Effects of thirty-times chewing per bite on secretion of glucagon-like peptide-1 in healthy volunteers and type 2 diabetic patients

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Abstract. Glucagon-like peptide 1 (GLP-1) is secreted from the small intestine to the blood in response to glucose intake during a meal; however, it is not known whether mastication affects GLP-1 secretion. Here, we examined the relationship between mastication and GLP-1 secretion, along with postprandial blood glucose and insulin concentrations. We compared the levels of blood glucose, serum insulin, and plasma active GLP-1 concentrations after young healthy volunteers ate a test meal either by usual eating (control) or in one of three specified ways: 1. unilateral chewing, 2. quick eating, 3. 30-times chewing per bite. Ten volunteers participated in each of the three groups. Plasma active GLP-1 concentrations did not change by unilateral chewing or quick eating, but did increase by the third method, without affecting the concentrations of blood glucose or serum insulin. Next, we tested whether 30-times chewing per bite increased plasma active GLP-1 concentrations in 15 patients with type 2 diabetes mellitus, but there was no difference in results between usual eating and 30-times chewing per bite. This is a pilot trial with a small number of subjects, but is the first study to investigate the relationships between various styles of mastication and the GLP-1 secretion in young healthy volunteers and type 2 diabetic patients.

Key words: Glucagon-like peptide-1, Mastication, Japanese, Type 2 diabetes, Ghrelin
vent rapid postprandial blood glucose rises in diabetic patients, were reported to enhance the GLP-1 response to a standard breakfast or oral sucrose load in healthy subjects [4-6], probably because αGI moves the absorption site of carbohydrates to the GLP-1-producing lower intestine [7, 8]. These reports suggested that the secretion of GLP-1 was affected by the change of glucose absorption, and prompted us to compare the GLP-1 secretion by various patterns of mastication with that by usual eating in healthy subjects for the purpose of clarifying which type of mastication changes the postprandial GLP-1 secretion through glucose absorption.

In Japan, the practice of thorough mastication such as 30-times chewing per bite has been applied to obesity treatment and is effective as a behavior approach to curbing obesity [9], because mastication-induced activation of histamine neurons suppresses physical food intake through the H1-receptor in the hypothalamic paraventricular nucleus and the ventromedial hypothalamus, which are known as satiety centers [10]. Therefore, we employed 30-times chewing per bite and its opposite, quick eating, as two patterns of mastication that have the potential to change glucose absorption in healthy subjects. In addition, because it was reported that unilateral chewers presented significantly worse masticatory performance than that of bilateral chewers [11], unilateral chewing was also examined. We compared all three to usual eating, which was defined as bilateral chewing at the subject’s usual pace. On this occasion, as a reference to the anorexigenic hormone GLP-1, we also measured the orexigenic hormone ghrelin, which rises in the fasted state and decreases rapidly after food ingestion [12], because it was reported that quick eating tended to result in a higher ghrelin level at 120 minutes after a meal compared to eating slowly [3].

As a result of the investigation in healthy subjects, 30-times chewing per bite increased the GLP-1 response, but the other two patterns did not; therefore, we next examined whether 30-times chewing per bite in type 2 diabetic patients increased plasma active GLP-1 concentrations compared with usual eating, as enhancement of endogenous GLP-1 secretion may have great benefit for type 2 diabetic patients [13].

Subjects and Methods

Subjects
Thirty healthy third-year student volunteers from Kyushu Dental University and 15 outpatients with type 2 diabetes mellitus who regularly visit the Internal Medicine Department at the Kyushu Dental University Hospital participated in this study. The study protocol was approved by the Human Ethics Committee of Kyushu Dental University (No. 09-09 for healthy volunteers and No. 10-17 for diabetic patients), and written informed consent for study participation was obtained from all healthy volunteers and diabetic patients. The study was conducted in 2009 on healthy volunteers and in 2010 on diabetic patients in accordance with the ethical principles stated in the Declaration of Helsinki (2000) of the World Medical Association.

Study protocol
After an overnight fast, healthy volunteers came to the Internal Medicine Department at the Kyushu Dental University Hospital at 9 a.m. Their weight, height, and blood pressure were measured, and they were interviewed regarding their past history and health condition. Then, they were advised to eat all of a test meal in their usual manner and at their usual rate, together with drinking 100 mL of water. The total energy content of the test meal, which consisted of chicken cream stew, crackers, and pudding, and which was developed for the assessment of postprandial hyperglycemia and hyperlipidemia simultaneously in diabetic subjects by the Japan Diabetes Society (Janef E460F18, Kewpie Corporation, Tokyo, Japan), was 460 kcal, with 56.5 g of carbohydrate, 18.0 g of fat, and 18.0 g of protein. This meal supplies a total of 49.1 energy % (E%) from carbohydrate, 35.2 E% from fat, and 15.7 E% from protein. Venous blood was drawn just before eating (0 minutes), and at 30, 60, and 120 minutes after the subject began eating the meal. A few days later, the individuals were gathered again after an overnight fast and instructed to eat the same test meal in one of three specified ways, as randomly assigned: 1. unilateral chewing, 2. quick eating, or 3. 30-times chewing per bite. The choice of chewing side for unilateral chewing was made by the subject according to personal preference. The meal duration from the start of eating to the end of eating was measured, and in the case of quick eating, subjects were instructed to eat the test meal in half the time it took for their control trial of usual eating.

As in the case with healthy volunteers, after an overnight fast, 15 type 2 diabetic outpatients came to the Internal Medicine Department at the Kyushu Dental University Hospital at 9 a.m and were advised to eat all of the same test meal in their usual manner at their usual
rate, together with drinking 100 mL of water. Venous blood was drawn just before eating (0 minutes), and at 30, 60, and 120 minutes after patients began eating the meal. At the next visit, which was ordinarily one month later, patients arrived again at 9 a.m. after an overnight fast and were instructed to eat the same test meal by 30-times chewing per bite. Venous blood was drawn again. Two patients were being treated with diet therapy alone and 13 patients were being treated with oral hypoglycemic agents (sulfonylureas 9, voglibose 10, metformin 7, thiazolidines 3) other than DPP-4 inhibitors. Before and during eating the test meal and drawing the blood, they were prohibited from taking any medicine.

Laboratory analysis
We outsourced the testing of blood cell count and blood biochemistry, including blood glucose and serum insulin before and after meals, to SRL, Inc. (Tokyo, Japan). The value for hemoglobin Alc (HbAlc) (%) was estimated as a National Glycohemoglobin Standardization Program (NGSP) equivalent value (%) calculated by the formula HbAlc (NGSP) (%) = HbAlc as defined by the Japan Diabetes Society (%) + 0.4%. The active GLP-1 [GLP-1-(7-36 amide) and GLP-1-(7-37)] and the desacyl-ghrelin were analyzed by a commercially available enzyme-linked immunosorbent assay kit (Millipore Corporation, Billerica, MA for GLP-1; Mitsubishi Kagaku Iatron, Inc., Tokyo, Japan for ghrelin). The intra- and inter-assay variations for the active GLP-1 were 7-9 % and <1-13 %, respectively, for five human plasma samples. The intra- and inter-assay variations for the desacyl-ghrelin were 2.1-4.7 % and 4.2-8.6 %, respectively, for three human plasma samples. At the time of blood collection for GLP-1, 2 mL of collected blood in an EDTA-plasma tube was immediately (<30 seconds) spiked with 20 μL of DPP-4 inhibitor (Millipore Corporation), vortexed, and centrifuged at 1000×g for 10 minutes according to the manufacturer’s directions. The specimen was stored at -80 ºC until measurement. The blood for ghrelin was collected in a tube containing aprotinin and EDTA and then vortexed immediately and centrifuged; the resulting supernatant was kept at -80 ºC until measurement.

Statistical analysis
Data are presented as means±SDs. The male-to-female ratio and other parameters were compared among the 3 specified eating groups by the χ²-test and one-way ANOVA followed by Bonferroni/Dunn as a post hoc test, respectively, using the software StatView-J, version 5.0 (SAS Institute Japan, Tokyo, Japan). Comparison of the changes of blood glucose, serum insulin, and plasma active GLP-1 concentrations between usual eating and each specified eating method was carried out by repeated measures ANOVA. If a significant interaction of eating method and time was documented (p<0.05), values of plasma active GLP-1 concentrations at single time points for usual eating and each specified eating method were compared by paired t-test, and we calculated the incremental area under the curve (IAUC) according to the trapezoid rule to compare usual eating with each specified eating method by paired t-test. The relationships between pairs of parameters were investigated by Pearson’s correlation coefficient, and the average meal duration for usual eating and each specified eating method was compared by paired t-test.

Results
Table 1 shows the clinical characteristics of the healthy volunteers, broken down into the 3 specified eating groups, and the type 2 diabetic patients. There were no differences in parameters among the 3 specified eating groups of healthy volunteers. Table 2 shows the comparison of plasma glucose, serum insulin, plasma active GLP-1 and ghrelin concentrations between usual eating and unilateral chewing upon eating the test meal. In both eating methods, plasma glucose and active GLP-1 concentrations peaked at 30 minutes after eating, and the maximum levels were equivalent. Repeated measures ANOVA showed no significant difference between the two eating methods. Likewise, the changes of serum insulin concentrations were not different between the two eating methods. Plasma ghrelin concentrations at 120 minutes after both eating methods were significantly decreased compared with before eating.
the mean plasma active GLP-1 concentration from 30-times chewing per bite was significantly higher than that from usual eating (9.9±3.3 pmol/L for 30-times chewing per bite vs. 8.6±2.1 pmol/L for usual eating, \(p<0.05\)). Furthermore, 30-times chewing per bite significantly increased the GLP-1 IAUC (91.3±61.7 pmol・min/L for 30-times chewing per bite vs. 56.1±49.8 pmol・min/L for usual eating, \(p<0.05\)). Since eating slowly increased the GLP-1 secretion without affecting blood glucose and serum insulin concentrations [3] and in this study, 30-times chewing per bite extended the average mealtime from 9.8±0.9 minutes to 15.0±1.6 minutes (\(p<0.001\)), we investigated the correlative relationship between the meal duration and the GLP-1 IAUC of usual eating and 30-times chewing per bite by Pearson’s correlation coefficient. However, there was no relationship between them. The significant increase of plasma active GLP-1 concentration at 30 minutes after eating and GLP-1 IAUC by 30-times chewing per bite appeared to be unaffected by quick eating as compared to usual eating. Plasma ghrelin concentrations were significantly decreased even in the case of quick eating.

Table 3, which shows the comparison of plasma glucose, serum insulin, plasma active GLP-1 and ghrelin concentrations between usual eating and quick eating, plasma glucose, serum insulin, and plasma active GLP-1 concentrations appeared to be unaffected by quick eating as compared to usual eating. Plasma ghrelin concentrations were significantly decreased even in the case of quick eating.

Table 4 shows the comparison of plasma glucose, serum insulin, plasma active GLP-1 and ghrelin concentrations between usual eating and unilateral chewing in healthy volunteers. Repeated measures ANOVA of plasma glucose and serum insulin concentrations were not different statistically between the two eating methods; however, plasma active GLP-1 concentrations were significantly different (\(p<0.05\)). Therefore, we compared the values of plasma active GLP-1 concentrations between the two eating methods at each measuring point by paired t-test; at 30 minutes after eating, the mean plasma active GLP-1 concentration from 30-times chewing per bite was significantly higher than that from usual eating (9.9±3.3 pmol/L for 30-times chewing per bite vs. 8.6±2.1 pmol/L for usual eating, \(p<0.05\)). Furthermore, 30-times chewing per bite significantly increased the GLP-1 IAUC (91.3±61.7 pmol・min/L for 30-times chewing per bite vs. 56.1±49.8 pmol・min/L for usual eating, \(p<0.05\)). Since eating slowly increased the GLP-1 secretion without affecting blood glucose and serum insulin concentrations [3] and in this study, 30-times chewing per bite extended the average mealtime from 9.8±0.9 minutes to 15.0±1.6 minutes (\(p<0.001\)), we investigated the correlative relationship between the meal duration and the GLP-1 IAUC of usual eating and 30-times chewing per bite by Pearson’s correlation coefficient. However, there was no relationship between them. The significant increase of plasma active GLP-1 concentration at 30 minutes after eating and GLP-1 IAUC by 30-times chewing per
Thorough chewing affects GLP-1 secretion

bite led us to investigate whether the increase of GLP-1 from usual eating to 30-times chewing per bite affected the initial insulin secretion shown as the insulinogenic index, which is calculated by dividing the increment in insulin during the first 30 minutes by the increment in glucose over the same period at 75g OGTT. The difference in GLP-1 increment during first 30 minutes (ΔGLP-1_{30-0}) between usual eating and 30-times chewing per bite was positively correlated to the difference in insulinogenic index between usual eating and 30-times chewing per bite by Pearson’s correlation coefficient (Fig. 1, r=0.806, p<0.01).

Since plasma active GLP-1 concentrations were increased by 30-times chewing per bite in the healthy volunteers compared to usual eating, we next investigated whether this eating method increased plasma active GLP-1 concentrations in type 2 diabetic patients. As shown in Table 5, repeated measures ANOVA of plasma glucose, serum insulin and plasma active

Table 3 Comparison of glucose, insulin, GLP-1 and ghrelin concentrations between usual eating and quick eating in healthy volunteers

<table>
<thead>
<tr>
<th>Glucose (mg/dL)</th>
<th>0 min</th>
<th>30 min</th>
<th>60 min</th>
<th>120 min</th>
<th>IAUC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Usual eating</td>
<td>85.1±6.4</td>
<td>105.0±17.2††</td>
<td>99.1±28.8</td>
<td>81.5±10.8</td>
<td>1422±1201</td>
</tr>
<tr>
<td>Quick eating</td>
<td>82.1±6.9</td>
<td>102.8±14.7††</td>
<td>86.8±20.9†</td>
<td>80.7±10.7†</td>
<td>1108±841</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Insulin (µU/mL)</th>
<th>0 min</th>
<th>30 min</th>
<th>60 min</th>
<th>120 min</th>
<th>IAUC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Usual eating</td>
<td>8.8±6.9</td>
<td>42.8±21.6††</td>
<td>37.6±21.6††</td>
<td>22.7±17.2††</td>
<td>2745±1450</td>
</tr>
<tr>
<td>Quick eating</td>
<td>6.9±5.2</td>
<td>56.9±32.5††</td>
<td>51.9±48.4†</td>
<td>22.2±20.0†</td>
<td>3983±3133</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>GLP-1 (pmol/L)</th>
<th>0 min</th>
<th>30 min</th>
<th>60 min</th>
<th>120 min</th>
<th>IAUC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Usual eating</td>
<td>8.0±1.9</td>
<td>9.1±1.6†</td>
<td>8.9±2.6†</td>
<td>8.6±1.7†</td>
<td>93.1±75.1</td>
</tr>
<tr>
<td>Quick eating</td>
<td>7.8±1.5</td>
<td>9.0±1.7†</td>
<td>8.6±1.5†</td>
<td>8.5±1.3†</td>
<td>91.8±61.9</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Ghrelin (fmol)</th>
<th>0 min</th>
<th>30 min</th>
<th>60 min</th>
<th>120 min</th>
<th>IAUC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Usual eating</td>
<td>195.4±124.8</td>
<td>-</td>
<td>-</td>
<td>106.8±54.4†</td>
<td></td>
</tr>
<tr>
<td>Quick eating</td>
<td>179.8±118.0</td>
<td>-</td>
<td>-</td>
<td>110.5±62.7†</td>
<td></td>
</tr>
</tbody>
</table>

Table 4 Comparison of glucose, insulin, GLP-1 and ghrelin concentrations between usual eating and 30-times chewing per bite in healthy volunteers

<table>
<thead>
<tr>
<th>Glucose (mg/dL)</th>
<th>0 min</th>
<th>30 min</th>
<th>60 min</th>
<th>120 min</th>
<th>IAUC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Usual eating</td>
<td>86.4±6.2</td>
<td>114.4±16.7††</td>
<td>105.9±19.9††</td>
<td>78.8±11.9</td>
<td>1609±811</td>
</tr>
<tr>
<td>30-times chewing</td>
<td>84.9±5.4</td>
<td>120.4±18.9††</td>
<td>101.0±28.9</td>
<td>80.9±10.6</td>
<td>1625±1162</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Insulin (µU/mL)</th>
<th>0 min</th>
<th>30 min</th>
<th>60 min</th>
<th>120 min</th>
<th>IAUC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Usual eating</td>
<td>5.5±0.6</td>
<td>40.5±5.4††</td>
<td>46.7±5.3††</td>
<td>18.9±5.1†</td>
<td>3300±1211</td>
</tr>
<tr>
<td>30 times chewing</td>
<td>5.3±0.7</td>
<td>54.4±7.7††</td>
<td>53.8±7.1††</td>
<td>26.9±6.4†</td>
<td>4308±1939</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>GLP-1 (pmol/L)</th>
<th>0 min</th>
<th>30 min</th>
<th>60 min</th>
<th>120 min</th>
<th>IAUC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Usual eating</td>
<td>8.2±2.9</td>
<td>8.6±2.1†</td>
<td>8.6±3.2†</td>
<td>8.7±2.7†</td>
<td>56.1±49.8</td>
</tr>
<tr>
<td>30 times chewing</td>
<td>8.0±2.1</td>
<td>9.9±3.3†*</td>
<td>8.5±2.0†</td>
<td>8.4±1.9†</td>
<td>91.3±61.7*</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Ghrelin (fmol)</th>
<th>0 min</th>
<th>30 min</th>
<th>60 min</th>
<th>120 min</th>
<th>IAUC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Usual eating</td>
<td>159.1±73.8</td>
<td>-</td>
<td>-</td>
<td>98.0±56.9†</td>
<td></td>
</tr>
<tr>
<td>30 times chewing</td>
<td>153.2±59.5</td>
<td>-</td>
<td>-</td>
<td>101.8±41.1†</td>
<td></td>
</tr>
</tbody>
</table>

Data are means±SDs. †p<0.05, ††p<0.01 vs 0 min, *p<0.05 vs usual eating.

Fig. 1 Positive correlation between the increase of GLP-1 from usual eating to 30-times chewing per bite and changes of initial insulin secretion. The x axis represents the difference in GLP-1 increment during first 30 minutes (ΔGLP-1_{30-0}) between usual eating and 30-times chewing per bite (x-axis) while the y-axis shows the difference of insulinogenic index between usual eating and 30-times chewing per bite; i.e., the difference in initial insulin secretion.
GLP-1 concentrations did not respond differently to the two eating methods. On the other hand, plasma ghrelin concentrations significantly decreased in both eating methods. Chronic treatment with αGI [14] or metformin [15] in type 2 diabetic patients has been reported to affect GLP-1 secretion; therefore, we compared plasma active GLP-1 concentrations between patient groups treated with and without the αGI voglibose (n=9 and 6, respectively) and between patient groups treated with and without metformin (n=7 and 8, respectively). Repeated measures ANOVA of plasma active GLP-1 concentrations revealed a significant difference between the groups treated with and without voglibose in eating a test meal by 30-times chewing per bite (Fig. 2B, \( p<0.05 \)) but not by usual eating (Fig. 2A); however, the values of plasma active GLP-1 concentrations at each measuring point and the GLP-1 IAUC between the groups treated with and without voglibose (184.9\pm152.6 \text{ pmol} \cdot \text{min/L} for the groups treated with voglibose vs. 160.7\pm95.9 \text{ pmol} \cdot \text{min/L} for the groups not treated with voglibose) were not different by unpaired \( t \)-test. In addition, both those treated with and without voglibose showed no difference between changes of plasma GLP-1 concentrations for each of the two eating methods by repeated measures ANOVA. Unlike voglibose, metformin treatment had no effect on plasma active GLP-1 concentrations.

**Discussion**

In the present study, we found that GLP-1 secretion did not change by unilateral chewing or quick eating compared to usual eating, but increased significantly by 30-times chewing per bite compared to usual eating.
ing at 30 minutes after the ingestion of a test meal in healthy young volunteers. Furthermore, it is suggested that the increase of GLP-1 secretion by 30-times chewing per bite contributed to the increase of initial insulin secretion, due to positive correlation between the difference of GLP-1 increment during first 30 minutes and the difference in the insulinogenic index between usual eating and 30-times chewing per bite.

GLP-1 is one of the gut hormones secreted from L-cells in the distal parts of the small intestine in response to ingested nutrients. The main meal components that act as potent stimulants of GLP-1 secretion are glucose and triacylglycerol [13]; furthermore, the carbohydrate rather than the fat has been shown to stimulate GLP-1 secretion [16]. The results of an earlier study demonstrated that a liquid form of the meal stimulated significantly more GLP-1 secretion than the identical isocaloric solid meal in 6 healthy volunteers, suggesting that the physical form of a meal alters the GLP-1 response [17]. In addition, in a crossover study of seventeen healthy male adults, eating slowly—namely, taking 30 min to ingest exactly 300 mL ice cream (675 kcal)—increased the postprandial response of GLP-1 compared to eating the same portion in 5 minutes [3]. These reports suggest the importance of glucose absorption in the intestine on the regulation of GLP-1 secretion. Therefore, 30-times chewing per bite in our study also may have augmented GLP-1 secretion compared to usual eating by changing the glucose absorption in the small intestine. One possibility is that 30-times chewing per bite increased the volume of glucose absorption by thorough mastication and abundant breakdown of carbohydrate. In such case, the secretion of glucose-dependent insulinotropic polypeptide (GIP) is also expected to increase; regrettably, we did not measure the plasma GIP concentrations together with GLP-1.

On the other hand, in the group performing 30-times chewing per bite in our study, the lack of relationship between the meal duration and the GLP-1 IAUC suggests that other mechanisms may have promoted the GLP-1 response, although our study had shorter meal durations than those described in the report about eating slowly [3]. Eating slowly by performing thorough mastication leads to decreases in energy intake within meals in healthy women [18], and when rats were fed with either hard or soft pellets, the meal size and eating speed increased in rats fed with soft pellets compared to those fed with hard pellets [19]. In the case of rats, it was explained that mastication-induced activation of histamine neurons via the mesencephalic sensory trigeminal nucleus suppressed physiological food intake through H1-receptors in the hypothalamic paraventricular nucleus (PVN) and the ventromedial hypothalamus (VMH), which are known as satiety centers [10]. In parallel, this histamine neuron activation accelerated lipolysis in the visceral adipocytes and up-regulated gene expression of the uncoupling protein (UCP) family through the sympathetic efferent nerve to regulate peripheral energy expenditure [10]. The existence of the efferent signals evoked by mastication led us to assume the involvement of neurohormonal mechanisms to explain the enhancement of GLP-1 secretion from L-cells of the intestine by mastication, although to date, the existence of neurohormonal mechanisms has not been defined in humans [7].

It was regrettable that 30-times chewing per bite did not increase GLP-1 secretion in the type 2 diabetic patients. One possible cause is GLP-1 deficiency related to diabetes mellitus; earlier studies have demonstrated an impairment of GLP-1 secretion in type 2 diabetic patients after the ingestion of a meal [20, 21], but recent measurements of intact GLP-1 have revealed no decreases in the intact GLP-1 concentrations in type 2 diabetic patients [22, 23]. It thus remains debatable whether GLP-1 deficiency exists in type 2 diabetic patients. By contrast, αGI voglibose was reported to enhance GLP-1 response to a mixed meal in Japanese type 2 diabetic patients, as well as another αGI, miglitol [14]. In type 2 diabetic patients of this study, changes in plasma GLP-1 concentrations were significantly different between patients treated with and without voglibose by repeated measures ANOVA, although the increase of GLP-1 secretion was not proved statistically, probably due to the small number of subjects. Therefore, our results supported the idea of the voglibose’s enhancement of GLP-1 response in type 2 diabetic patients, although, to be exact, a comparison of the patient group treated with neither voglibose or metformin (n =2) to the patient group treated with voglibose only (n=7) is needed to exclude the effects of metformin.

Recently, it was reported that intact GLP-1 levels determined by immunoassay with ethanol or solid-phase extractions were lower than those determined without extraction, because ethanol or solid-phase extractions remove interference for intact GLP-1 immunoassay [24-26]. The Japan Diabetes Society proposed an extraction procedure for plasma samples before
measuring active GLP-1 in 2011. However, since this study was conducted in 2009 and 2010, and there was no information about ethanol or solid-phase extractions in the manufacturer’s instructions for the active GLP-1 test kit, we could not apply the extraction procedure. Therefore, the values of plasma active GLP-1 concentration at 0 minutes in this study seem to be higher than those in other reports after meal ingestion in Japanese healthy controls and patients with type 2 diabetes [25, 26]. Furthermore, it was reported that intact GLP-1 levels remained low with no significant peak unlike in this study, although the reduced GLP-1 response could be explained by meal size as well as meal composition, which has been shown to be critical to GLP-1 response [26]. Accordingly, measuring active GLP-1 levels without ethanol or solid-phase extractions and evaluating the secretion of GLP-1 by active GLP-1 levels not by total GLP-1 levels, which are considered to be suitable for the evaluation of GLP-1 secretion rather than active GLP-1 levels [24], are both limitations in this study.

In conclusion, although the current study was a pilot trial with a small number of subjects, we observed that when healthy young adults ate a test meal, the eating style of 30-times chewing per bite increased the endogenous GLP-1 compared with usual eating, without affecting the concentrations of blood glucose or serum insulin. However, in type 2 diabetic patients, this eating style did not augment the GLP-1 secretion; this is unfortunate, as behavioral enhancement of endogenous GLP-1 secretion might have great benefit for type 2 diabetic patients [13].

Acknowledgements

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Declaration of Interest

The authors have no conflicts of interest to declare.

References


