Occlusal interference causes an increase in salivary protein

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Occlusal interference may influence the functional balance of the autonomic nervous system. Salivary amylase levels are related to plasma noradrenaline concentrations and are increasingly being used as a marker for the reaction of the sympathetic nervous system to stress. We examined the effect of occlusal interference on salivary amylase activity (SAA). Saliva samples were obtained from 10 healthy volunteers (9 males and 1 female, who had an average age of 29 years). They all had normal occlusion and no history or signs of medical disorders. Each subject was instructed to tap his teeth with and without the experimental occlusal inference of metal foil on the occlusal surface of the mandibular first molar on the habitual masticatory side. We assayed SAA ten times each for metal foil thicknesses of 12.7, 25.4, 38.1 and 50.8 μm. There was an immediate and significant increase in the SAA with all foil thicknesses compared with the control. Furthermore, the SAA returned to the control level after removal of the foil.

There results suggest that SAA is a useful indicator of occlusal balance because it sensitively reacts to differences in the thickness of the metal foil. The measurement method of occlusal contact using BiteEye has high reproducibility and reliability. In this study, both occlusal contact area and the number of occlusal contact points were calculated using the BiteEye, and the occlusal contact area per point on the experimental teeth and both adjacent teeth was calculated. We found that it was possible to objectively evalvate the occlusal state by measuring SAA with tapping. (J Osaka Dent Univ 2017 ; 51 : 63-72)

**Key words : Salivary protein ; Salivary amylase activity ; Occlusal examination**

INTRODUCTION

It is important to accurately grasp the state of occlusal contacts in prosthetic treatment. Various occlusal test methods (articulating paper, the pull-out test, the Dental Prescale Occlusal System (GC, Tokyo, Japan), the occlusal contact pressure distribution measurement system, and the check bite method using wax and silicone rubber) are currently used in prosthetic treatment and occlusial therapy. However, these methods have several disadvantages.

The artificial paper method has low reproducibility because of changes in the material at the point of occlusal contact, surface roughness and moisture, as well as the number of times the contact is cycled.[1] The pull-out test has poor reproducibility and objectivity because the presence or absence of contact and the degree of tightness are evaluated from the resistance when pulling out plastic, metal strips, or thin film between the upper and lower teeth.[2-4] With the Prescale method, there is a possibility of measurement leakage on the front teeth, and the storage management of the pressure sensitive sheet after color development affects the result. With the T scan, since the surface of the teeth does not coincide with the actual dentition, it is impossible to measure the occlusal force and the contact area per tooth.[5, 6]

Stress is classified as human body stress and
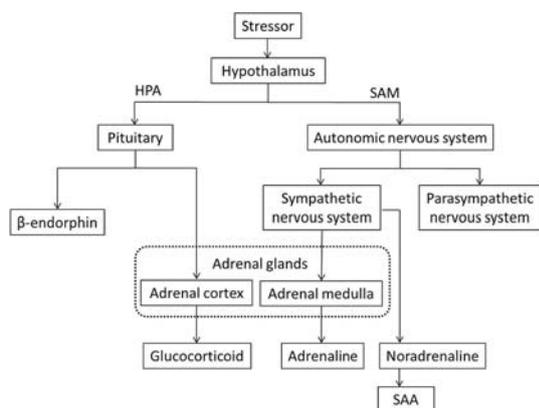
Fig. 1 Physiological response to stress by the sympathetic nervous-adrenomedullary system (SAM) and by the hypothalamic-adrenocortical system (HPA).

cell stress, as well as psychological stress and physiological stress. Two areas that reflect changes in human stress are the hypothalamus-pituitary-adrenocortical (HPA) system, with cortisol secretion that is regulated by adrenocorticotropic hormone (ACTH) from the pituitary gland, and the sympathetic adrenomedullary (SAM) system, with secretion of catecholamine (Fig. 1).

Salivary α-amylase activity (SAA) have been used to determine pain-induced stress levels, having shown good correlation with scales for the subjective analysis of pain intensity.[7] It has been demonstrated that secretion of salivary amylase is regulated by the sympathetic nervous-adrenomedullary system, which is controlled by noradrenalin, in the salivary glands.[8, 9] This happens because the production of salivary α-amylase reflects the activity of the sympathetic adrenomedullary system (SAM) in individuals under stress. In order to quantify psychological stress and distinguish comfortable and uncomfortable physical stress, we attempted to establish a method that can quantify SAA in the saliva.[10, 11]

On the other hand, the predominant secretory immunoglobulin on mucosal surfaces is immunoglobulin A (IgA). In the mouth, it is a component of the saliva secreted by the major and minor salivary glands.[12] Secretory immunoglobulin A (SIgA) is present in salivary secretions and, along with other glycoproteins such as mucin, lactoferrin and peroxi-

dase, is responsible for helping maintain the integrity of mucosal surfaces against infectious agents.[13] SIgA is the main component of the adaptive immune system.[14]

Lactoferrin is one of the most abundant antimicrobial proteins and plays a key role in mucosal immunity against pathogenic infection.[15] Previous studies have reported that salivary lactoferrin concentrations are modulated immediately after strenuous exercise.[16, 17] Lactoferrin is thought to play a role in innate defense and exhibits a diverse range of biological activities, including antimicrobial activities, antiviral activities, antioxidant activities, immunomodulation, modulation of cell growth, and binding of several bioactive compounds, such as lipopolysaccharides.[18] Lactoferrin seems to be the available stress marker for the oral physiological response. Although it is well known that saliva is secreted by pressure stimulation to the periodontal ligament, this stimulation may affect the secretion of stress proteins.

We investigated how occlusal interference affects the autonomic nervous function, i.e. secretion of salivary amylase, SIgA and lactoferrin concentration. Occlusal interference not only affects the periodontal tissue and the temporomandibular joint, but also affects the functions of the jaw, face, head and neck, as well as the neuromuscular system, and it can cause various systemic disorders It has been shown that occlusal interference can have a major influence as a stressor of the autonomic nervous system. It is a possible that occlusal interferences might influence the functional balance of the autonomic nervous system. Salivary amylase levels correspond to plasma noradrenaline concentrations and are increasingly utilized as an accessible measure of sympathetic nervous system reactivity to stressors. Therefore, we examined whether the concentration of SAA, SIgA and lactoferrin fluctuate during experimental occlusal interference induced by tapping and placement of metal foil on teeth. Although it is well known that saliva is secreted by pressure stimulation to the periodontal ligament, this stimulation may also affect the secretion of stress proteins.

MATERIALS AND METHODS

The experimental procedures were approved by the Ethics Committee of Osaka Dental University (Approval No. 110772), and all subjects gave informed consent. All subjects consented to participate following verbal and written explanation regarding the goals and the design of the study.

Subjects

Ten healthy, volunteer subjects with no occlusal abnormalities participated in this study. They were all dentists working at Osaka Dental University Hospital (9 males and 1 female) between 24 and 34 years of age with an average age of 29 years.

Study design

We collected saliva before and after having the subjects tap their teeth with and without occlusal interference and detected the SAA activity, SIgA concentration, and lactoferrin. The subjects were seated in a dental chair for ten minutes before the start of the experiment. The first saliva collection was done before tapping as a control. Saliva collection was done after the subjects tapped ten times with experimental occlusal interference on their habitual masticatory side (Fig. 2).

Experimental occlusal interference

We made an experimental occlusal interference by putting metal foil (Occlusal Registration Strips of 12.7 μm ; Artus, Humburg, Germany) on the lower first molar of the subject's habitual masticatory side. The experimental simulation of an occlusal interfer-
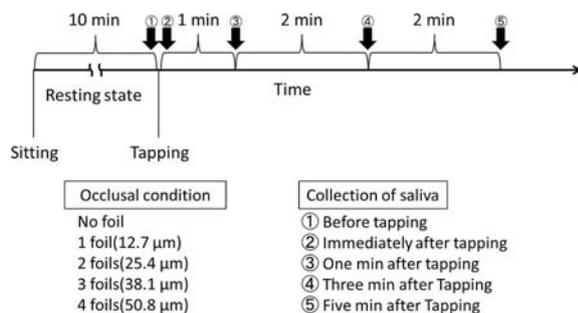
ence was created by having the subject tap ten times without the foil (control), then tapping with one, two, three and four foils (Figs. 3 and 4).

Saliva collection

We collected saliva using a saliva collection tube (Salivette Cotton, Sarstedt, Nümbrecht, Germany)

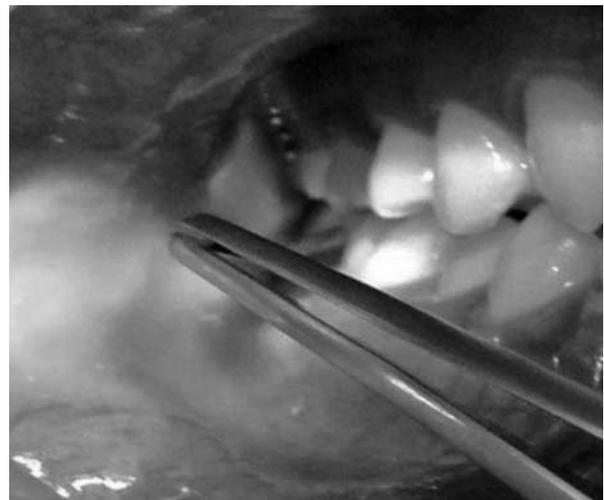**Fig. 3**   Placement of a metal foil on the subject's tooth.

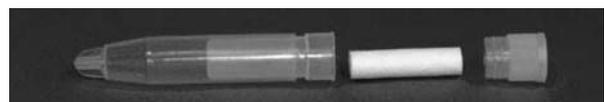**Fig. 4**   After the subject had been instructed to tap with the foil placed on the tooth.

Fig. 5   Saliva collection tube(Salivette Cotton, Sarstedt, Nümbrecht, Germany).

Fig. 2   Experimental times.

(Fig. 5). Saliva samples were collected for 1 minute just before tapping, immediately after tapping, and at 1, 3 and 5 minutes after tapping.

Assay of salivary proteins

The concentration of salivary SIgA was measured by enzyme-linked immunosorbent assay (ELISA). Primary (anti-human IgA) and secondary antibodies (Peroxidase-conjugated anti-human IgA, Sigma, Poole, UK) using a rabbit anti-rat IgA (Serotec, Oxford, UK). The assays were calibrated using serial dilutions of human colostrum IgA (Sigma). The concentrations of salivary lactoferrin was measured using a commercial ELISA assay kit (DRG Diagnostics, Marburg, Garmany) according to the manufacturer's instructions.

Salivary α-Amylase activity (SAA) was measured using a hand-held salivary amylase monitor (Nipro, Osaka, Japan) (Fig. 6). This analyzer enables automatic measurement of salivary amylase activity using a dry-chemical system within 1 min from collection to completion of the measurement. The tip of the testing strip was set under the tongue for 30 sec to collect saliva. Then, the strip was immediately inserted into the analyzer, which displays the result automatically.

Calculation of both of occlusal contact area and the score of occlusal contacts

The contact condition of both of the test teeth and adjacent teeth was recorded using conformity test materials (Blue Silicone, GC, Tokyo, Japan), when we instructed the subjects to occlude gently. The area of each contact point of the test tooth and the adjacent teeth were analyzed using a tooth contact analyzer (BiteEye BE- I, GC, Tokyo, Japan) (Fig. 7).

Statistical analysis

One-way ANOVA was performed with the significance level set at 5%. In order to reveal the relationship between SAA with the occlusal contact area per point, the correlation coefficient was calculated. The statistical analysis was done using IBM SPSS Statistics 19.0 (IBM, Tokyo, Japan).

RESULTS

Salivary amylase activity

Figures 8-12 show the relation between SAA level and the metal foil thickness. Immediately after tap-

Fig. 7   BiteEye BE-I® (GC, Tokyo, Japan).

Fig. 6   Portable salivary amylase activity monitor(Nipro, Osaka, Japan).
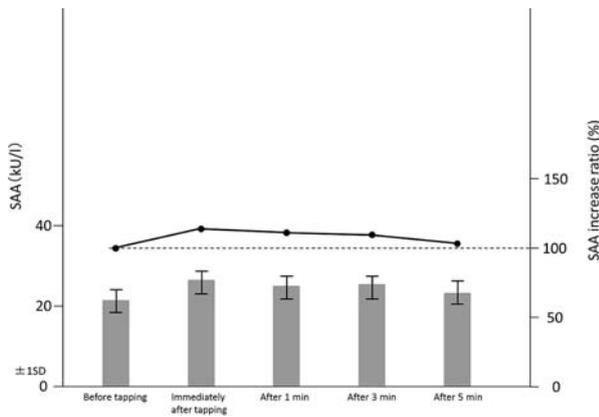
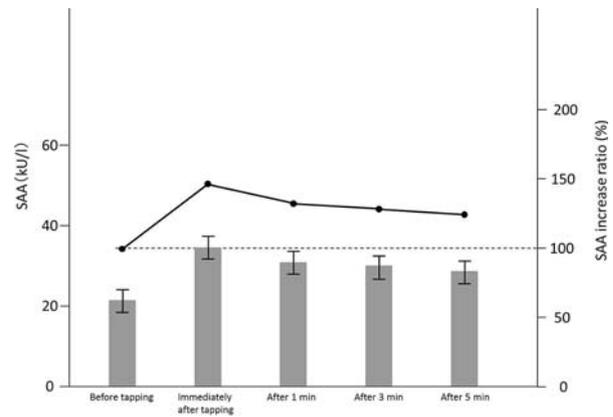**Fig. 8** Changes in SAA without the metal foi l (Ⅰ : ±SD).
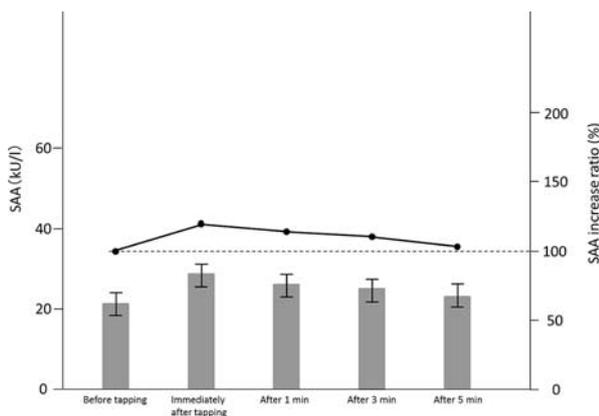

Fig. 11 Changes in SAA with 3 metal foils.
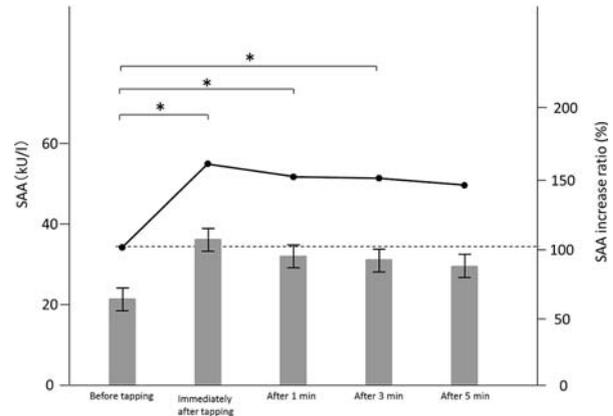

Fig. 9 Changes in SAA with 1 metal foil.

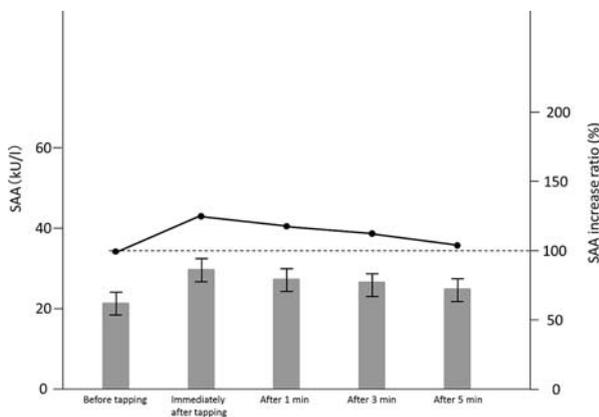**Fig. 12** Changes in SAA with 4 metal foils (*p＜0.05).

Fig. 10 Changes in SAA with 2 metal foils.

12).

The SAA level increased as the thickness of the metal foil increased. SAA was greater with tapping compared to the control, and it increased as the number of metal foils increased. In addition, the SAA level immediately decreased to the control level when the metal foil was removed for all metal thicknesses. One minute after tapping, the SAA level was significantly increased by 109.8% in the control, by 122.2% with 1 metal foil, by 137.6% with 2, by 150.4% with 3, and by 163.9% with 4. There minutes after tapping the SAA level was increased by 111.0% in the control, by 119.3% with 1 metal foil, by 129.8% with 2, by 138.0% with 3, and by 157.5% with 4. Five minutes after tapping the SAA level was increased by 104.4% in the control, by 112.4% with 1, by 117.4% with 2, by 128.3% with

ping, the SAA level was significantly increased by 110.8% in the control (Fig. 8), by 127.4% with 1 metal foil (Fig. 9), by 135.8% with 2 (Fig. 10), by 157.1% with 3 (Fig. 11), and by 174.4% with 4 (Fig.

3, and by 141.8% with 4. The SAA level increased as the thickness of the metal foil increased. It was greater with tapping compared to the control and further increased as the number of metal foils increased. Further, SAA level immediately decreased to the control level when the metal foil was removed for all metal thicknesses.

Concentration of SIgA

Figure 13 shows how SIgA concentration is related to the number of metal foils. It was 45.78±4.00 µg/mL in the control, 46.42±4.25 µg/mL immediately after tapping, 46.11±3.87 µg/mL at 1 minute, 45.71±4.13 µg/mL at 3, and 45.71±4.13 µg/mL at 5. There was no significant difference between the SIgA concentration and the number of metal foils. The concentration of SIgA after tapping with 1 metal foil was 45.48±19.56 µg/mL in the control, 46.13±3.74 µg/mL immediately after tapping, 46.17±4.16 µg/mL at 1 minute, 46.18±4.61 µg/mL at 3, and 46.06±4.52 µg/mL at 5. There was no significant difference between the SIgA concentration and the number of metal foils.

The concentration of SIgA after tapping with 2 metal foils was 46.34±3.26 µg/mL in the control, 46.05±3.39 µg/mL immediately after tapping, 46.59±3.68 µg/mL at 1 minute, 46.60±4.05 µg/mL at 3, and 46.42±3.61 µg/mL at 5. There was no significant difference between the SIgA concentration and the number of metal foils.

After tapping with 3 metal foils the concentration of SIgA was 47.20±4.07 µg/mL in the control, 47.41±4.07 µg/mL immediately after tapping, 47.13±4.15 µg/mL at 1 minute, 47.34±4.55 µg/mL at 3, and 47.21±3.95 µg/mL at 5. There was no significant difference between the SIgA concentration and the number of metal foils. The concentration of SIgA after tapping with 3 metal foils was 46.80±3.61 µg/mL in the control, 47.22±3.98 µg/mL immediately after tapping, 47.10±3.94 µg/mL at 1 minute, 47.23±4.10 µg/mL at 3, and 47.60±4.29 µg/mL at 5. There was no significant difference between the SIgA concentration and the number of metal foils.

Concentration of lactoferrin

Figure 14 shows the relation of lactoferrin concentration to the numbers of metal foils. The concentration of lactoferrin after tapping without metal foil was 5.37±0.68 µg/mL in the control, 5.37±0.67 µg/mL immediately after tapping, 5.41±0.66 µg/mL at 1 minute, 5.41±0.64 µg/mL at 3, and 5.49±0.65 µg/mL at 5. The concentration of lactoferrin after tapping with 1 metal foil was 5.32±0.79 µg/mL in the control, 5.34±0.77 µg/mL immediately after tapping, 5.36±0.72 µg/mL at 1, 5.38±0.75 µg/mL at 3, and 5.42±0.75 µg/mL at 5. The concentration of lactoferrin after tapping with 2 metal foils was 5.35±0.90 µg/mL in the control, 5.38±0.86 µg/mL immediately after tapping, 5.38±0.85 µg/mL at 1 minute, 5.47±0.90 µg/mL at 3, and 55.57±0.90 µg/mL at 5. The concentration of lactoferrin after tap-
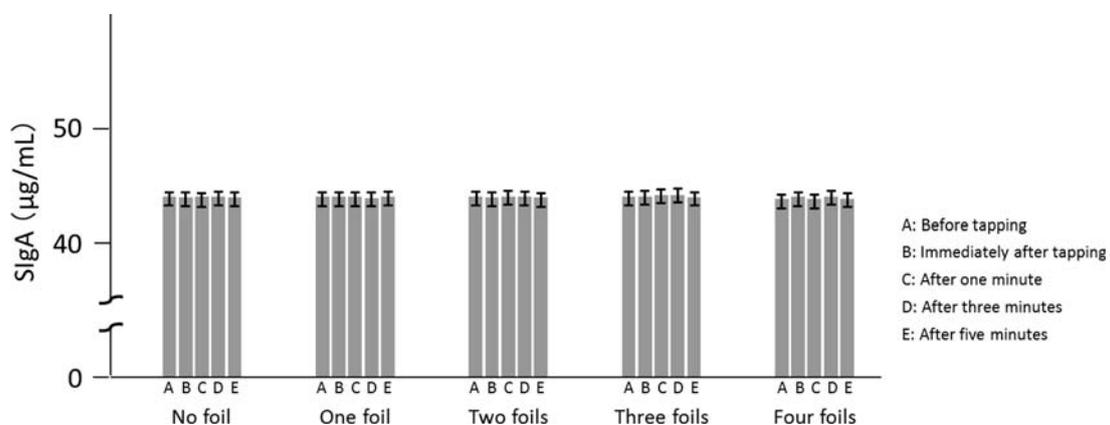

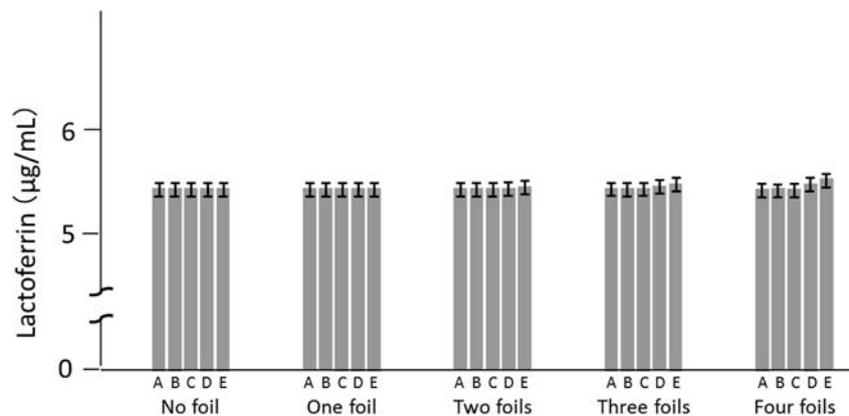**Fig. 13**   Relation of SIgA concentration and the numbers of metal foils.

Fig. 14 Relation of lactoferrin concentration and the number of metal foils.

ping with 3 metal foils was 5.30 ± 0.90 µg/mL in the control, 5.34 ± 0.89 µg/mL immediately after tapping, 5.41 ± 0.89 µg/mL at 1 minute, 5.63 ± 0.91 µg/mL at 3, and 5.75 ± 0.95 µg/mL at 5. The concentration of lactoferrin after tapping with 4 metal foils was 5.37 ± 0.68 µg/mL in the control, 5.37 ± 0.72 µg/mL immediately after tapping, 5.48 ± 0.77 µg/mL at 1 minute, 5.72 ± 0.77 µg/mL at 3, and 5.86 ± 0.75 µg/mL at 5. Although there was no significant difference between the lactoferrin concentration and the number of metal foils, the concentration had a tendency to rise after tapping with more than 3 foils and at 3 minutes.

Relation between occlusal contact area per point and SAA

Figures 15 and 16 show the correlation coefficient of the relation between SAA and occlusal contact. The coefficient was calculated to understand how occluding on the interference relates to increases in SAA. Figure 15 shows a scatter diagram of the correlation between the occlusal contact and increases in SAA. There was a positive correlation of the occlusal contact score of the examined tooth with the contact area and the contact area per point (area : $r = 0.53$, points : $r = 0.39$, points/area : $r = 0.31$). Figure 16 shows a scatter diagram for the correlation between the occlusal contact and the increase in the rate of SAA after tapping.

We found a positive correlation between the occlusal contact score of the examined tooth and the
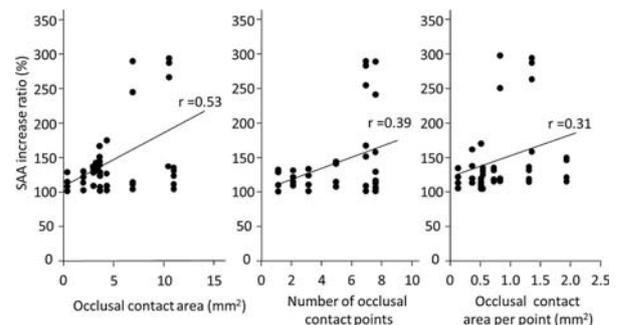
Fig. 15 Correlation between the occlusal contact of the examined tooth and the SAA increase rate after tapping.
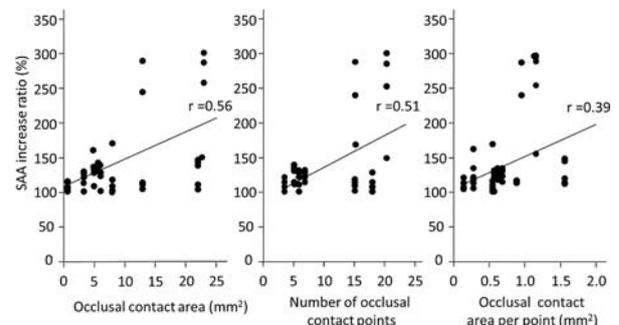

Fig. 16 Correlation of the occlusal contact of the examined tooth and adjacent teeth with the SAA increase rate after tapping.

adjacent teeth, the area, and the area per contact point. (area : $r = 0.56$, points : $r = 0.39$, points/area : $r = 0.39$). It was noteworthy that there was a moderate positive correlation between the occlusal contact area and the rate of increase in the SAA.

DISCUSSION

We examined the effect of occlusal inference on the constituents of saliva after tapping of the teeth. Saliva sampling has the advantage of being non-invasive, making multiple sampling easy, and being stress free. To rule out the confounding effects of additional stress induced by the SAA, SIgA and lactoferrin concentrations were assayed as indexes of the sympathetic nervous systems. We also compared the characteristics of these parameters. Unpleasant experiences, which may raise stress levels, are reflected by an increase in the concentration of the salivary stress markers, SAA, SIgA and lactoferrin. We created an experimental occlusal interference with metal foil of thicknesses between 12.7 μm and 50.8 μm, because it has been reported that if the amount of interference is less than 30 μm, it does not affect the periodontal tissue.[19]

Salivary α-amylase is produced by the salivary glands and has as the main function of the enzymatic digestion of carbohydrates.[20] These glands have a large number of noradrenalin-stimulated β-adrenergic receptors.[21,22] SAM activation during stress increases plasma noradrenalin, with consequent increases in plasma noradrenalin, resulting in an increase in the production and release of α-amylase.[23, 24]

SAA levels increased after tapping during normal occlusion in the control experiment. Marked increases in SAA occurred in response to experimental occlusal inference. The SAA level increases significantly with the thickness of the interference. SAA was increased with tapping compared to the control, and further increased as the number of metal foils increased. The response to the difference in thickness of the metal foil after tapping was marked, and significantly increased at a thickness of 12.7 μm. Since the discrimination threshold of the thickness by the periodontal ligament is 33 μm, this indicates that the difference in thickness can be measured at a level below this threshold value. Furthermore, the SAA level immediately decreased to the control level when the metal foil was removed for all thicknesses. Because the SAA level

indicates changes in real time, it is useful for evaluating the state of the occlusion.

Secretion of saliva is dependent upon stimulus from autonomic nerves that are the effector arms of reflexes activated predominantly by taste and periodontal baroreceptors.[25] We found in this study that SAA secretion of saliva responded sensitively to pressure stimulation in the periodontal membrane. There was a positive correlation between the occlusal contact relationship and the rate of increase in SAA. This suggests that the larger the occlusal contact area, the greater the rate of increase in SAA.

The major antibody in saliva is IgA, which is actively transported by parenchymal cells within the salivary glands. The autonomic nerves supplying the glands *in vivo* regulate the rate of IgA secretion in the saliva.[26] SIgA secretion by salivary glands is increased by stimuli from autonomic nerves. There have been some reports concerning whether psychological stress increases or decreases salivary SIgA concentration. Several studies have reported lower than normal concentration,[27, 28] while others have reported higher values.[29, 30] Our results did not agree. There was no significant difference in SIgA concentration between the controls and the subjects with experimental occlusal inferences. Neither of these experiments required a long period of time, but rather were achieved immediately after stimulation. SIgA secretion may require some time after stimulation to appear. In addition, SIgA is not secreted despite stimulation to sympathetic nerves.

Changes in the lactoferrin concentration level have a time lag response. Lactoferrin is one of the antimicrobial factors in saliva.[31] Previous studies have reported that salivary lactoferrin concentrations are modulated immediately after strenuous exercise.[16, 17] An increase in lactoferrin concentration was observed with 3 or more metal foils and after more than 3 minutes of tapping. Lactoferrin is a component of saliva and is suspected of being a defense factor against oral pathogens. Its level was only exceeded by salivary amylase in our study. Increases in both the SAA and lactoferrin levels were observed lagging in time behind increases in the

SAA levels. These results seem to indicate that the concentrations both of the SAA and lactoferrin level increases considerably in the salivary gland.

These results indicate that amylase and lactoferrin sensitively respond to mechanical stimulation of the periodontal membrane, even with limited stimulation. Both salivary amylase and lactoferrin are suitable stress markers for stimulation of the periodontal membrane. Lactoferrin may be an important stress marker for the oral physiological response. The SAA level was enhanced by prolonged occlusal interference. Recently, salivary issues have become important in psychoneuroendocrinological research. Brown suggested that changes in saliva parameters could be regarded as an "index of specific states of psychopathology."[32] Salivary components are considered meaningful physiological markers in psychoneuroendocrinological research.[7, 8, 33] Stress is a multifaceted phenomenon that requires a multidimensional measurement approach. As a consequence, research can benefit by taking into consideration a large number of psychological parameters. One parameter that has been suggested to reflect stress-related changes in the body is the SAA.[9, 34]

Our results suggest that analysis of salivary amylase activity and lactoferrin concentration may be valuable for evaluating the degree of stress experienced with occlusal inference. We observed increases in the salivary amylase and lactoferrin levels during the experiment. Our data in this study indicate that not only salivary amylase but also lactoferrin may be used as stress markers of orthodontic treatment. Commonly used methods of mechanical stimulation invoke local autonomic reflex activity, shifting the balance from submandibular secretion (modest salivary amylase and lactoferrin levels) to salivary secretion independent of central sympathetic adrenomedullary regulation. We believe that both salivary amylase and lactoferrin are biomarkers for malocclusion. However, it is extremely difficult to obtain a value from stimulated salivary amylase and lactoferrin that can confidently be attributed to the central effects on local sympathetic adrenomedullary activity.

In this study, we examined a method for objectively measuring normal occlusion. Our data suggest that it is possible to grasp the sense of pressure in the periodontal ligament very accurately. The dry clinical chemistry analyzer (Saliva Amylase Monitor ; Nipro) can measure salivary amylase activity non-invasively in only 60 seconds, with minimum stress on the subject. Measuring the occlusal contact using the BiteEye has high reproducibility and reliability. We determined both the occlusal contact area and number of occlusal contact points using this device, as well as the occlusal contact area per point on the experimental teeth and both adjacent teeth. We found that it is possible to objectively evaluate the state of occlusion by measuring SAA.

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