

Title	Influence of chewing and clenching on salivary cortisol levels as an indicator of stress
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Abstract

Purpose The purpose of this study was to investigate the effects of chewing and experimental clenching on stress condition by measuring the salivary cortisol levels, as an indicator of stress condition.

Materials and Methods Seventeen normal dentulous subjects were given arithmetic exercises to be performed within 20 minutes, in order to elicit stress. For the first experiment (chewing), subjects were asked to chew a paraffin wax while reading some printed materials (books, magazines, etc) in silence for 10 minutes. The same procedure without the wax chewing was carried out for control purposes. In the second experiment (light clenching), 5 seconds of experimental light clenching followed by 5 seconds of resting were performed and repeated in this order for 3 minutes. The control data was obtained by measuring resting without experimental clenching performing. Saliva specimens were collected before and immediately after experimental stress loading and after each procedure for 1 minute to measure cortisol levels.

Results In the chewing experiment, the salivary cortisol levels were significantly reduced in the chewing condition in comparison to the control ($P < 0.05$). Relaxation of stress occurred during chewing for 10 minutes after stress loading. In the clenching experiment, the salivary cortisol levels also showed a significant reduction in the clenching condition in comparison to the control ($P < 0.05$). Once again, stress relaxation was observed to occur during the intermittent experimental clenching for 3 minutes after stress loading.

Conclusions Based on these results we suggest that chewing and experimental clenching can be useful to achieve stress relaxation.

Introduction

In general, bruxism has been considered as one of the physical responses to emotional stress,¹⁻³ though this relation has been the target for controversial discussions and it still remains unclear. Rao and Glaros⁴ concluded that frustration and anxiety make the masseter muscles tense and suggested that there is a relationship between diurnal clenching and such muscular tension. Moreover, Yemm⁵ reported that the activity of the masseter muscle increased due to experimental stress loading in humans. Butler and Stallard⁶ observed that patients under stress condition have more frequent and long-lasting teeth contact than patients without stress. A similar phenomenon has been recognized in rats, where the stress-loaded rats demonstrated bruxism-like activity of the masseter muscle.⁷ In contrast to this other study had reported that there is not a significant relation between the frequency of bruxism and daily stress.⁸

In order to elucidate the possible relation between stress and chewing, Morita⁹ reported that chewing gummi candies and chewing gum result in relaxation of mental stress in humans as assessed by changes in adrenaline, noradrenalin and adrenocorticotrophic hormone in plasma, serum cortisol levels, electrodermal activity, facial skin temperature, blood pressure, pulse rate, SpO₂, and electrocardiogram. Also, Ohtsuka et al.¹⁰ reported an increase in the frequency of alpha waves after gum chewing. Although there have been several reports

describing the possible association between the stomatognathic system and stress, it is still not well understood.

Until now, several methods to objectively measure the stress condition have been introduced, including different types of scales, questionnaire, electroencephalogram and biochemical assessment of blood and urine specimens.

As it is known, in human beings in response to a stress situation, the hypothalamus-pituitary-adrenal (HPA) axis is activated and consequently there is an increase in the cortisol secretion ¹¹. Based on this fact, several studies have used the changes in the salivary cortisol levels as indicator of stress, ¹²⁻¹⁴ and it has been reported that these levels increase under acute stress, for example from performing arithmetic calculations in a noisy environment,¹⁵ from taking examination ,¹⁶ and watching suspense films.¹⁷

The purpose of the present study was to clarify the effects of chewing and experimental clenching on stress relaxation by measuring the salivary cortisol levels in whole saliva, which can be collected easily and noninvasively.

Materials and Methods

Subjects

The subjects consisted of 17 normal dentulous males (mean age, 26 ± 2 years) without subjective or objective abnormalities in the stomatognathic system. They were healthy and none of them had previous history of medical disease. All subjects were fully informed about the experimental procedures and they gave informed consent to participate in the study.

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 the circadian rhythm.¹¹ To avoid the effect of food intake on salivary secretion, subjects were asked to refrain from taking caffeine and alcohol the day before and on the day of the experiments. Also, eating, drinking, and 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 movements such as stretching throughout the experiments.

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

Chewing experiment (Fig.1)

First, subjects were asked to rest in the shield experimental room for 30 minutes and then the first saliva specimens (referred as R in Fig. 1) were collected. Subsequently, as stress loading, subjects were given a series of arithmetic calculations and were instructed to work out them within 20 minutes and immediately afterwards, the second saliva specimens (referred as S in Fig.1) were collected.

Then, subjects were asked to chew a paraffin wax (for clinical examination) while reading in silence their favorite book or any other printed material for 10 minutes. Once finished this period the third saliva specimens (referred as Ch1 in Fig. 1) were collected. Same procedure was repeated and the fourth saliva specimens (referred as Ch2 in Fig. 1) were collected. Within the context of this study, these were referred as chewing condition. The second part of this experiment was carried out under the same conditions but the third and fourth saliva specimens (referred as r1 and r2 in Fig. 1) were collected after each sessions of silence reading for 10 minutes without chewing. The data obtained from this experiment served as control.

As previously mentioned, paraffin wax was used to perform the chewing condition. The amount used was 1.0 g and it was softened to the appropriate hardness prior to the experiment. Each subject underwent both reading with chewing (chewing condition), and reading without chewing (control) on different days.

Clenching experiment (Fig.2)

As for the chewing experiment, subjects were asked to rest in the shield room for 30 minutes and then to perform some arithmetic calculation within 20 minutes. Saliva specimens were collected immediately after each session (referred as R and S in Fig. 2). Following subjects were instructed to perform intermittent experimental clenching for 3 minutes and repeat this session three times. This intermittent experimental clenching was defined as 5 seconds of clenching followed by 5 seconds of rest repeated for 3 minutes. For the strength of clenching, subjects were instructed to clench with “light strength” while the EMG activity from the center area of the masseter muscle from both sides was monitored. After each clenching session saliva specimens (referred as C11, C12 and C13 in Fig. 2) were collected. A total of five saliva specimens were obtained. Within the context of this study, these

measurements were referred as clenching condition. The second part of this experiment was carried out under the same conditions but after stress loading, subjects were asked to stay rest for 3 minutes and repeated three times. After each 3 minutes session saliva specimens were collected. The data obtained from this experiment served as control. The saliva specimens collected after each rest session are referred as R1, R2 and R3 in Fig. 2.

Each subject underwent both experimental intermittent clenching (clenching condition), and rest (control) on different days.

Measurements and recording equipments.

Salivary cortisol levels

In order to assess the changes occurred in the cortisol level during different conditions, the whole saliva was collected by keeping a cotton roll intraorally for 1 min, and the saliva collection equipment Salivette (SARSTED Inc., Rommelsdorf, FRG) was used. The saliva specimen obtained was centrifuged at 3000 rpm for 15 min and the supernatant was frozen to -20°C for preservation.

Subsequently, salivary cortisol levels were analyzed with GammaCoatTMCortisol (DiaSorin Inc, Oklahoma, USA), according to the radioimmunoassay.

Electromyogram (EMG)

Muscle Tester ME3000p (Mega Electronics Ltd., Kuopio, Finland) was used to measure the 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 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. The skin impedance between the electrodes was lower than 8k Ω .

First of all, subjects were instructed to perform clenching with a light strength. Since the clenching strength is an individual level it was considered necessary to monitor the clenching strength by means of a visual biofeedback during the experimental clenching. However, it could make feel subjects uneasy and stressed. Then, in order to avoid this situation subjects were instructed to perform light strength clenching and were asked to practice it many times prior to the experiment while the muscle activity was monitored. Then, the maximal voluntary clenching (MVC) of each subject was recorded at the end of each clenching experiment. The mean muscle activity during light clenching was calculated by

averaging the data obtained from the 3 sets of experimental intermittent clenching. The EMG results were expressed as a percentage of MVC.

Statistical analysis

Changes in salivary cortisol levels immediately after experimental stress loading and the time of each saliva collection were calculated, a paired t-test was used. Statistical significance was defined as P-value of $<0 \cdot 05$ by using statistical analysis software SPSS 11.0J for Windows (SPSS, Illinois, USA).

Experimental ethics

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

Results

Chewing experiment

Five out of 17 subjects did not show any increase in their salivary cortisol level after the experimental stress loading (arithmetic calculations within 20 min) and they were excluded from the statistical analysis. Then, statistical analysis were performed on 12 subjects. The mean and standard deviation of the salivary cortisol levels in subjects in the chewing experiment are shown in Figs 3 and 4.

When the changes in salivary cortisol levels between the second (S) and third saliva collection (Ch1 and r1), in both chewing condition and control were recognized a reduction of 15.4% in the chewing condition in contrast to -3.0% in control. Moreover, between the second (S) and fourth saliva collection (Ch2 and r2) a reduction of 24.6% and 7.1% in the chewing condition and control respectively was observed. Significant differences in the salivary cortisol levels were recognized in the chewing condition in comparison to the control (Fig. 5).

Clenching experiment

Four out of 17 subjects did not show any increase in their salivary cortisol levels after experimental stress loading. For this reason, as for the chewing experiment 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 Figs. 6 and 7. In regard to the salivary cortisol levels, changes between the second (S) and third saliva collection (C11 and R1) in both clenching condition and control were compared and recognized a reduction of 11.2% in the clenching condition in contrast to -2.9% in control. Same tendency was observed when salivary cortisol levels between the second (S) and fifth saliva collection (C13 and R3) were compared. A reduction of 23.4% and -2.7% in the cortisol level was recognized in the clenching condition and control respectively. These results showed significant difference in the salivary cortisol levels in the clenching condition in comparison to control (Fig. 8). However, when the changes in salivary cortisol levels between the second (S) and fourth saliva collection (C12 or R2) were compared, there were not significant differences between the clenching condition and control (Fig. 8).

Discussion

In regard to the assessment of stress conditions, some of methods have been introduced in previous studies include invasive procedures (sample collection of blood, etc) which not only induce stress in subjects but also are difficult to perform. Moreover, such as urinalysis are limited in time and not always reflect the changes in cortisol level. In contrast to this, saliva collection was considered to be an easy, non-invasive and effective method. Salivary cortisol levels highly correlate with serum cortisol levels.¹⁸⁻²⁰ Moreover, cortisol in serum is known to be rapidly transferred to saliva within 5 minutes , without been affected by the salivary flow rate.^{19,20} Therefore, for the purpose of this study measurement of salivary cortisol level was selected as the method to properly assess the stress condition in subjects.

In this study arithmetic calculations were used as experimental stress loading and were given to subjects to be done within a limited time (20 minutes) and with body movement and stretching restriction. When the salivary cortisol levels were measured immediately after the experimental stress loading, significant difference of these levels were recognized in almost all the subjects regardless their arithmetic skills. However, 5 and 4 out of 17 subjects during the chewing experiment and the clenching experiment respectively did not show any increase on the salivary cortisol level after 20 minutes of stress loading. The reasons why

these subjects were not affected by the stress loading are not well understood. However, we presume that they probably did not fully understand the instruction given how to perform the arithmetic calculations and consequently they tried to solve them at their own pace. These subjects were excluded from further statistical analysis.

As previously mentioned in both chewing and clenching experiments the salivary cortisol levels were significantly reduced in the chewing condition and the clenching condition in comparison to control. With regard to the chewing experiments, our results concur to those reported by Morita⁹ and Ohtsuka.¹⁰ The changes of salivary cortisol levels between the second and third saliva collection were significant difference between the chewing condition and the control ($P < 0.05$). Meanwhile these changes between the third and fourth saliva collection were not significant difference between the chewing condition and control ($P > 0.05$). From these results we presume that stress relaxation occurred predominantly during the first 10 minutes of experimental chewing. Taking this into consideration, for the clenching experiment the salivary cortisol levels were decided to be measured within the first 10 minutes after stress loading. Moreover, in the clenching experiment the same tendency was observed and changes on salivary cortisol levels were

significant differences during the first set of 3 minutes of intermittent clenching.

With regard to the clenching strength, the “light strength” of experimental clenching ranged from 11.3% MVC to 45.5% MVC among the subjects. Piquero and Sakurai²¹ reported clenching event defined as the more than 10% MVC of at least 3 seconds duration during silent reading for 10 minutes. In the clenching experiment of present study, the muscle activity of more than 10% MVC observed. Therefore, experimental clenching in present study was considered appropriate clenching.

Bruxism has been defined as an oral parafunction which has harmful effects on the stomatognathic system, such as facial pain, abnormal tooth wear, periodontal pain, increased tooth mobility, muscle tenderness to palpation, increased muscle tonus and hypertrophy and TMJ discomfort, among others. These effects are related to the intensity, frequency and duration of the parafunction activity. In the present study, however, was observed that light intermittent clenching (one type of bruxism) effectively helps in the reduction of stress, which was observed as a reduction in the salivary cortisol level. Based on these results we suggest that within bruxism, the intermittent light clenching might be an ortho-function directly related to stress, which has the functional objective to elicit stress relaxation.

Conclusions

The results obtained in this study showed that chewing and the intermittent light clenching have a direct effect on the reduction of stress condition. However, for future investigation, it would be necessary to assess how the intensity, duration and frequency of clenching could have influence on the relaxation of daily stress.

Acknowledgment

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Figure Legends

Fig.1 Chewing experimental schedule

R: First saliva collection (Before experimental stress loading)

S: Second saliva collection (Immediately after experimental stress loading)

Ch1: Third saliva collection (After first wax chewing with silently reading for 10 minutes)

Ch2: Fourth saliva collection (After second wax chewing with silently reading for 10 minutes)

r1: Third saliva collection (After first silently reading for 10 minutes)

r2: Fourth saliva collection (After second silently reading for 10 minutes)

Fig.2 Clenching experimental schedule

R: First saliva collection (Before experimental stress loading)

S: Second saliva collection (Immediately after experimental stress loading)

Cl1: Third saliva collection (After first intermittent experimental clenching for 3 minutes)

Cl2: Fourth saliva collection (After second intermittent experimental clenching for 3 minutes)

Cl3: Fifth saliva collection (After third intermittent experimental 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)

Fig.3 Changes of salivary cortisol levels in chewing condition

Fig.4 Changes of salivary cortisol levels in control

Fig.5 Comparison between the chewing condition and the control with respect to the changes of salivary cortisol levels

Fig.6 Changes of salivary cortisol levels in clenching condition

Fig.7 Changes of salivary cortisol levels in control

Fig.8 Comparison between the clenching condition and the control with respect to the changes of salivary cortisol levels

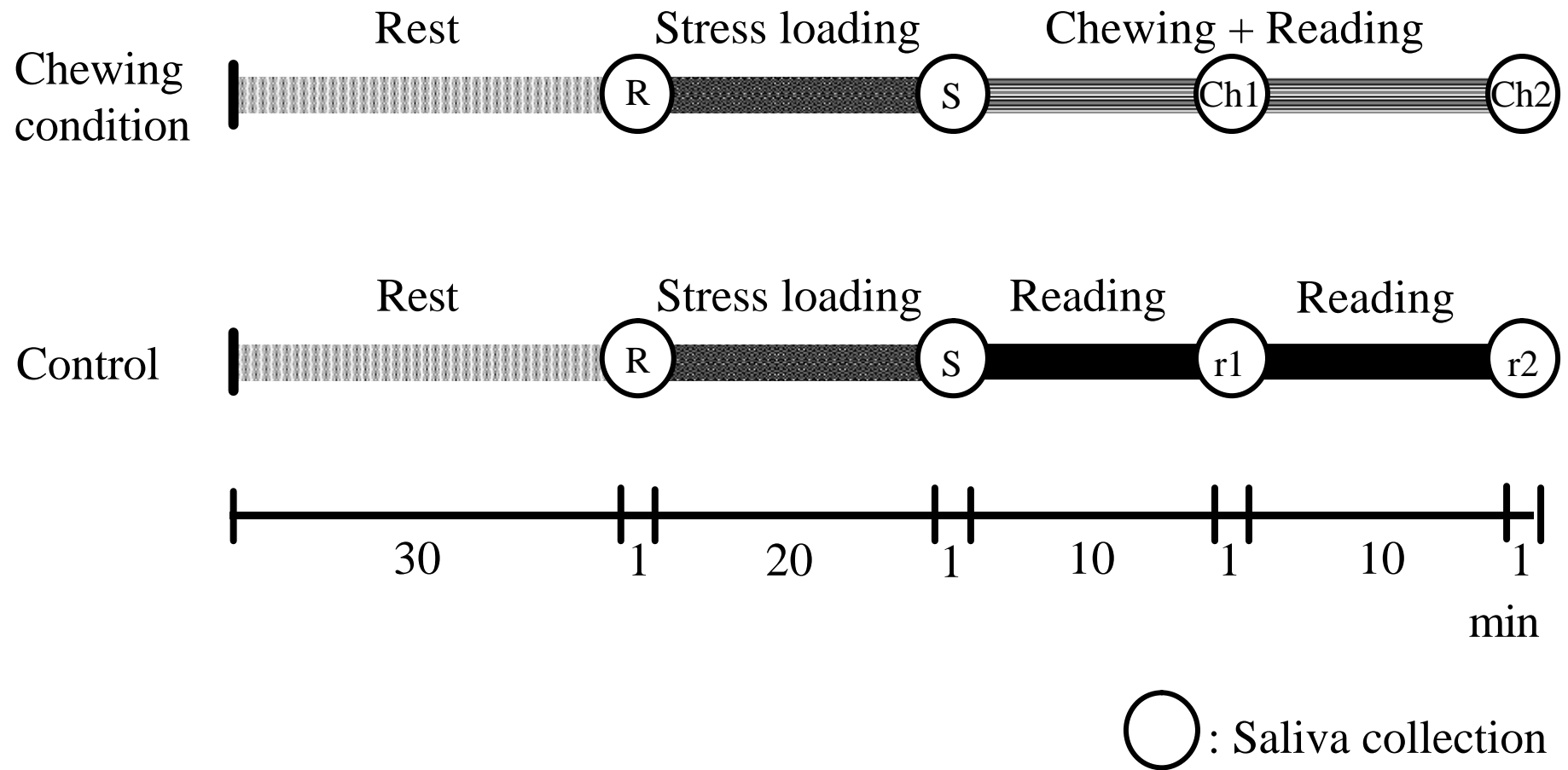


Fig.1 Chewing experimental schedule

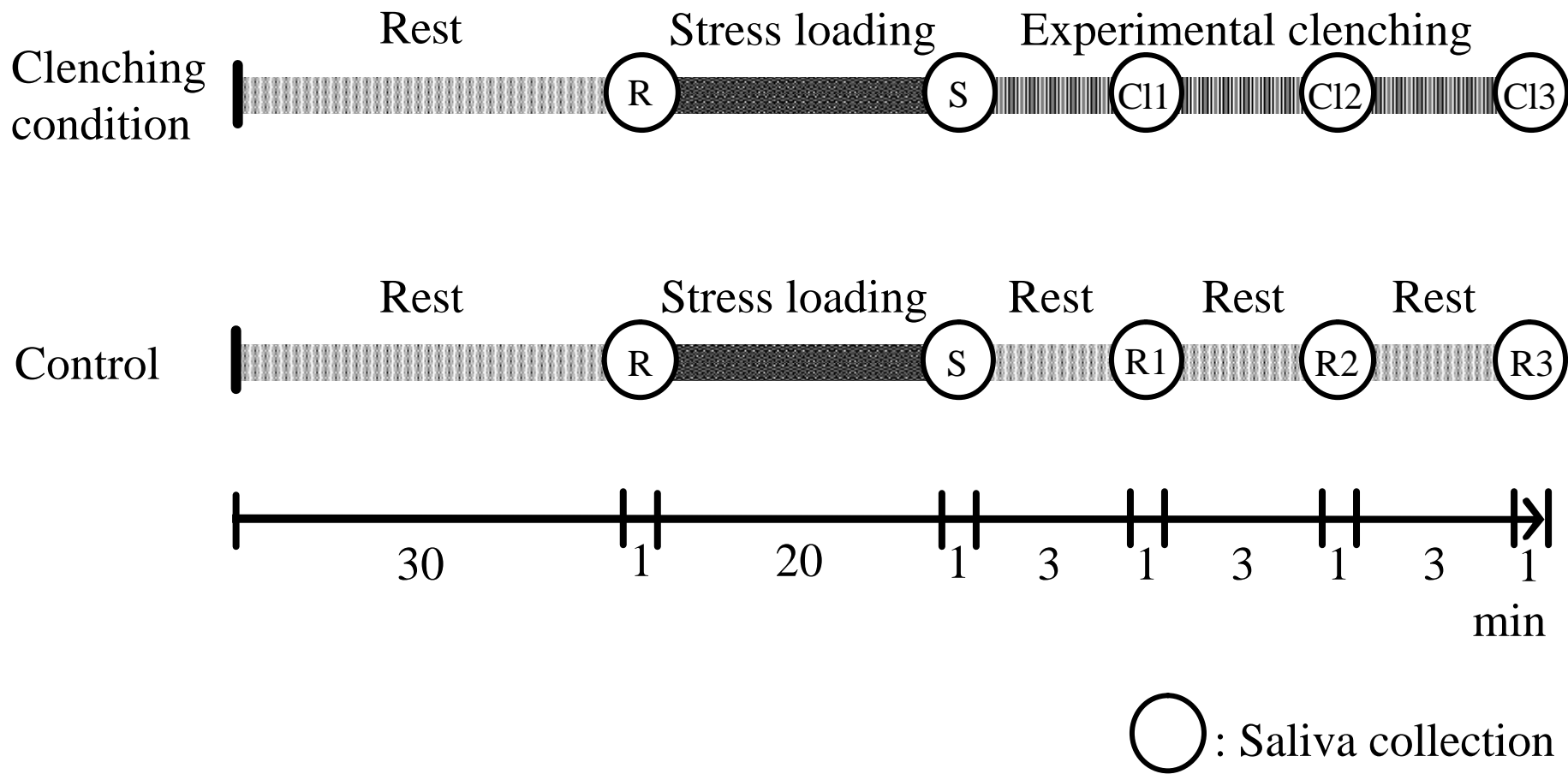


Fig.2 Clenching experimental schedule

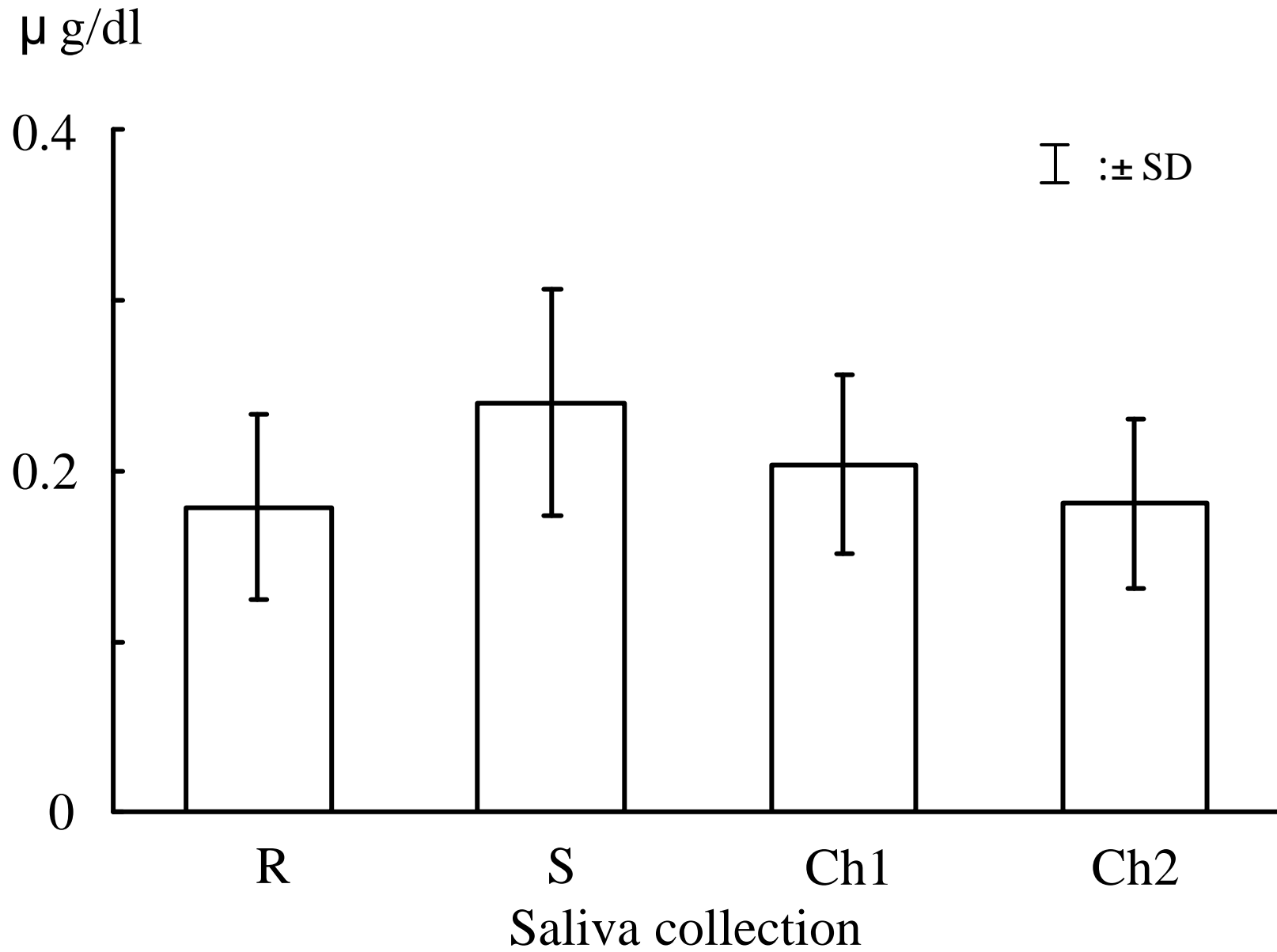


Fig.3 Changes of salivary cortisol levels in chewing condition

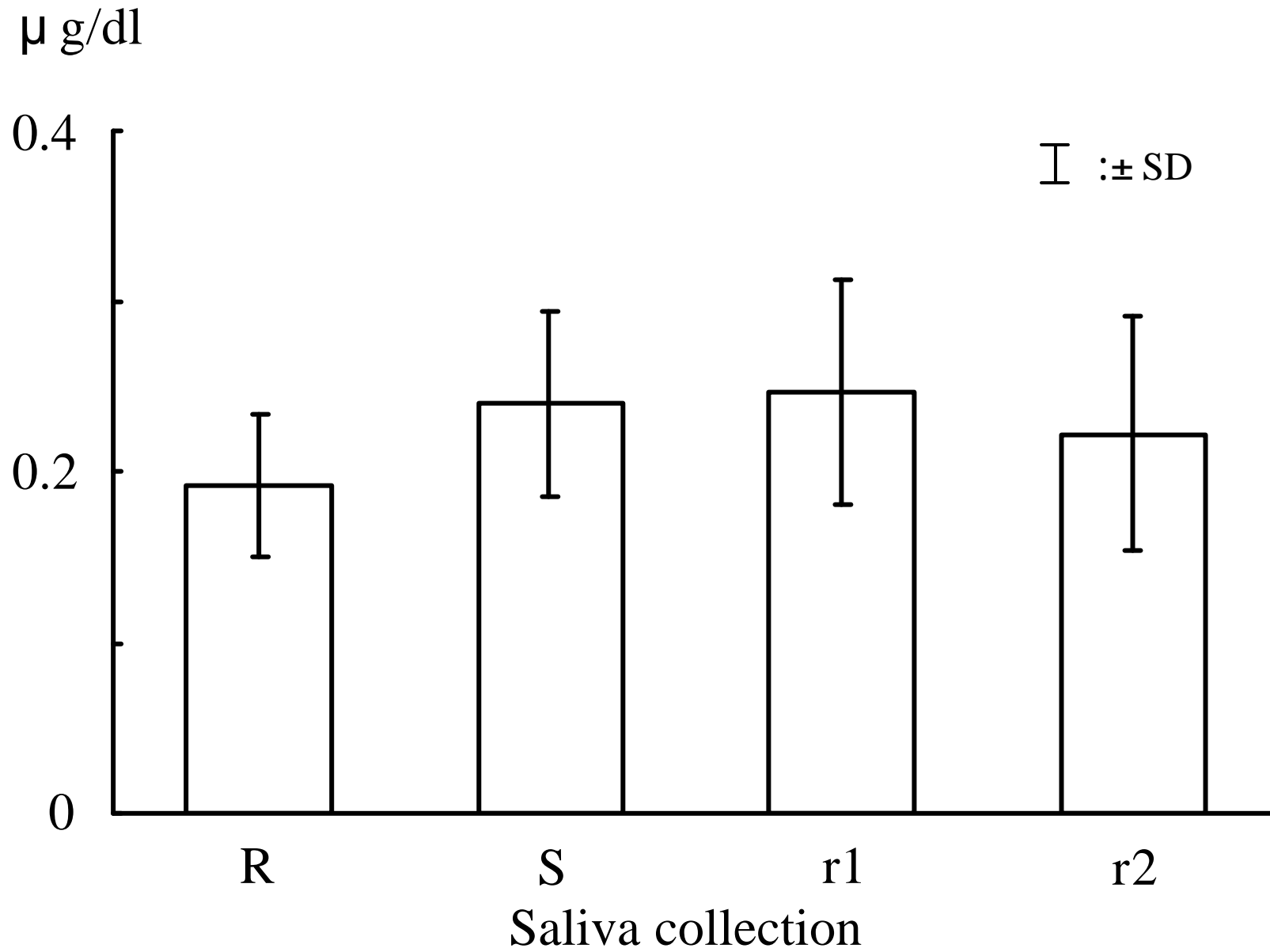
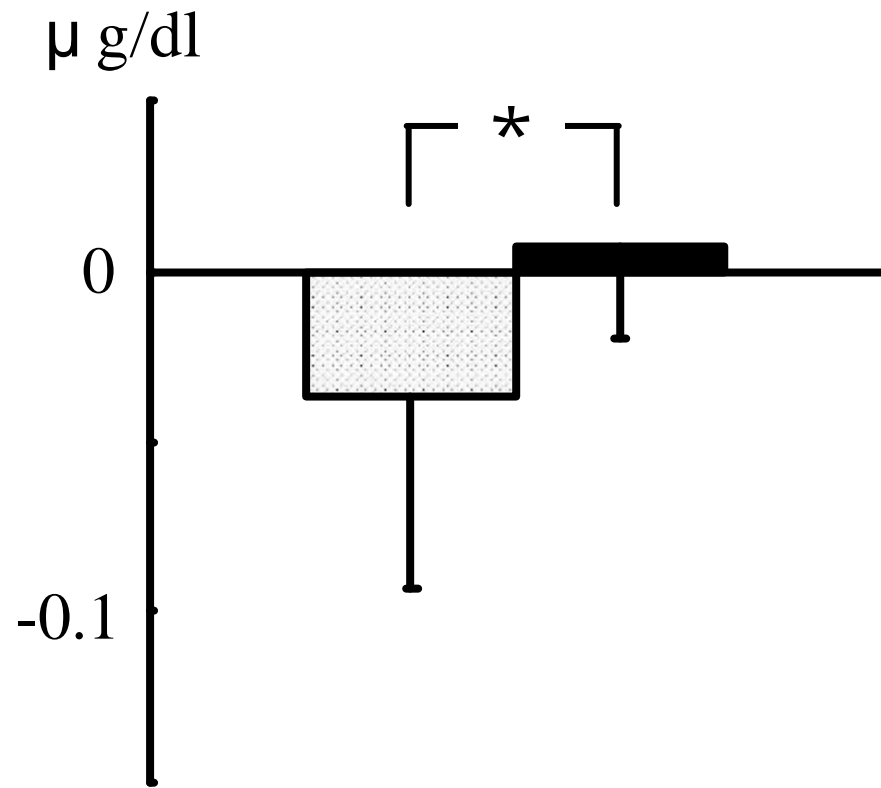
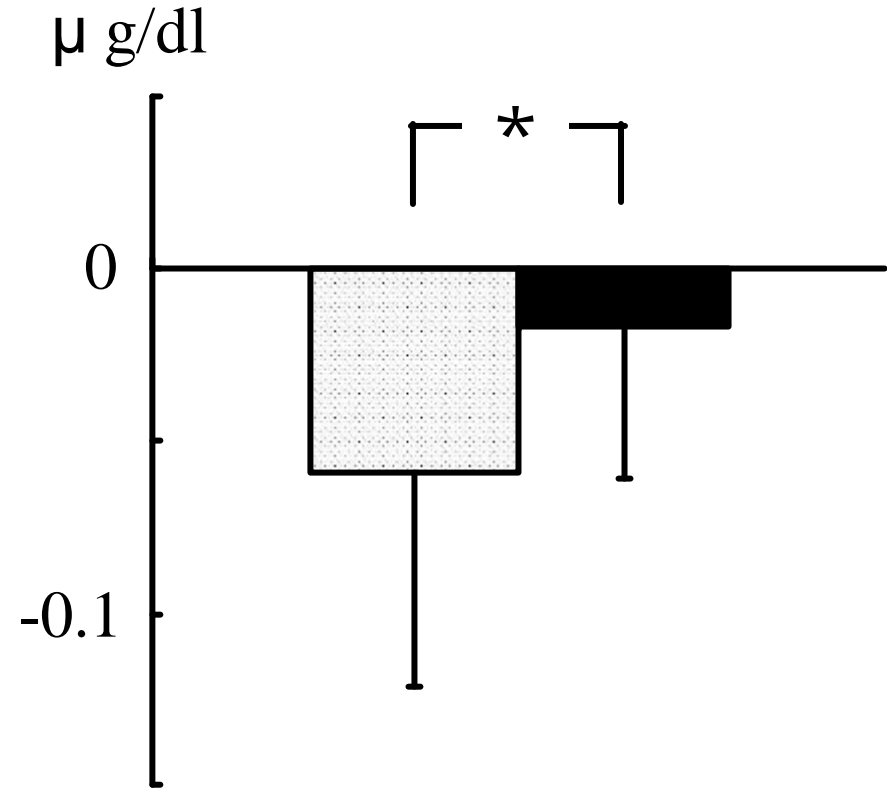


Fig.4 Changes of salivary cortisol levels in control



The changes between
S and Ch1 or r1
salivary cortisol levels



The changes between
S and Ch2 or r2
salivary cortisol levels



:Chewing condition



:Control

* P < 0.05

⊥ :SD

Fig.5 Comparison between the chewing condition and the control with respect to the changes of salivary cortisol levels

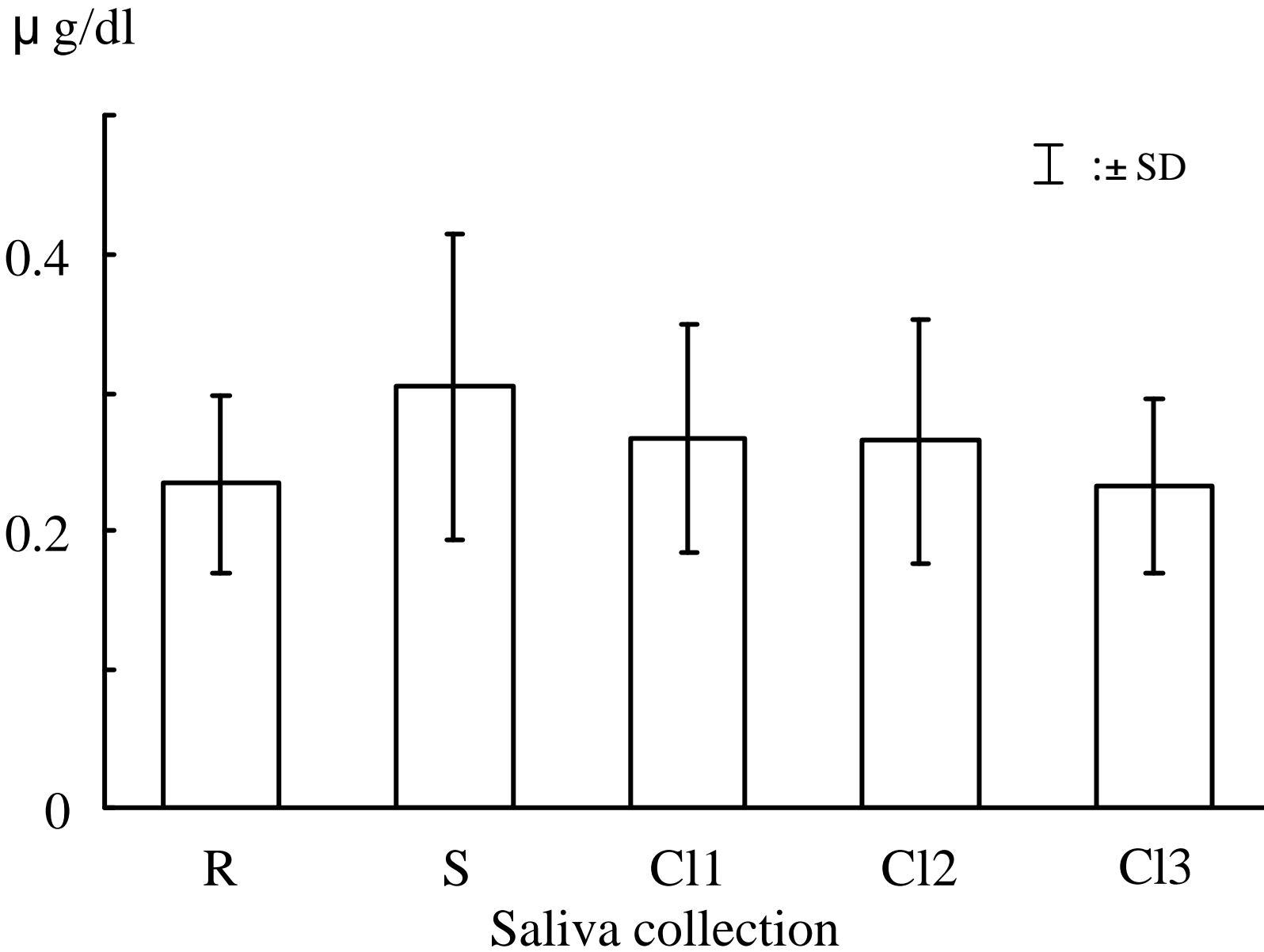


Fig.6 Changes of salivary cortisol levels in clenching condition

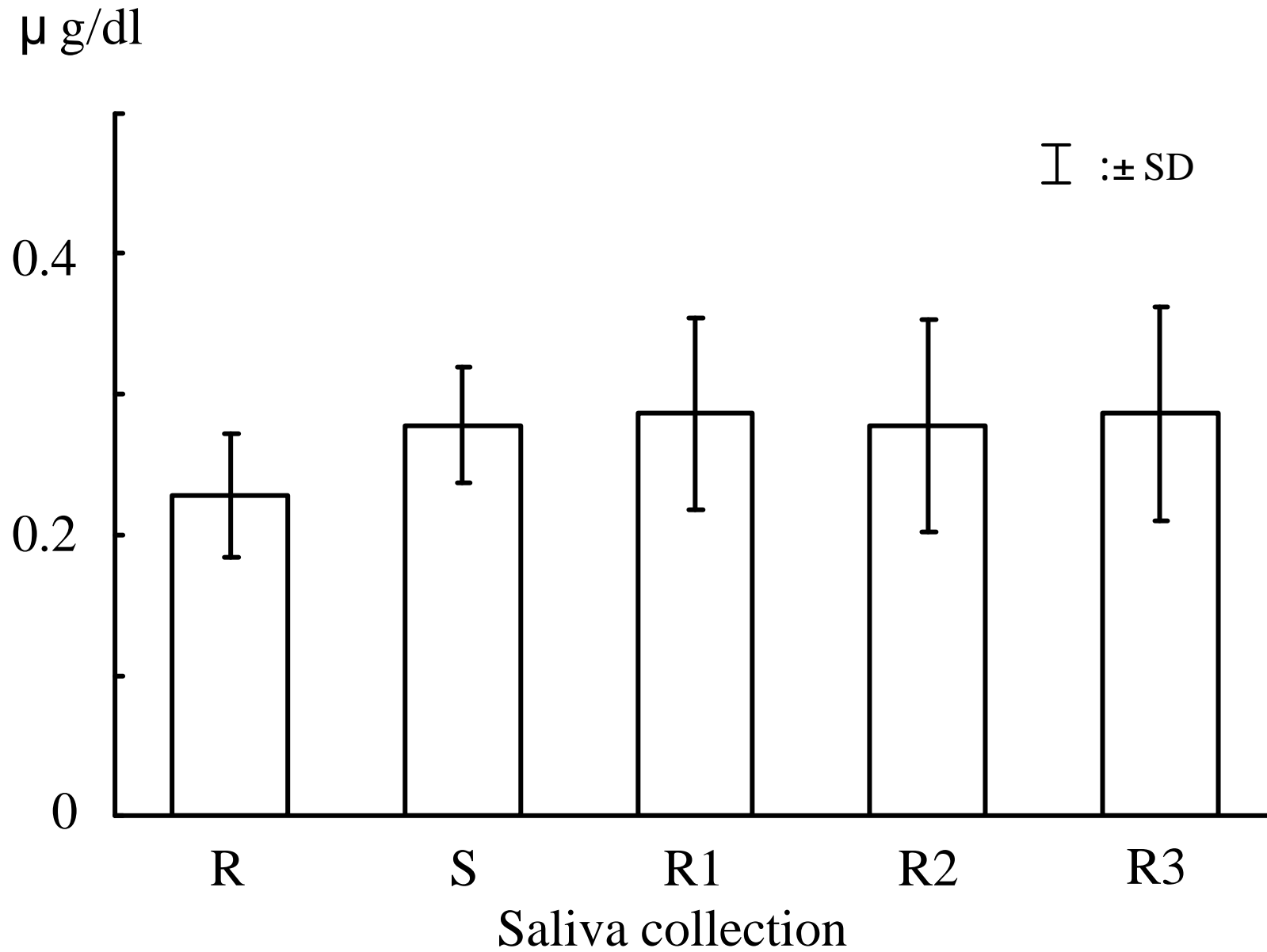


Fig.7 Changes of salivary cortisol levels in control

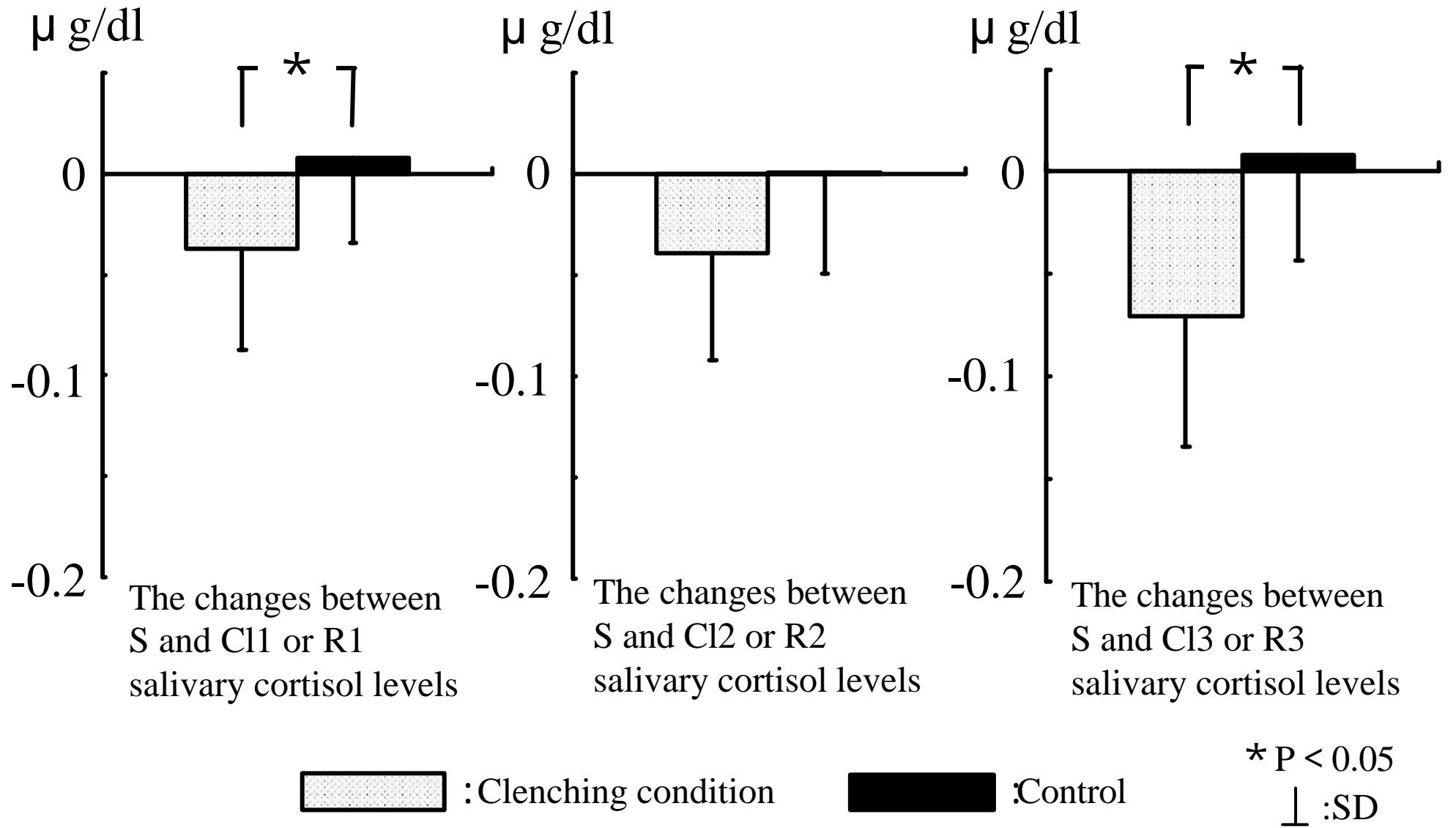


Fig.8 Comparison between the clenching condition and the control with respect to the changes of salivary cortisol levels