Gingival Stimulation: An Important Metabolic Regulator?

Yildirim A, Atmaca H, Bedir A and Devrim I

Objective: This study aimed to determine whether a relationship exists between gingival stimulation and the levels of leptin, ghrelin, insulin and glucose, which are important regulators of energy homeostasis.

Materials and Methods: Blood samples for ghrelin, leptin, glucose and insulin were taken from 15 male volunteers (mean age 25.5±2.3 years; mean body mass index 24.4±2.79 kg/m²), who did not brush their teeth for one day, after a 12 h-long overnight fasting and before standard breakfast (0 min) and thereafter at 30, 60, 120 and 180 min after breakfast. After tooth brushing after dinner and after a 12 h-long overnight fasting, blood samples were taken again before standard breakfast (0 min) and then after at the same time points following tooth brushing.

Results: A significant reduction was found in the leptin levels measured at 0, 30, 60, 120 and 180 min after tooth brushing (p < 0.005). The ghrelin levels also declined at these time points but were significant at 0, 30 and 120 min (p < 0.05). Despite the reduced insulin levels at 120 and 180 min after tooth brushing (p < 0.05), no significant change was observed in the glucose levels.

Conclusion: Mechanical stimulation of the gingiva by tooth brushing may play a role in appetite regulation by influencing the ghrelin, leptin and insulin signalling pathways.

Keywords: Appetite; Ghrelin; Gingival Stimulation; Insulin; Leptin; Tooth Brushing

List of abbreviations: BMI: Mean Body mass index; CNS: Central nervous system; HOMA: Homeostasis model assessment; GI: Gastrointestinal; GHS: Growth hormone secretagogue; MS: Metabolic syndrome; NPY: Neuropeptide Y

Ghrelin and leptin regulate energy balance primarily through the hypothalamic neurons in the central nervous system (CNS). Ghrelin stimulates the activity of arcuate neuropeptide Y (NPY) neurons. However, the appetite-promoting NPY neurons are inhibited by leptin. Therefore, both function as mutual antagonists in the hypothalamus, which regulates feeding behaviour [1]. Insulin and glucose are also important co-regulators of energy homeostasis [1]. As a processing centre for appetite, the hypothalamus integrates signals from the brain, the peripheral circulation and the gastrointestinal (GI) tract to regulate energy intake and expenditure [2]. Neurons expressing these neuropeptides communicate with each other and with many peripheral signals, including nutrients such as glucose, GI peptide hormones such as ghrelin, and other hormones such as insulin and leptin, to influence appetite level and feeding [3]. Therefore, the GI tract is an important and active part of energy regulation. However, the stomach and intestine usually attract more attention than other parts of the GI tract in terms of appetite regulation [2]. The implication of the mouth in energy regulation has rarely been studied [1].

Sakata et al. showed that the mastication-induced activation of histamine neurons suppressed physiological food intake through the H1-receptor in the hypothalamic paraventricular nucleus and the ventromedial hypothalamus in rats [4]. Oral proprioceptive signals received at the mesencephalic trigeminal sensory nucleus (Me5) could modulate the hypothalamic histamine neurons through the ascending pathway from Me5 to the posterior hypothalamus [5]. Moreover, histamine neurons were found to be
concordant with the leptin signalling system through a negative feedback loop. Leptin infusion elevated the turnover rate of neuronal histamine in the hypothalamus. Similar to mastication, whether gingival stimulation can affect the histaminergic neuron system should be elucidated.

The dental pulp and gingiva are highly vascularised connective tissues. Studies have shown that these tissues are active in terms of ghrelin and leptin production and are also a target of these hormones [6-10].

This study specifically investigated the possible role of tooth brushing as an initial part of energy regulation. This work is the first so far to evaluate leptin, ghrelin, insulin and glucose levels in relation to tooth brushing.

Materials and Methods

Participants

The study included 15 male volunteers (mean age: 25.5±2.3 years; age range: 23–31), who were final-year medical students or research assistants at the Faculty of Medicine, Ondokuz Mayis University. The participants did not have any systemic diseases, experience recent weight loss, participate in any dieting behaviour, smoke, or receive periodontal treatment in the previous six months. As sex hormones may affect the leptin levels, the participants were chosen to be all male [11]. Moreover, to form a homogeneous group in terms of insulin sensitivity, the participants with similar lifestyles, who were either medical students or research assistants at the Faculty of Medicine, were recruited. The study protocol was approved by the local ethics committee of Ondokuz Mayis University. All subjects signed an informed written consent prior to participation.

Protocol

This study aimed to investigate the possible effects of tooth brushing on appetite hormones. The investigated hormones and glucose are known to be affected by fasting and eating, but the effect of gingival stimulation in the form of tooth brushing is unknown. If such an effect exists, then its onset and duration should be determined. This work was a physiological study, and we attempted to simulate a normal lifestyle in subjects during the study period. For this reason, the hormones were evaluated before and after tooth brushing and meals. Breakfast in this study contained approximately 430 Kcal (56% carbohydrates, 17% protein and 27% fat).

The long-term exposure of the gingiva to microbial dental plaque may cause gingival inflammation [12]. The lack of tooth brushing, which is known to accelerate plaque formation and inflammation, could interfere with the study. Thus, the participants brushed their teeth regularly before the study. They were only allowed not to brush their teeth for one day at the beginning of the study. Blood samples were taken from the subjects who did not brush their teeth for one day, after a 12 h-long overnight fasting and before standard breakfast (0 min) and thereafter at 30, 60, 120 and 180 min after breakfast.

To simulate a normal life condition, the subjects were allowed to brush their teeth after the following dinner and breakfast. After tooth brushing following dinner and a 12 h-long overnight fasting, blood samples were taken again before standard breakfast (0 min) and then after at 30, 60, 120 and 180 min following tooth brushing without toothpaste. Ghrelin, leptin, glucose and insulin levels were measured from the blood samples. The study scheme is illustrated in detail in Figure 1.

![Figure 1](image-url)
The estimate of insulin resistance by the homeostasis model assessment (HOMA) score was calculated with the following formula: fasting serum insulin (µU/ml) × fasting serum glucose (mmol/L)/22.5 [13]. Leptin, ghrelin, glucose and insulin levels were expressed as the area under the curve (AUC) estimated by the trapezoidal rule. Each subject served as his own control for the effect of tooth brushing on serum hormones.

Blood sampling and processing
Venous blood samples were drawn into plain vacutainers and kept at room temperature until clotting. Serum samples were separated by centrifugation at 3000x g for 10 min and stored at –80 °C until assay. Serum leptin and total ghrelin levels were measured by commercial ELISA kits (DIAsource Leptin-EASIA Kit, Belgium and RayBio Human/Mouse/Rat Ghrelin Enzyme Immunoassay Kit, Germany). The precision performance of ELISA kits was evaluated with low and high levels of the control material included. Fasting blood glucose and insulin levels were measured on a Cobas 8000 modular analyser (Roche Diagnostics, Indianapolis, USA) by dedicated reagents.

Statistical analysis
Data were analysed by SPSS 15.0 software. Normality test was performed for continuous variables and parametric (paired-t test and Pearson correlation analysis) and nonparametric tests (Wilcoxon signed rank test, Friedman analysis of variance and Bonferroni adjusted Wilcoxon signed rank test) were used to evaluate the data according to whether they were distributed normally or not. A value of p < 0.05 was accepted as statistically significant for the paired-t test, Wilcoxon signed rank test and Friedman analysis of variance, and a value of p < 0.01 was considered statistically significant for the Bonferroni-adjusted Wilcoxon signed rank test. Data distributed normally were presented as the mean ± SD, whereas those that were not distributed normally were presented as the median (minimum–maximum).

Results
The mean body mass index (BMI) of the participants was 24.4±2.79 kg/m² (range: 19–29). A significant reduction was found in the leptin levels measured at 0 (p=0.01), 30 (p=0.002), 60 (p=0.001), 120 (p=0.004) and 180 (p=0.001) min after tooth brushing (Figure 2). The ghrelin levels also declined at these time points, which were significant at 0 (p=0.032), 30 (p=0.009) and 120 min (p=0.011) (Figure 3). Similarly, both the AUC_{leptin} (p=0.001) and AUC_{ghrelin} (p=0.006) levels were lower in the post-brushing period than in the pre-brushing period (Table 1). Despite the reduced insulin levels at 120 (p=0.027) and 180 (p=0.016) min after tooth brushing (Figure 4), no significant change was observed in the glucose levels (p>0.05) (Figure 5). Although the AUC_{insulin} levels declined in the post-brushing period, these differences were statistically insignificant (p=0.053). Moreover, the AUC_{glucose} levels did not change between the pre and post- brushing periods (p=0.828) (Table 1). The HOMA scores did not differ between pre-brushing (0.87±0.73) and post-brushing (1.35±1.40) periods (p=0.281).

![Figure 2: Mean (± SD) leptin levels in the pre-brushing and post-brushing periods. A significant reduction was found in the leptin levels in the post-brushing period for all time points measured at 0 (p=0.001), 30 (p=0.002), 60 (p=0.001), 120 (p=0.004) and 180 (p=0.001) min](image)

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<tr>
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<th>Pre-brushing</th>
<th>Post-brushing</th>
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<tbody>
<tr>
<td>AUC_{leptin} (ng/ml x180 min)</td>
<td>447 ± 198</td>
<td>86 ± 114</td>
<td>0.001</td>
</tr>
<tr>
<td>AUC_{ghrelin} (ng/ml x180 min)</td>
<td>3228 ± 955</td>
<td>2556 ± 506</td>
<td>0.006</td>
</tr>
<tr>
<td>AUC_{insulin} (µU/ml x180 min)</td>
<td>3796 ± 1915</td>
<td>3226.4 ± 1621.9</td>
<td>0.053</td>
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<tr>
<td>AUC_{glucose} (mg/dl x180 min)</td>
<td>14777 ± 1786</td>
<td>14728.0 ± 1555</td>
<td>0.828</td>
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Values are the mean±SD
Table 1: Area under the curve (AUC) values of leptin, ghrelin, insulin and glucose in the pre-brushing and post-brushing periods
Discussion

We found that gingival stimulation in the form of tooth brushing decreases the leptin, ghrelin and, partly, insulin levels in non-obese healthy adults males. The mechanism for these alterations is not clear.

Gingival tissues have been shown to produce both ghrelin [6] and leptin [7], and both plasma levels increased [8,9] in patients with chronic periodontitis. Gingival tissues also express leptin receptor as well as ghrelin mRNA and growth hormone secretagogue
Bedir A analysed and interpreted the data. Devrim I was responsible for the conception and design of the study. Sultan AT performed the statistical tests, interpreted the data and revised the article. Yildirim A designed and conducted the study as well as analysed and interpreted the data. Atmaca H designed the study, analysed and interpreted data and drafted the article. Sunter AT performed the statistical tests, interpreted the data and revised the article.

Authorship Contributions

Yildirim A designed and conducted the study as well as analysed and interpreted the data. Atmaca H designed the study, analysed and interpreted data and drafted the article. Sultan AT performed the statistical tests, interpreted the data and revised the article. Bedir A analysed and interpreted the data. Devrim I was responsible for the conception and design of the study.

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References


