLC, Shizuoka, Japan) were used in the present study. The animals were housed individually in stainless steel cages with wire-mesh floors at room temperature (23˚C) and with relative humidity of 55%. These animals were fed solid feed (LRC-4; Oriental Yeast Co., Ltd., Tokyo, Japan) and tap water *ad libidion*. The animals were allocated to the following five groups (each with 15 animals) (Figure 2). For each animal in each group, electrodes were placed subcutaneously on the left femur and right shoulder for ECG test under anesthesia (*i.p.* administration with 30 mg/kg of sodium pentobarbital) before treatment of teeth. (i) Control group (untreated group): The teeth and occlusal position were not affected throughout the study period. (ii) Decreased OVD group: The 8 posterior maxillary teeth, *i.e.* the 4 right and 4 left, of the anesthetized guinea pigs were ground approximately 3 mm to the cervical area with a dental diamond bur, resulting in the removal of the crown, not in exposure of the dental pulp in the area. The teeth were ground carefully at low speed to minimize heat generation and damage to the tongue or buccal mucosa. (iii) Slit group: The occlusal surface of the 4 right and left posterior maxillary teeth of guinea pigs were grooved to the 3 mm in depth under anesthesia in order to investigate the effects of tooth stimulation without changing the occlusal height. (iv) Restored VOD group: The VOD was reduced by grinding the teeth of guinea pigs as described in the section for the reduced VOD group. Twenty-four hours after grinding the teeth, the ground part was raised. One layer of light-curing acrylic resin was placed to make it flat on the ground surface of the bilateral maxillary posterior teeth of the anesthetized guinea pigs, and an acrylic resin pellet (thickness: 3 mm, width: 2.5 mm and length: 4 mm) was adhered to the surface with a self-curing resin to restore the OVD to its original level. (v) Increased VOD group: The VOD was raised by approximately 3 mm by adhering an acrylic resin pellet to the occlusal surface of the posterior maxillary teeth of the untreated guinea pigs anesthetized as described above.

ECG recordings. The electrodes were placed subcutaneously on the left femur and right shoulder of guinea pigs and the cords were fixed subcutaneously on the parietal sub maxilla. ECG lead II in the four extremities was recorded for 20 min in unrestrained animals after awakening from the anesthesia (AC-601G: Nihon Kohden, Tokyo, Japan). After recording the waveforms *via* a computer, the waves and heart rate fluctuations were analyzed using Fluclet computer software (Dainippon Sumitomo Pharmaceutical Co., Ltd., Suita, Japan). The ECG findings were recorded on a once-a-day basis, unless otherwise stated. The number of ECG tests was increased when abnormal changes, such as T-wave reversion, appeared.

Biochemical examination of blood. The blood was collected by heart puncture before and after tooth grinding and allowed to clot at room temperature for 30 min; the serum was separated into the supernatant fraction by centrifugation. CPK isozyme was analyzed by cellulose acetate membrane electrophoresis, according to the method of Ikegami *et al*. (12). Lactate dehydrogenase (LDH) isozyme was analyzed by a modification (13) of cellogel-methylthiazoletetrazolium method (14).

Statistical analysis. Experimental values are expressed as the mean±standard error (SE) (n=15). Statistical analysis was performed by using Student's *t*-test. A *p*-value <0.05 was considered to be significant. Multiple comparison testing was performed by the Dunnett method (15).

Results

Control group. No apparent changes, including heart rate fluctuations were observed throughout the study period of about two weeks. The findings in the control group were almost the same as those in the reduced VOD group described below.

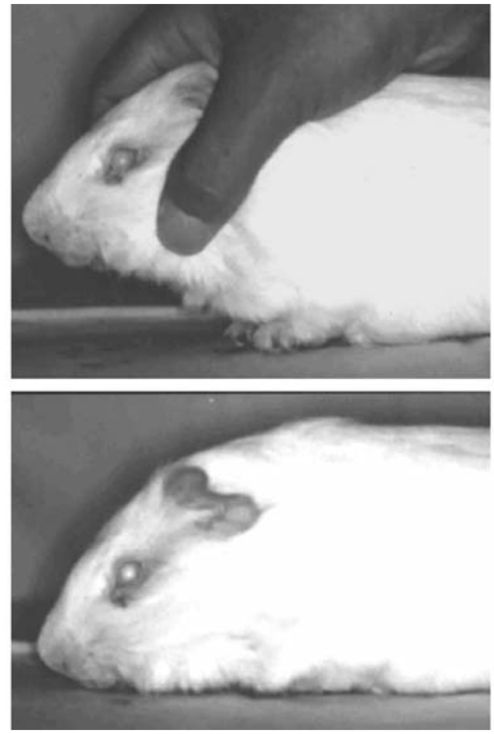

Figure 1. *The posture of head drop to the ground observed from 5 days after tooth grinding. The cranium of the guinea pig drops to the floor immediately after the held cranium is released. This posture indicates the motor ataxia.*

Reduced VOD group. The body weights of guinea pigs decreased continuously throughout the experimental period, after grinding the posterior maxillary teeth to the cervical area (Figure 3). Abnormal postures, including the main symptom of the head drop to the ground (Figure 1), and abnormal waveforms on ECG, including T-wave inversion, were observed 6 to 8 days after the tooth grinding (11). The heart rate fluctuations increased 1 or 2 days after the tooth grinding, returned once to the normal level, and then increased again, with a peak at 4 or 5 days after the tooth grinding (Figure 4A). The first and second peaks of the heart rate fluctuations were analyzed by Student's *t*-test. The increase in these fluctuations was found to be significantly greater in the reduced VOD group than in the control group ($p<0.05$). On the other hand, the multiple comparison tests for the first and second heart rate fluctuations performed by the Dunnett method gave no significant differences between the two groups.

Slit group. The body weights of the animals in the slit group decreased immediately after tooth grinding, and the rate of decrease of body weights was much milder than the one observed in the reduced VOD group, beginning to increase

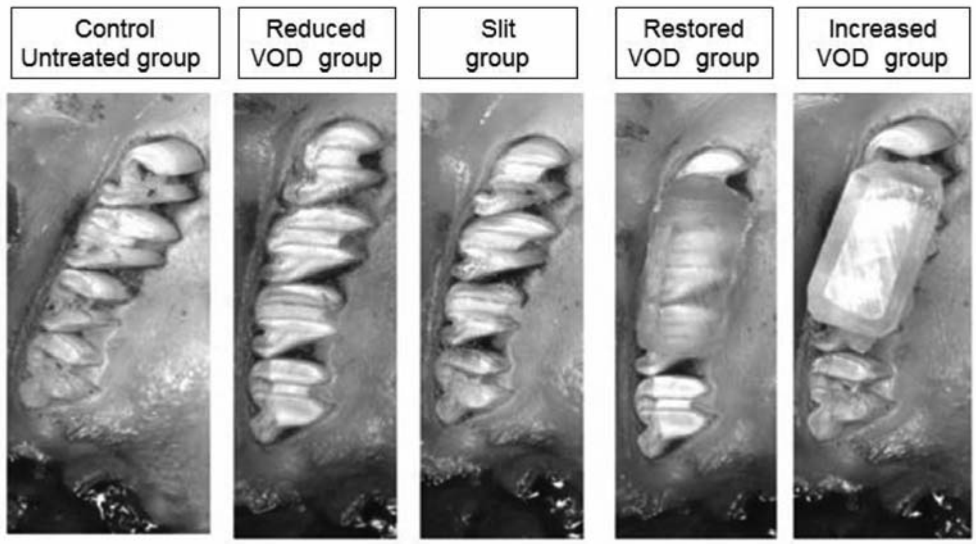

Figure 2. *Dental destruction state of five groups of guinea pigs. Treatment of the right and left posterior maxillary teeth. The right posterior maxillary teeth are shown.*

5 days after the tooth grinding and continuing to increase thereafter (Figure 3). Heart rate fluctuations in the slit group were comparable with those in the first peak of heart rate fluctuation (at 1 or 2 days after the tooth grinding), observed in the reduced VOD group (Figure 4B). However, there was neither a second peak (Figure 4B), nor the corresponding abnormality in the posture or the waveforms on ECG.

Restored VOD group. The body weights of animals in the restored VOD group after tooth restoration decreased at a rate comparable with the one of the reduced VOD group, but they began to increase from 6 days after the restoration and continued to increase thereafter (Figure 3). There was only the first peak of heart rate fluctuations, without the second peak (Figure 4C), nor any abnormality in the posture or the waveforms on ECG.

Increased VOD group. The body weights of the animals in the increased VOD group after tooth restoration declined at a rate comparable with the one of the reduced VOD group, but they began to increase 7 days after the elevation and continued to increase thereafter (Figure 3). There was only a slight increase in the heart rate fluctuations 1 to 3 days after the bite raising, without any apparent first and second peaks (Figure 4D), nor any abnormality in the posture or the waveforms on ECG.

Blood biochemical analysis. The blood of the reduced VOD group was subjected to biochemical analysis. CPK, an indicator of myocardial disorder, was increased transiently in the reduced VOD group (Figure 5A). The type of CPK isozymes involved was investigated to clarify the mechanism of the increase in the CPK concentration (16, 17). Three kinds of isozymes of CPK, namely skeletal muscle type isozyme (MM), cardiac muscle type (MB) and brain type (BB) were detected in guinea pigs. In the cardiac muscle of guinea pigs, MM isozyme accounted for more than 90% of total CPK, whereas MB and BB isozymes were present at much lower amounts (data not shown). In the other organs, BB and MM isozymes accounted for more than 25% and more than 30%, respectively (data not shown). We found that the majority of the increased CPK isozymes in the blood were of the MM type (Figure 5B). No clear-cut changes in LDH isozyme pattern were observed (data not shown).

Discussion

The present study demonstrated that the changes of the occlusal conditions of the guinea-pig, *i.e.* the reduction of VOD, induced a continuous decrease in body weight, changes in posture (the head drop to the ground), abnormal waveforms, including T-wave inversion (11), and a two peak waveforms of heart rate fluctuations. On the other hand, abnormal behavior and abnormal waveforms on ECG, or a second peak of the increase in the heart rate fluctuations disappeared and body weight loss became greatly milder when the dental occlusion was restored after tooth grinding and the even in the slit group.

The heart rate fluctuations were analyzed by wavelet analysis (18) using computer software (Fluclet). The peaks of the two major components, called 'low-frequency components' (LF component: 0.27-0.74 Hz) and 'high-

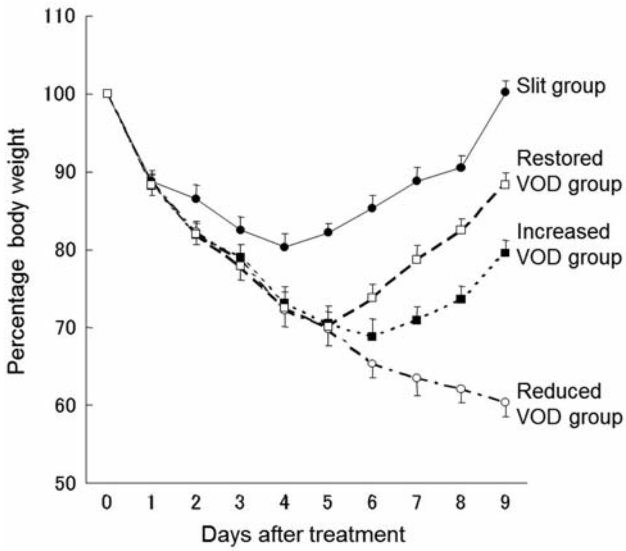

Figure 3. *Decrease rate of body weights in the treated groups, calculated on the basis of the body weight in the control group. Vertical bars represent the standard error (SE), n=15.*

frequency components' (HF component: 0.74-2.00 Hz) were observed in the spectra of the heart rate fluctuations at rest (19). The HF component reflects cardiac vagal nervous activity, indicating the excitation of the cardiac vagal nerve. However, the LF component was increased in nearly the same patterns and extents as the HF component. The LF component is a mixture of both sympathetic and cardiac vagal nervous activities, and sympathetic nervous activity has been evaluated by the analysis of the LF component, but no appropriate evaluation method for sympathetic nervous activity in heart rate fluctuations has been established.

Analysis of the heart rate fluctuations in the reduced VOD group demonstrates that the both LF and HF activities were increased with the first peak at 1 or 2 days, and the second peak at 4 or 5 days after tooth grinding in the HF component, indicating excitation of the cardiac vagal nerve. However, the LF component was increased in nearly the same patterns and extents as the HF component.

The first peak of the heart rate fluctuations were observed in the reduced VOD, the slit and the restored VOD groups (Figure 4A-C). It was suggested that the increase in the activities of the autonomic responses is caused by pulpal stimulation that is well known as a stressor. The second peak of the heart rate fluctuations was observed only in the reduced VOD group (Figure 4A). It is highly probable that the second peak is closely related to the reduced VOD. In this condition, the masticatory muscles should be contracted further to occlude after tooth grinding. In order to contract the masticatory muscles, the excessive efferent impulse from the central nervous system to the masticatory muscles in

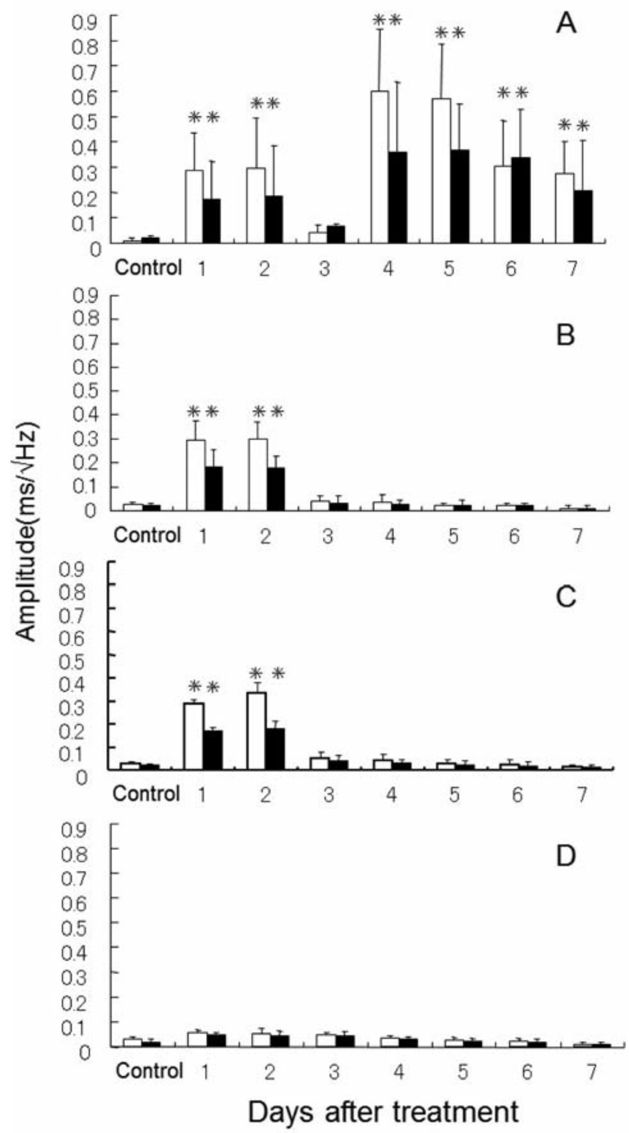

Figure 4. *Changes in heart rate fluctuations after reduced VOD (A), the slit formation (B), occlusal restoration (C) and increased VOD groups (D). High frequency (HF): closed columns, low frequency (LF): open columns, n=15. Vertical bars represent the standard error (SE); *p<0.01 vs. corresponding control value.*

needed. The autonomic system and motor nervous system are included in the excessive efferent impulse and these outputs are more enhanced (*i.e.* the brain works harder). Therefore, the autonomic response adjusting the cardiac function in response to the occlusal height was investigated in the present study.

Analysis of the heart rate fluctuations in the slit and restored VOD groups demonstrated the appearance of only the first peak of the heart rate fluctuations, without the second peak shown in reduced VOD group. This suggests

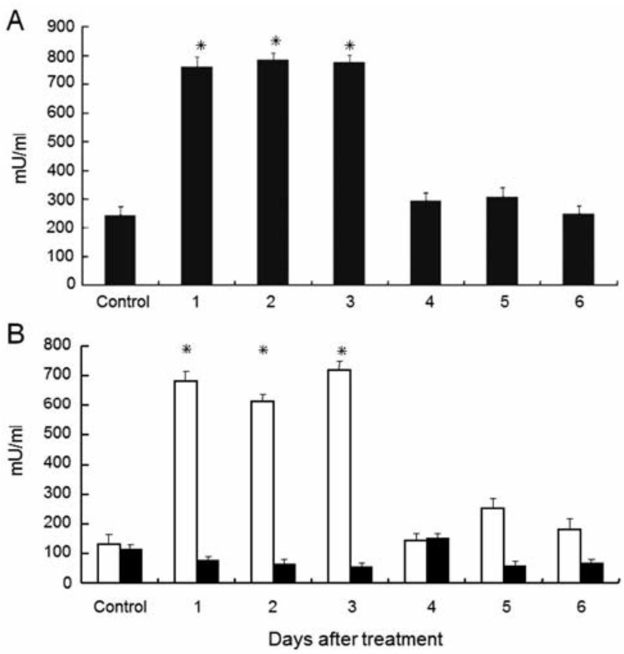

Figure 5. *Changes in creatine phosphokinase (CPK) activity in the reduced VOD group. A: Total CPK activity; n=15; B: The activity of CPK isozymes: brain type BB (closed columns), skeletal muscle type MM (open columns); n=20. Vertical bars represent the standard error (SE); * p<0.01 vs. corresponding control value.*

that the first peak was common to these three groups and was due to the efferent impulse generated by the pulpal stimulation due to tooth grinding. On the other hand, the second peak is induced by the reduction in occlusal height and furthermore the resultant over closure of the masticatory muscles. It was expected that the second peak may be increased due to the occlusal raising, but we did not observe such an increase in the heart rate fluctuations. This suggests that the extended stimulation in the masticatory muscles, is the single synaptic reflex of the trigeminal mesencephalic nucleus and the motor nucleus of the trigeminal nerve (20-22) and this showed that the biological response is quite different from the excitation of the trigeminal spinal nucleus (mainly pulpal stimulation) associated with tooth grinding, as in the case of the reduced VOD, the restored group and the slit groups. Furthermore, since it is clear from the heart rate fluctuations that the excessive contraction order affects the autonomic nervous system, the output abnormality may be an important factor in causing heart failure. When the occlusal position is raised as in the restored VOD group and increased VOD group, the occlusion is completed so fast that the contraction order to the masticatory muscles is not required. In other words, the excessive output information from the central nervous system, such as the motor output system and autonomic nervous system, is not required. Therefore, it may not be necessary for the brain to adjust the occlusal position.

We performed the biochemical examination of the blood to investigate the etiology of heart failure in the reduced VOD group. We did not observe an abnormality of electrolytes, considered as one of the causes of T-wave inversion (11). We confirmed that the activity of CPK, an index of cardiac disorder, was increased, with a peak at 1 or 2 days after tooth grinding (Figures 4 and 5A). Among CPK isozymes, higher expression of the MM type was found (Figure 5B). This strongly suggests that the increased CPK may derive from the cardiac muscle. However, the skeletal muscle has nearly the same isozyme component as in the cardiac muscle; further study is needed to identify the origin of the increased CPK. There was another possibility that LDH isozyme may have caused the elevation of CPK activity. Since LDH isozyme component in the cardiac muscle is clearly different from the one of the skeletal muscle, we thought it might be possible to distinguish the component from the cardiac muscle from that of the skeletal muscle, but since the fluctuations of total LDH after tooth grinding were slight, no conclusion is reached. However, it is highly probable that the increased CPK results from the cardiac muscle, judging from the transient increase in CPK and appearance of abnormal waveforms on ECG.

These results strongly suggest that the reduction in VOD is not a so-called stress but the excessive output of the central nervous system, resulting in the appearance of the malposture and systemic adverse effects, including heart failure, through the autonomic nervous system

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