

Masticatory performance alters stress relief effect of gum chewing

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5 **Abstract**

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7 Purpose: We evaluated the effects of gum chewing on the response to **psychological**
8 stress induced by a calculation task and investigated the relationship between this
9 response and masticatory performance.
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14 Methods: Nineteen healthy adult volunteers without dental problems undertook the
15 Uchida–Kraepelin (UK) test (30 min of reiterating additions of one-digit numbers).
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19 Before and immediately after the test, saliva samples were collected from the sublingual
20 area of the participants. Three min after the UK test, the participants were made to chew
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22 flavorless gum for 3 min, and the final saliva samples were collected 10 min after the UK
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27 test. The experiment was performed without gum chewing on a different day.
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30 Masticatory performance was evaluated using color-changing chewing gum.
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32 Results: Salivary CgA levels at immediately and 10 min after the UK test were
33 compared with and without gum chewing condition. Two-way repeated measures
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35 analysis of variance revealed significant interaction between gum chewing condition
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37 and changes in CgA levels during post 10 min UK test period. A significant correlation
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39 was found between changes in CgA levels and masticatory performance in all
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44 participants.
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47 Conclusion: Our results indicate that gum chewing may relieve stress responses;
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49 however, high masticatory performance is required to achieve this effect.
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54 **Keywords**

55 Stress, Saliva, Gum chewing, Masticatory performance, Chromogranin-A
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1. Introduction

Mastication is one of the most important oral functions. Recently, however, several studies have revealed a significant role of mastication in maintaining mental health. Reportedly [1-5], habitual gum chewing relieves anxiety and mental stress. Several studies [6-13] evaluating salivary markers of stress showed that gum chewing decreases the level of salivary cortisol after experimental stress loading. Cognitive function, memory, and attention may also be improved by gum chewing [14-16].

When humans experience stress, the hypothalamic–pituitary–adrenal axis (HPA) and sympathoadrenal system (SAS) are activated, inducing a stress response. The HPA facilitates the release of cortisol, whereas the SAS induces the secretion of catecholamine, both of which enhance the human body’s ability to deal with stress. Because SAS activation precedes HPA activation, catecholamine responds more quickly to stress compared with cortisol, and its measurement is therefore suitable for rapid detection of low stress. Conversely, cortisol can be detected in saliva, blood, and urine. It can be sampled easily and is frequently adopted as the standard index for evaluating stress levels. Although catecholamine exhibits a better response to stress compared with cortisol, it is difficult to detect this stress hormone in saliva samples.

Chromogranin A (CgA) is an acidic glycoprotein released with catecholamine by the adrenal medulla and sympathetic nerve endings. Because CgA can be detected in saliva samples, it represents a suitable stress index substitute for catecholamine [17-19]. The validity of salivary CgA levels as an indicator of stress has been confirmed by experimental stress tests, including cognitive tests, noise exposure, and venipuncture [20-22].

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4 Many previous studies [6-13] on the stress-relieving effects of gum chewing measured
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7 salivary cortisol as the stress index. Because the response to stress mediated by the HPA
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10 is affected by the menstrual cycle [23], some of these reports included only male
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12 participants [7-9,12]. However, CgA is an SAS index that can be expected to respond
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14 quickly to psychological stress in both male and female participants.
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17 Soeda *et al.* [12] evaluated the effects of gum chewing on experimental stress loading by
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19 recording surface electromyographic (EMG) activity of the masseter during gum
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21 chewing and concluded that forceful chewing relieves stress more effectively compared
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23 with weak chewing. The detailed mechanism underlying the stress-relieving effects of
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25 gum chewing remains to be identified. However, this report showed that, qualitatively,
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27 gum chewing produces a stress-relieving effect.
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32 Several approaches have been utilized to evaluate chewing quality. Objective methods
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34 such as measurement of maximum occlusal force and/or occlusal contact at the
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36 maximum intercuspal position have been used to evaluate chewing function. These
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38 parameters are known to contribute to **masticatory performance**, although they may not
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40 completely reflect chewing function [24-26]. Direct analysis of chewed food samples is
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42 effective for investigating chewing function. Recently, various materials such as gummy
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44 jelly, wax cubes, and gum have been used to assess chewing quality [27-30]. Numerical
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46 analysis of experiments using these materials indicates masticatory performance [24]. In
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48 this study, we verified the ability of gum chewing to relieve acute experimental stress by
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50 evaluating salivary CgA levels in response to the Uchida–Kraepelin (UK) test with and
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52 without gum chewing and investigated the relationship of masticatory performance and
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54 masticatory muscle activity to the stress-relieving effects of gum chewing.
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2. Materials and Methods

2.1. Participants

Nineteen adult volunteers (nine males, 10 females; mean age, 25.9 years) participated in this study. All participants were healthy; none had any dental problems or were taking any medication. Participants with missing teeth (except for the third molar), pathological malocclusion, full-veneer restoration of molars, or a smoking habit were excluded. Before they provided consent to participate, the participants were informed about the procedures and experimental stress test. This research was approved by the Research Ethics Committee of Tokushima University Hospital, Tokushima, Japan (No. 1424).

2.2. Measurements

The salivary stress marker CgA was measured to evaluate acute physiologic responses to experimental stress. Resting saliva from the sublingual area was obtained with an oral swab and cryopreserved. Saliva samples were analyzed by enzyme-linked immunosorbent assay for the quantitative measurement of CgA levels.

Surface EMG activity of the masseter muscle during gum chewing was recorded to evaluate the magnitude of chewing force. Miniature biomedical waveform recorders (Actiwave®; CamNtech Ltd., Cambridge, UK) were used to record the EMG activity of the masticatory muscles.

Masticatory performance was assessed using color-changing chewing gum (Masticatory Performance Evaluating Gum XYLITOL®; Lotte Co., Ltd., Saitama, Japan), which changes color with chewing. Color change was measured using a colorimeter (CR-13;

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4 Konica Minolta, Inc., Tokyo, Japan) after 80 chewing cycles.
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10 *2.3. Procedure*

11 All participants undertook the Uchida–Kraepelin (UK) test [31-41], which is a
12 psychodiagnostic examination involving reiterative additions of one-digit numbers for
13 30 min after speech guidance. Experiments were initiated between 13:00 and 14:00 h.
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15 From the night before the experiment, participants were asked to refrain from
16 consuming alcohol, caffeinated drinks, and spicy foods. Experiments were performed in
17 a quiet laboratory isolated from the external environment.
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20 A disposable electrode was attached to the skin over the masseter muscle on the habitual
21 masticatory side and connected to the EMG lead. Participants were then instructed to sit
22 on a chair and try to relax for 30 min. After this relaxation period, initial (pre-UK) saliva
23 samples were collected; subsequently, participants undertook the UK test. Immediately
24 after the UK test, further (post-UK) saliva samples were collected. Three minutes after
25 the UK test, the participants were instructed to chew flavorless gum (Check Buff
26 Salivary Gum; HORIBA, Ltd., Kyoto, Japan) for 3 min using regular chewing force.
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29 Rhythmic audio signals were used to regulate the **chewing rate** at 1.5 Hz. After gum
30 chewing, participants were asked to relax for 4 min, following which the final saliva
31 samples were collected (10 min after the UK test). At the end of the experiment, the
32 participants were asked to perform maximum voluntary clenching for 3 s three times at
33 1-min intervals to obtain a calibration signal for EMG analysis. To ensure the exact
34 timing of each experimental step, all procedures were performed according to
35 prerecorded audio guidance (Figure 1).
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4 Taking over a month interval precede or follow the experiment, the same procedures
5 were performed without gum chewing. This time, after collecting the post-UK saliva
6 sample, the participants were asked to relax until the final saliva sample was collected 10
7 min later. Each participant performed both experiments. The order of these two
8 experiments was randomly assigned and counterbalanced for all participants.
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17 Masticatory performance was evaluated using color-changing gum on a different day.
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22 *2.4. Data analysis*

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24 *Chromogranin A:* Defrosted saliva samples were extracted using a refrigerated
25 centrifuge, and salivary CgA levels were quantified using a Human Chromogranin A
26 EIA Kit (YK070; Yanaihara Institute Inc., Shizuoka, Japan) according to the
27 manufacturer's instructions.
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34 *Masticatory muscle activity:* Root mean square conversion of EMG signals was
35 performed with a 60-ms time constant. The rectified signal was standardized by dividing
36 it by the signal amplitude at maximum voluntary contraction. Subsequently, the average
37 magnitude of EMG signals during the 3-min gum-chewing period was calculated to
38 yield the average EMG activity for each participant.
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47 *Masticatory performance:* Immediately after 80 chewing strokes at the rate of 1.5 Hz,
48 the color-changing chewing gum was extracted and flattened between glass plates in a
49 polyethylene film. Then, the change in color of the chewed gum was measured
50 according to the CIE-L*a*b* color system. The following equation was used to
51 determine the degree of color change [30]:
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$$\Delta E = \sqrt{(L^* - 72.3)^2 + (a^* + 14.9)^2 + (b^* - 33.0)^2}$$

10 This procedure was repeated five times, and the average ΔE value was adopted as the
11 masticatory performance of the participant.
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14 15 16 17 *2.5. Statistical analysis*

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19 This was a cross-over study with one intervention factor (gum chewing). On each
20 experimental day, saliva samples were collected before, immediately after, and 10 min
21 after the UK test from each participant. To avoid the effect of the inter individual
22 difference on CgA levels, CgA level change that was standardized with the levels after
23 the resting period was analyzed (subtraction of resting period CgA levels from the levels
24 after the UK test). To assess the stress-relieving effects of gum chewing, two-way
25 ANOVA with repeated measures for one factor—with or without gum chewing—was
26 performed. To investigate our hypothesis that masticatory performance contributes to
27 the stress-relieving effects of gum chewing, Spearman's rank correlation coefficients
28 between changes in CgA levels following gum chewing and average EMG activity
29 and/or masticatory performance were obtained.
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47 A 5% significance level was adopted, and all analyses were undertaken using JMP
48 statistical software (SPSS-15.0J for Windows; SPSS Japan, Inc., Tokyo, Japan).
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55 **3. Results**

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57 Figure 2 exhibit the transition of the salivary CgA level after UK test with and without
58 gum chewing. Two-way ANOVA with repeated measures for these CgA data did not
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4 exhibited significant effect of gum chewing ($F = 0.06$; $P = 0.809$) and post UK test
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6 salivary samples ($F = 3.418$; $P = 0.081$) independently, but revealed significant
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8 interaction effect of gum chewing \times post UK test salivary samples ($F = 5.284$; $P = 0.034$,
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11 Table 1).

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15 Figures 3 and 4 present the correlation of changes in CgA levels with average EMG
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17 activity and masticatory performance. The horizontal lines in both graphs show the
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19 differences between CgA levels immediately after and 10 min after the UK test with gum
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21 chewing. A negative correlation was found between changes in CgA levels and
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23 masticatory performance, whereas average EMG activity did not exhibit a specific
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25 correlation.
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32 **4. Discussion**

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35 To induce experimental stress, we used the UK test, in which participants perform
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37 monotonous and reiterative single-digit additions. The original purpose of this test was
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39 to evaluate the character and attitude of participants from the pattern of their work over 1
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41 min. Because this test requires lengthy numerical work and places considerable
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43 psychological burden on the participant, a number of studies adopted this test for the
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45 purpose of experimental stress loading [31-41]. Heart rate, respiration, and salivary and
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47 plasma stress indicators have all been evaluated to investigate the stress response to the
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49 UK test. In this study, all participants undertook the UK test on two different days.
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52 However, our results showed no remarkable increase in salivary CgA levels after the UK
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54 test. Before obtaining the first saliva sample, participants were made to relax for 30 min.
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57 Because this sample was obtained immediately before the UK test, participants may
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4 have been experiencing stress in anticipation. CgA responds quickly to mental stress;
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6 therefore, we consider that CgA levels were increased before initiation of the test,
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8 because of which they did not present a clear increase immediately after the UK test.
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10 Before participating in this study, none of the participants had undergone the UK test.
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12 However, before obtaining informed consent, all participants were provided with a
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14 detailed explanation of the experimental procedure, which may have increased their
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16 psychological stress. Kanamaru *et al.* [22] evaluated salivary CgA levels in response to
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18 psychological stress during a cognitive test and reported that CgA levels increased
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20 before the test.
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27 Result of ANOVA did not exhibit independent effect of gum chewing and post UK test
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29 salivary CgA levels (Table 1). However, change in salivary CgA levels immediately
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31 after and 10 min after the UK test revealed a significant interaction with and without
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33 gum chewing condition that consistent with findings of previous reports on salivary
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35 cortisol levels [6-13]. These findings confirm that gum chewing could have relief effect
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37 for the experimental acute stress. Since CgA levels exhibited relatively high
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39 inter-individual deviation and independent effect of gum chewing and post UK test were
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41 not significant, we suspected the stress-relieving effect of gum chewing was affected by
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43 differences among individuals.
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50 The detailed mechanism underlying the stress-relieving effects of gum chewing remains
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52 unclear. Nutrients in the gum base, such as glucose and/or flavoring, may affect stress
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54 levels [9]; therefore, we used flavorless gum. We consider that the decrease in CgA
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56 levels after gum chewing was elicited by chewing action itself.
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59 Reflex saliva and unstimulated saliva possess different properties. Therefore, the effects
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4 of reflex saliva secreted in response to chewing may have affected CgA levels. In this
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7 study, after the 3-min gum-chewing task, the participant relaxed for 4 min before the
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10 final saliva samples were collected. Therefore, we believe that reflex saliva secreted
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12 during gum chewing was already washed out by resting saliva during the resting period.
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14 This study did not demonstrate consistent decreases in CgA levels after gum chewing:
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16 seven of 19 participants showed a slight increase in CgA levels after gum chewing.
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18 Therefore, we speculate that the stress-relieving effects of gum chewing can be affected
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20 by several factors. Tasaka *et al.* [8] investigated the effects of gum chewing on salivary
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22 stress marker levels after experimental stress loading. They regulated the chewing rate
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24 using three steps and found that fast chewing relieved stress more effectively than slow
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26 chewing. In a similar experiment using three regulated chewing forces, Soeda *et al.* [12]
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28 reported that powerful chewing more effectively decreased salivary cortisol levels.
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30 These two studies evaluated salivary cortisol levels as an indicator of stress; both
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32 instructed participants to chew gum for 10 min and collected final saliva samples 30 min
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34 after experimental stress loading. Because we expected the changes in CgA to be quicker
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36 than those in cortisol, we instructed participants to chew for 3 min. In this study, the
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38 chewing rate was regulated at 1.5 Hz using an audio rhythm. Because no correlation was
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40 found between the average changes in CgA levels and EMG activity (Figure 3), the
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42 relationship between these dynamic chewing properties and the stress-relieving effects
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44 of gum chewing could not be confirmed by this study.

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47 Despite the lack of a relationship between chewing rate and force, we found a significant
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49 correlation between the changes in CgA levels and masticatory performance (Figure 4).

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52 As mentioned previously, salivary CgA levels reflect the stress response mediated by the
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4 SAS. Because the stress-relieving effects of chewing have been established by similar
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7 studies measuring indicators of HPA activity [6-13] and in a study using rats [42], it is
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10 undeniable that gum chewing may have such effects. It is also known that gum chewing
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12 increases cerebral blood flow [43,44]. These reports therefore provide evidence to
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14 support the hypothesis that certain parts of the brain show alterations in function during
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16 chewing. However, there is no detailed mechanism explaining why and how chewing
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18 has this effect on the central nervous system (CNS). Because mastication involves the
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20 coordination of multiple head and neck organs and supposedly has comprehensive
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22 effects on the CNS, it is challenging to identify its effects on a specific afferent pathway.
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24 Chewing is a basic action facilitating ingestion; therefore, we consider that efficient
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26 chewing satisfies instinctive desires and subconsciously promotes a stress-free state of
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28 mind.
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35 We found a significant correlation between decreased CgA levels after gum chewing and
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37 masticatory performance. This finding indicates that participants with higher
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39 masticatory performance may experience more efficient stress-relieving effects of gum
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41 chewing. Because masticatory performance is a comprehensive parameter influenced by
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43 multiple oral factors and organs, it is difficult to prove a causal relationship between
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45 gum chewing and the mechanisms of stress relief within the CNS using our data alone.
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48 However, if the stress-relieving effects of chewing derive from subconscious
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50 psychological promotion, it is possible to speculate that higher masticatory performance
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52 causes increased mental satisfaction, producing greater stress-relieving effects.
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60 **5. Conclusions**

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In conclusion, our findings indicate that chewing with stronger masticatory performance provides more effective stress relief compared with lower masticatory performance.

Conflict of interest

The authors declare that they have no conflicts of interest with respect to their authorship or the publication of this article.

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5 **Figure Legends**
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9
10 Figure 1

11
12 Experimental schedule

13
14 Saliva samples were collected six times on two different days.
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20 Figure 2

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22 Changes in mean salivary CgA levels resulting from two way two-way ANOVA with
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24 repeated measures. These samples were collected immediately after and 10 min after the
25
26 Uchida–Kraepelin test and standardized by subtracting resting salivary CgA level before
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28 UK test.
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35 Figure 3

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37 Correlation between changes in chromogranin A levels and average electromyographic
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39 activity during the gum-chewing period
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42 The horizontal line represents the difference between chromogranin A levels
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44 immediately and 10 min after the Uchida–Kraepelin test with gum chewing (4 min after
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46 gum chewing). The linear equation represents the regression line in the scatter plots ($n =$
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48 18; electromyographic data was missing for one participant; Spearman’s rank
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50 correlation coefficient).
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57 Figure 4

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60 Correlation between changes in chromogranin A levels and masticatory performance
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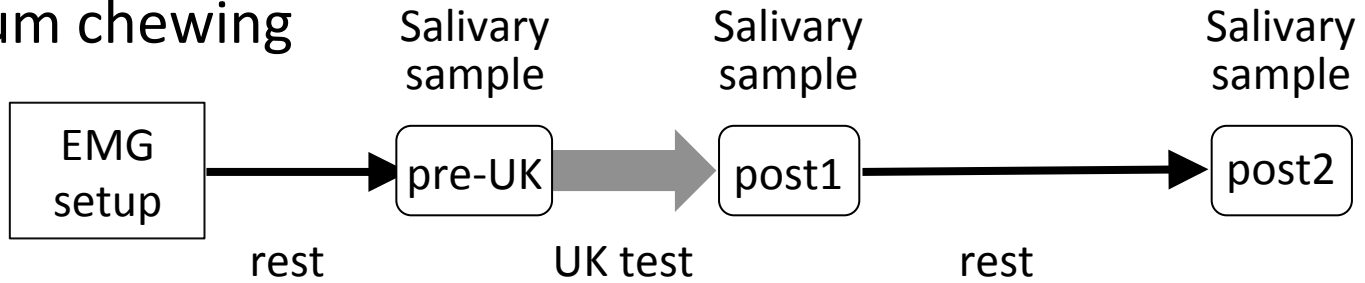
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The horizontal line represents the change in chromogranin A levels during the gum-chewing period. The linear equation shows the regression line in the scatter plots ($n = 19$; Spearman's rank correlation coefficient).

Table 1

Output of two-way ANOVA with repeated measures for salivary CgA data.

Without
gum chewing



With
gum chewing

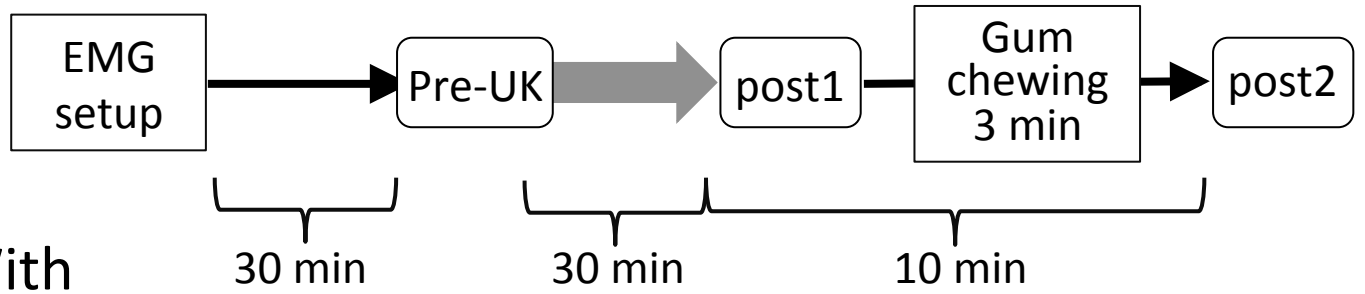


Figure 1

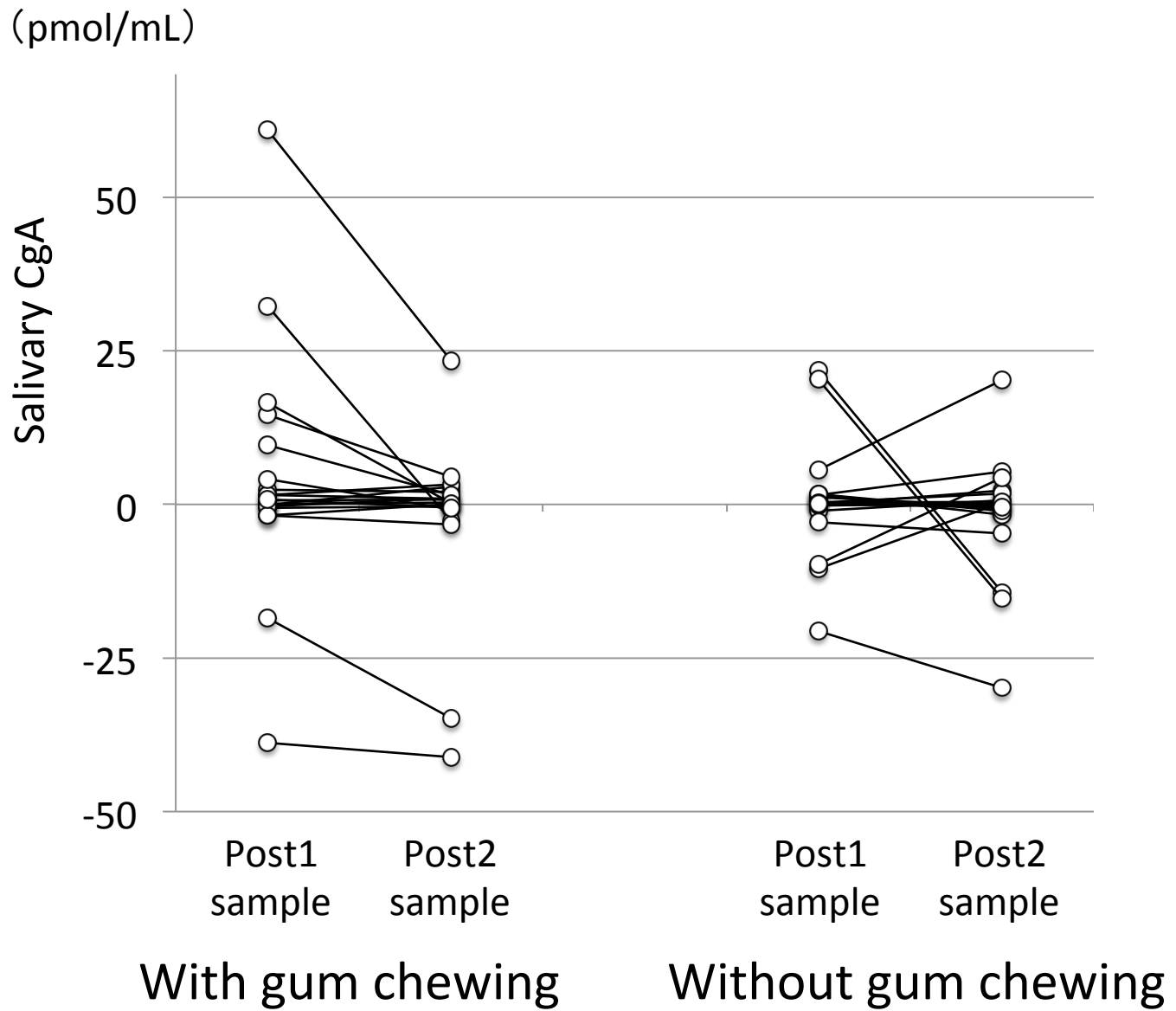


Figure 2

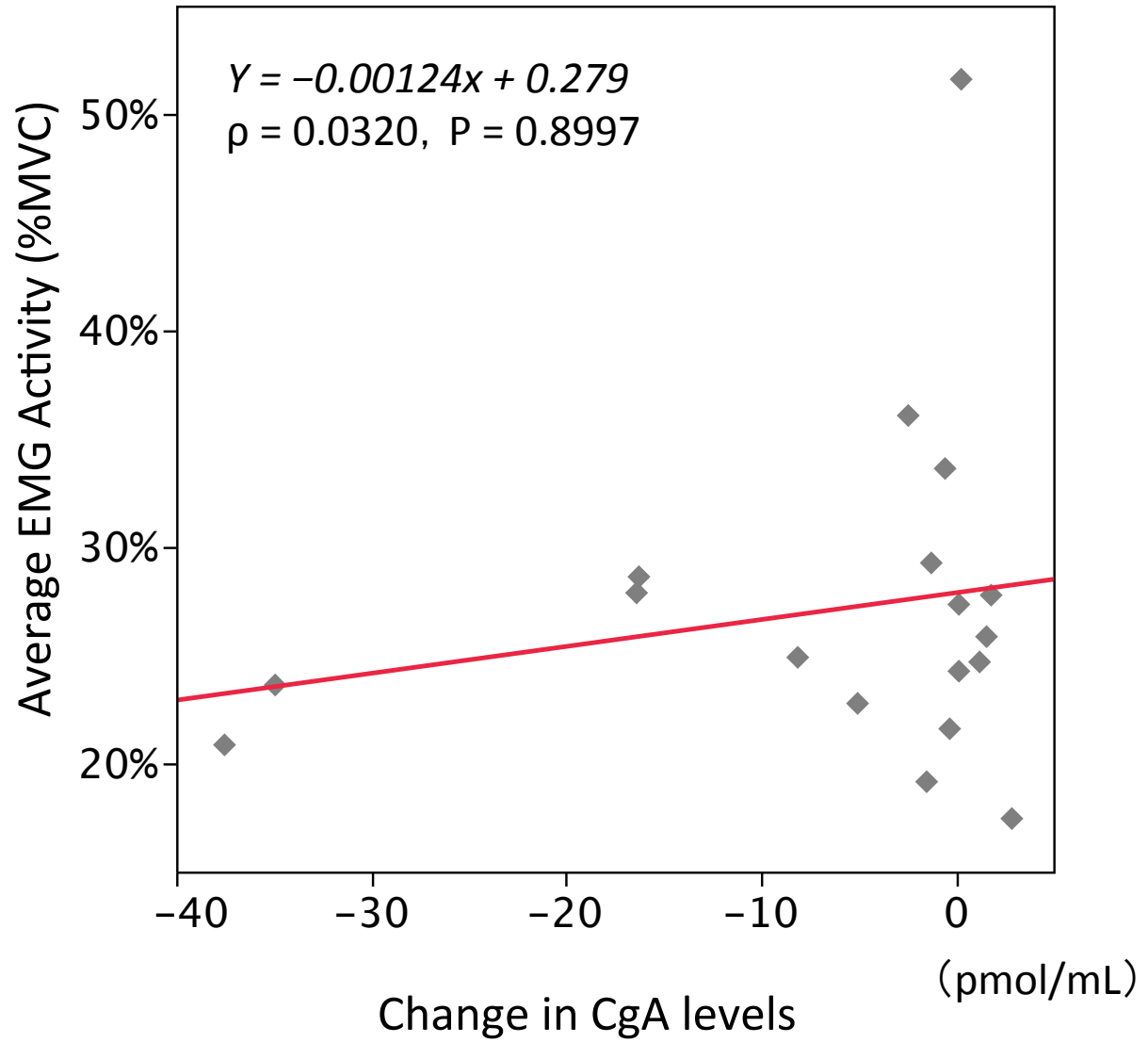


Figure 3

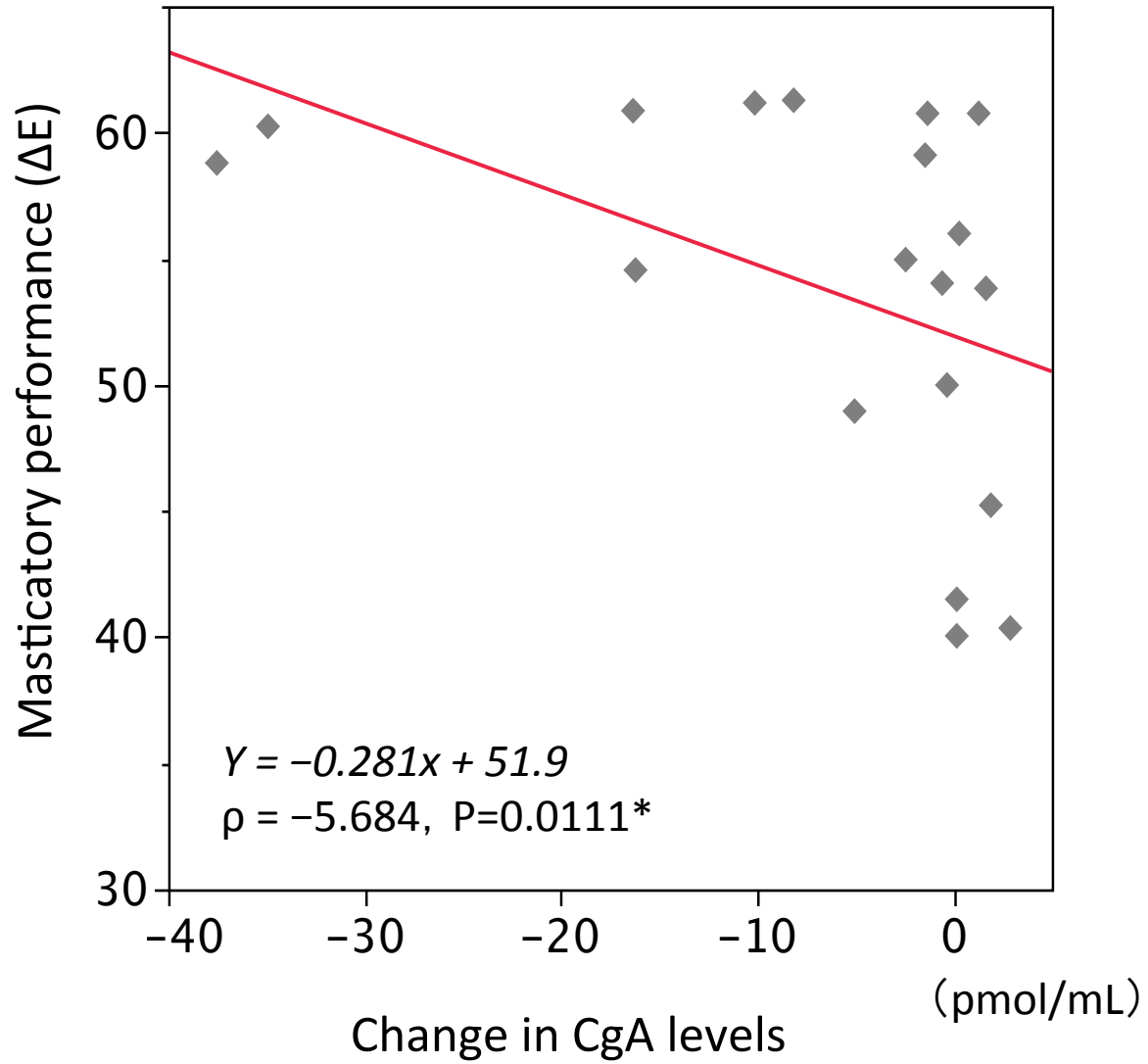


Figure 4

Source	Type III sum of squares	df	Mean square	F	Significance
Gum chewing	35.336	1	35.336	0.06	0.809
Error	10589.216	18	588.29		
Post UK test	335.849	1	335.849	3.418	0.081
Error	1768.483	18	98.249		
Gum chewing / Post UK test	112.597	1	112.597	5.284	0.034
Error	383.549	18	21.308		

Table 1