CLINICAL RESEARCH

Influence of Chewing and Clenching on Salivary Cortisol Levels as an Indicator of Stress

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Purpose: **The purpose of this study was to investigate the effects of chewing and clenching on salivary cortisol levels as an indicator of stress.**

Materials and Methods: **Seventeen healthy dentulous subjects were given arithmetic exercises to perform within a 20-minute time limit in order to elicit stress (stress loading). In the first experiment (chewing), after stress loading, the subjects were asked to chew a paraffin wax while reading printed material (books, magazines, etc.) in silence for 10 minutes. The same procedure was then carried out again for control purposes, but this time the subjects were not required to chew wax. In the second experiment (light clenching), after stress loading, the subjects were required to carry out 5 seconds of light clenching followed by 5 seconds of rest repeatedly over a 3-minute period. The whole 3-minute process was repeated a total of three times. The control data for this second experiment consisted of measurements taken during the rest periods. Saliva specimens were collected in both experiments both before stress loading and after each procedure during 1-minute intervals to measure cortisol levels.**

Results: **In the chewing experiment, salivary cortisol levels were significantly reduced by chewing, compared with those in the controls ($p < 0.05$). This reduction in salivary cortisol was observed during chewing over a 10-minute period following stress loading. In the clenching experiment, salivary cortisol levels also showed a significant reduction during clenching, compared with those in the controls ($p < 0.05$).**

Conclusions: **These results suggest that chewing and clenching promote relaxation in subjects under stress.**

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INDEX WORDS: chewing, clenching, stress, salivary cortisol, relaxation

BRUXISM is widely considered to be a physical response to emotional stress,[1–3] although this relationship remains controversial. Rao and Glaros[4] concluded that frustration and anxiety caused tension in the masseter muscles and suggested there was a relationship between diurnal clenching and this kind of muscular tension. Moreover, Yemm[5] reported that the activity of masseter muscles increased in response to experimental stress loading in humans. Butler and Stallard[6] observed that patients under stress exhibited more frequent and longer-lasting teeth contact than patients who were not under stress. A similar phenomenon has also been recognized in rats, where stress-loaded rats have exhibited bruxism-like activity of the masseter muscles.[7] On the other hand, another study has reported that there was no significant relation between frequency of bruxism and daily stress.[8] There have been a number of varying reports, but a consensus of opinion on this issue remains elusive.

In a study aimed at elucidating a possible relationship between stress and chewing, Morita[9] reported that chewing gummy candies and gum resulted in relaxation from mental stress in humans as assessed by changes in adrenaline, noradrenalin, adrenocorticotropic hormone in plasma,

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serum cortisol levels, electrodermal activity, facial skin temperature, blood pressure, pulse rate, SpO_2, and electrocardiograms. Furthermore, Ohtsuka et al[10] reported an increase in the frequency of alpha waves after gum chewing. Although there have been several reports describing a possible association between the stomatognathic system and stress, this association is still not well understood.

Various methods of objectively measuring stress have been introduced, including different types of scales, questionnaires, electroencephalograms, and biochemical assessments of blood and urine specimens.

When humans are placed under stress, the HPA is activated, leading to an increase in cortisol secretion.[11] Based on this fact, several studies have used changes in salivary cortisol levels as an indicator of stress,[12-14] and it has been reported that these levels increase under acute stress, for example, when subjects are required to perform arithmetic calculations in a noisy environment,[15] take an examination,[16] or watch a suspense film.[17]

The purpose of the present study was to clarify the effects of chewing and clenching on relaxation under stress by measuring salivary cortisol levels, which can be collected easily and non-invasively.

Materials and Methods

Subjects

The subjects consisted of 17 healthy dentulous males (mean age, 26 ± 2 years) without subjective or objective abnormalities of the stomatognathic system. None had any previous medical history of illness. The subjects were fully informed about the experimental procedures and informed consent was obtained from all of them.

Experimental Conditions

The experiments were performed between 14:00 and 19:00, when salivary cortisol levels are considered to be stable on the basis of circadian rhythm.[11] To avoid the effect of food intake on salivary secretion, subjects were asked to refrain from consuming caffeine or alcohol the day before and on the day of the experiments. Also, eating, drinking, oral prophylaxis, brushing, and flossing were prohibited within 2 hours before the experiments. Subjects were instructed to maintain the same posture and not to make any extraneous movements, such as stretching, during the experiments.

The following experiments were performed (Figs 1 and 2):

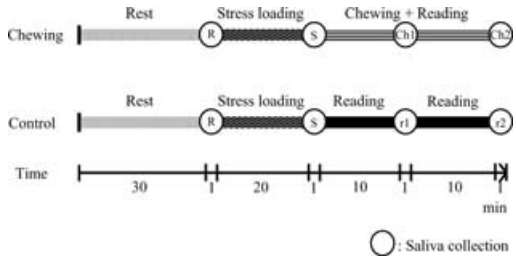

Figure 1. Chewing experiment schedule. R = First saliva collection (before stress loading); S = Second saliva collection (immediately after stress loading); Ch1 = Third saliva collection (after first wax chewing with silent reading for 10 minutes); Ch2 = Fourth saliva collection (after second wax chewing with silent reading for 10 minutes); r1 = Third saliva collection (after first silent reading for 10 minutes); and r2 = Fourth saliva collection (after second silent reading for 10 minutes).

Chewing Experiment (Fig 1)

First, the subjects were asked to rest in a shielded experiment room for 30 minutes in order to familiarize themselves with the environment, after which the first set of saliva specimens (referred to as R in Fig 1) was collected. Next, as stress loading, they were given a series of arithmetic calculations to perform within a 20-minute period. Immediately afterwards, the second set of saliva specimens (referred to as S in Fig 1) was collected.

Then, the subjects were asked to chew a paraffin wax (the type used for clinical examination) while reading their favorite book or any other printed material in silence for 10 minutes. After that, the third set of saliva

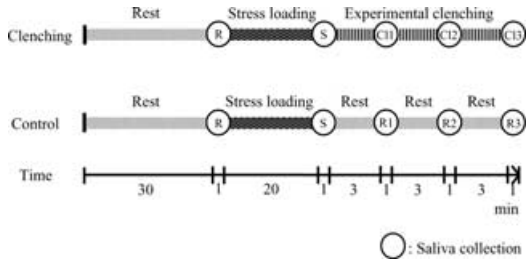

Figure 2. Clenching experiment schedule. R = First saliva collection (before stress loading); S = Second saliva collection (immediately after stress loading); Cl1 = Third saliva collection (after first periodic clenching for 3 minutes); Cl2 = Fourth saliva collection (after second periodic clenching for 3 minutes); Cl3 = Fifth saliva collection (after third periodic clenching for 3 minutes); R1 = Third saliva collection (after first rest for 3 minutes without clenching); R2 = Fourth saliva collection (after second rest for 3 minutes without clenching); R3 = Fifth saliva collection (after third rest for 3 minutes without clenching).

specimens (referred to as Ch1 in Fig 1) was collected. The subjects were then required to chew paraffin wax and read one more time, after which a fourth set of saliva specimens (referred to as Ch2 in Fig 1) was collected. The second part of this experiment was carried out under the same conditions, but with the third and fourth sets of saliva specimens (referred to as r1 and r2 in Fig 1) being collected after each session of reading in silence for 10 minutes without chewing. The data obtained from this experiment served as the control.

As previously mentioned, a paraffin wax was used as the material for chewing. The amount used was 1.0 g, and it was softened to the appropriate hardness prior to the experiment. Each subject performed both reading with chewing (chewing), and reading without chewing (control) on different days. Both chewing and control activities were assigned at random.

Clenching Experiment (Fig 2)

In the clenching experiment, the subjects were asked to rest in the shielded room for 30 minutes and were then required to perform some arithmetic calculations over a 20-minute period. Saliva specimens were collected immediately after each session (referred to as R and S in Fig 2). Next, the subjects were instructed to perform periodic clenching for 3 minutes. This periodic clenching was defined as 5 seconds of clenching followed by 5 seconds of rest to be repeated over a 3-minute period. The whole process was repeated a total of three times. Subjects were instructed to clench lightly, during which EMG activity in the central area of the masseter muscles was monitored on both sides. After each clenching session, sets of saliva specimens (referred to as Cl1, Cl2, and Cl3 in Fig 2) were collected. A total of five sets of saliva specimens were obtained. The second part of this experiment was carried out under the same protocol, but after stress loading, the subjects were asked to rest for 3 minutes and repeated three times. After each 3-minute session, sets of saliva specimens were collected. The data obtained from this experiment served as the control. The sets of saliva specimens collected after each rest session are referred to as R1, R2, and R3 in Figure 2.

Each subject performed both periodic clenching (clenching) and rest (control) on different days. Both clenching and control activities were assigned at random.

Measurements and Recording Equipment

Salivary Cortisol Levels. A Salivette (SARSTED Inc., Rommelsdorf, Germany) saliva collection kit was used for collection of the saliva specimens. Saliva was collected by requiring the subjects to keep a cotton roll intraorally for 1 minute. The saliva specimen obtained was centrifuged at 3000 rpm for 15 minutes and the supernatant was frozen to –20°C for preservation. Salivary cortisol levels were analyzed using radioimmunoassay with a GammaCoatTMCortisol (DiaSorin Inc, Stillwater, OK) RIA Kit in accordance with the manufacturer's instructions.

Electromyogram (EMG). A Muscle Tester ME3000p (Mega Electronics Ltd., Kuopio, Finland) was used to measure masseter muscle activity. Bipolar Surface electrodes (Blue Sensor P-00-S, Medicotest, Olstykke, Denmark) were placed in the direction of the muscle fibers over the main bulk of both sides of the masseter muscle as determined by palpation with an interelectrode distance of 20 mm. Before placing the electrodes, the skin was thoroughly cleansed using a specific skin cleansing gel (Skin pure Nihon Kohden, Tokyo, Japan) and ethanol-soaked gauze. Skin impedance between the electrodes was lower than 8 kΩ.

To obtain a uniform strength of light clenching, the subjects were required to practice periodic clenching many times prior to the actual experiment, with visual biofeedback being provided from a monitor. The maximal voluntary clenching (MVC) of each subject was recorded at the end of each clenching experiment. Mean muscle activity during light clenching was calculated by averaging the data obtained from the three sets of periodic clenching. The EMG results were expressed as a percentage of MVC.

Statistical Analysis

Changes in salivary cortisol levels immediately after experimental stress loading and time of each saliva collection were determined for each chewing and clenching experiment. A paired *t*-test was used for the statistical analysis. Statistical significance was defined as a p-value of <0.05 by using statistical analysis software SPSS 11.0J for Windows (SPSS, Chicago, IL).

Experimental Ethics

This protocol was approved by the Ethics Committee of Tokyo Dental College. All experiments were done in accordance with the Edinburgh Revision of the Helsinki Declaration.

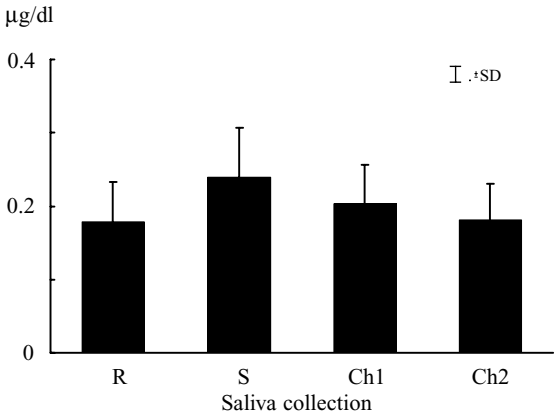

Figure 3. Salivary cortisol levels with chewing.

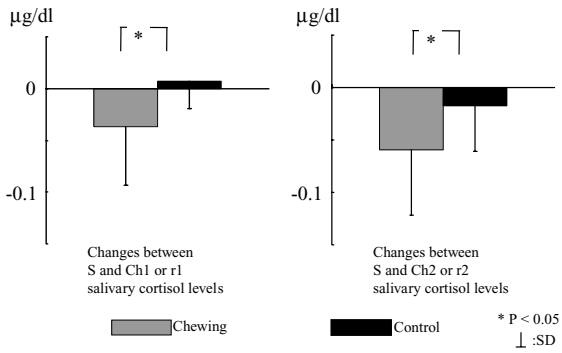

Figure 5. Comparison of changes in salivary cortisol levels between chewing and controls.

Results

Chewing Experiment

Five out of the 17 subjects showed no increase in their salivary cortisol levels after experimental stress loading (arithmetic calculations within a 20-minute period), and these were excluded from the statistical analysis. Therefore, statistical analysis was performed on data obtained from a total of 12 subjects. The mean and standard deviations of the salivary cortisol levels in the subjects in the chewing experiment are shown in Figures 3 and 4.

Salivary cortisol levels between the second (S) and third saliva collection (Ch1 and r1) with both chewing and in the controls showed a reduction of 15.4% with chewing, compared with −3.0% in the controls. Moreover, between the second (S) and fourth saliva collection (Ch2 and r2), reductions of 24.6% and 7.1% were observed with chewing and the controls, respectively. Significant differences in salivary cortisol levels were recognized with chewing compared with those in the controls (Fig 5).

Clenching Experiment

Four out of the 17 subjects showed no increase in their salivary cortisol levels after experimental stress loading. Therefore, as with for chewing, these subjects were excluded from further statistical analysis. The mean and standard deviation of the salivary cortisol levels in the clenching experiment are shown in Figures 6 and 7. Changes in salivary cortisol levels between the second (S) and third saliva collection (Cl1 and R1) in both clenching and the controls were compared. These showed a reduction of 11.2% with clenching, compared with –2.9% in the controls. The same tendency was

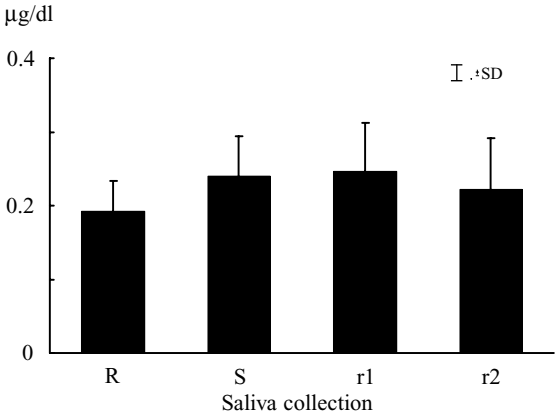

Figure 4. Salivary cortisol levels in controls.

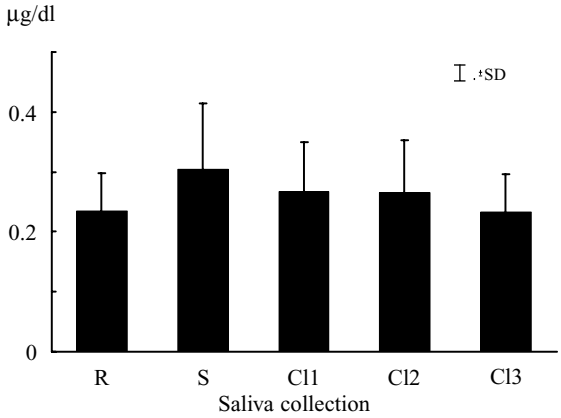

Figure 6. Salivary cortisol levels for clenching.

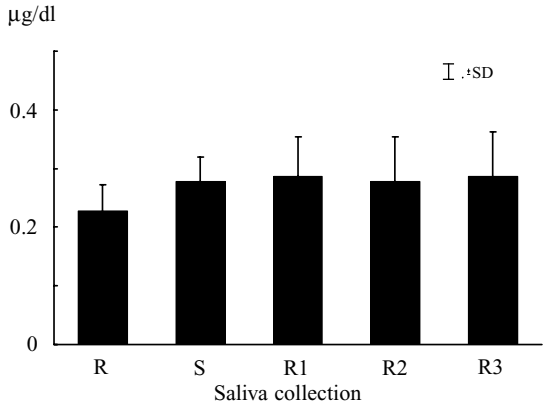

Figure 7. Salivary cortisol levels in controls.

observed when salivary cortisol levels between the second (S) and fifth saliva collection (Cl3 and R3) were compared. A reduction of 23.4% and –2.7% in cortisol levels was observed with clenching and in the controls, respectively. These results showed a significant difference in salivary cortisol levels with clenching compared with that in the controls (Fig 8); however, a comparison of the changes in salivary cortisol levels between the second (S) and fourth saliva collection (Cl2 or R2) showed no significant differences between clenching and the controls (Fig 8).

Discussion

In this study, the controls were required to read in silence in the chewing experiment and rest in the clenching experiment. Throughout the experiments, the subjects were instructed to maintain the same posture and not to make any extraneous

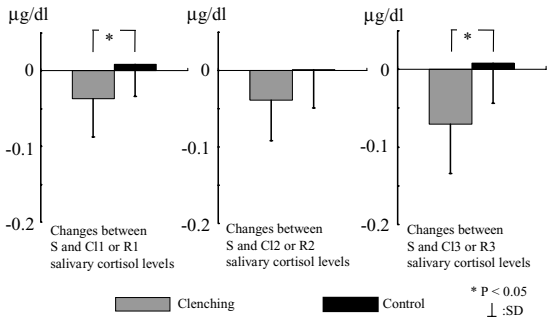

Figure 8. Comparison of changes in salivary cortisol levels between clenching and controls.

movements such as stretching or bruxism. No remarkable EMG activity in the masseter muscles, such as clenching, was recorded in any of the subjects when acting as controls. The purpose of this study was to clarify the effects of chewing and clenching on relaxation under stress. Therefore, we believe the conditions we set for the controls were appropriate.

Various methods have been introduced in previous studies for the assessment of stress. Some of these, however, include invasive procedures (collection of blood samples, etc.) which not only induce stress in subjects, but are also difficult to perform. Such methods, being stressful in themselves, carry the danger of eliciting skewed data. Other methods, such as urinalysis, carry time limitations, which may again skew data by not accurately reflecting changes in cortisol levels.

Saliva collection is considered to be an easy, non-invasive, and effective method, as salivary cortisol levels show a high correlation with serum cortisol levels.[18-20] Moreover, cortisol in serum is known to be rapidly transferred to saliva (within 5 minutes) and is not affected by salivary flow rate.[19,20] Therefore, for the purpose of this study, measurement of salivary cortisol levels was selected as the most appropriate method for properly assessing stress.

In this study, arithmetic calculations were used to elicit stress loading, with subjects being required to perform them within a limited time (20 minutes), and with restrictions on body movement and stretching. When the salivary cortisol levels were measured immediately after stress loading, a significant difference in these levels was recognized in almost all of the subjects, regardless of their arithmetic skills; however, five and four out of the initial total of 17 subjects showed no increase in salivary cortisol levels after 20 minutes of stress loading with chewing and clenching, respectively. Why these subjects were not affected by the stress loading remains unclear; however, we presume they probably did not fully understand the instructions on how to perform the arithmetic calculations, and consequently tried to solve them at their own pace, not realizing they were restricted to a 20-minute period. These subjects were excluded from further statistical analyses. After stress loading, salivary cortisol levels exhibited various responses, some showing an increase, some a decrease, and some hardly changing at all. We believe that this may have

occurred due to the following reasons: (1) in some cases, increased salivary cortisol levels occurring some time after chewing or clenching may have resulted from a muted response from the HPA system to stress loading, (2) in other cases, the high values for salivary cortisol levels after 30 minutes' rest (first set of saliva specimens) may have resulted from the stress of the experiment itself, and (3) in yet other cases, calculations for stress loading, chewing and clenching may have had no influence on the stress levels of the subjects at all, resulting in no changes in salivary cortisol levels.

Furthermore, with both chewing and clenching, the subjects were instructed to maintain the same posture and make no extraneous movements (no stretching or activities of the stomatognathic system such as bruxism) while performing the arithmetic calculations. As a result, no remarkable activity of the masseter muscles, such as clenching, was recorded during stress loading in any of the subjects.

As mentioned previously, with both chewing and clenching, salivary cortisol levels were significantly reduced compared with those in the controls. Our results for chewing concur with those reported by Morita [9] and Ohtsuka.[10] The changes in salivary cortisol levels between the second and third saliva collections showed a significant difference between chewing and the controls ($p < 0.05$). On the other hand, the changes that occurred between the third and fourth saliva collections showed no such difference ($p < 0.05$). These results suggest that relaxation under stress occurred predominantly during the first 10 minutes of chewing. Taking this into consideration, salivary cortisol levels were measured within the first 10 minutes after stress loading for the clenching experiment. Again, the same tendency was observed as with clenching, with changes in salivary cortisol levels showing significant differences during the first 3 minutes of periodic clenching.

In the clenching experiment, the subjects were requested to perform light clenching and were allowed to practice it as many times as they liked prior to the actual experiment. Practice involved periodic clenching, with 5 seconds of clenching followed by 5 seconds of rest repeated during a 3-minute period a total of three times, all the time maintaining a uniform strength of clenching. This practice included visual biofeedback on masseter muscle activity; however, there was concern that the subjects might feel stressed by being exposed to such feedback. Therefore, in order to avoid this, visual biofeedback was withheld during the actual clenching experiment.

Clenching strength ranged from 11.3% to 45.5% MVC among the subjects. We only instructed them to clench lightly, and did not establish an objective measure of clenching strength such as %MVC, for example. Therefore, this wide range of strengths may have resulted from differing subjective perceptions of strength.

Piquero and Sakurai [21] defined a naturally occurring clenching event as one of more than 10% MVC of masseter muscle at least 3 seconds during silent reading for 10 minutes. In the clenching experiment of the present study, we observed masseter muscle activity of more than 10% MVC lasting up to 5 seconds. Therefore, strength and duration of experimental clenching in the present study were considered as natural clenching. We did not find any relationship between salivary cortisol levels and clenching strength in this study; however, periodic light clenching, which the subjects themselves believed to be done with light strength, clearly ameliorated stress.

It appears that chewing and clenching following stress loading, both of which are activities of the masticatory muscles, stimulated the motor area of the cerebrum, thus muting the response of the endocrine HPA system, thereby decreasing cortisol levels.

We believe that stimuli to the periodontal membrane receptor oral mucosa and tongue brought about by chewing and clenching may also be transmitted to the brain, thus muting the response of the endocrine HPA system. The subjects were instructed to maintain the same posture and not make any extraneous movements during the experiment. Chewing and clenching may have provided a release from the stress that this would incur, thus stimulating the cerebral emotion mechanism. In other words, after stress loading, stimuli resulting from moderate chewing and clenching may be transmitted to the brain, thus activating the stress control mechanism; however, the precise mechanism of this process remains to be clarified.

Bruxism has been defined as an oral parafunction that has harmful effects on the stomatognathic system, such as facial pain, abnormal tooth wear, periodontal pain, increased tooth mobility, muscle tenderness palpation, increased muscle

tonus, hypertrophy, and TMJ discomfort, among others. These effects are related to the intensity, frequency, and duration of the parafunctional activity.

The results of the present study may not be of any direct use for the treatment of patients in a clinical setting; however, we did observe that light periodic clenching (one type of bruxism) effectively brought about a reduction in stress, as evidenced by a reduction in salivary cortisol levels. Therefore, these results may have some implications for giving temporomandibular disorder (TMD) patients information on their condition. In such cases, we suggest that it might be beneficial to explain that, within bruxism, periodic light clenching could be seen as an ortho-function directly related to stress, and that its functional objective was to elicit relaxation.

Conclusion

The results of this study showed that chewing and periodic light clenching reduced cortisol levels. We believe that this reduction may thereby also indicate a reduction in stress; however, for future investigation, it will be necessary to assess how intensity, duration, and frequency of clenching influence relaxation under daily stress.

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