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Morning Mastication Enhances Postprandial Glucose Metabolism in Healthy Young Subjects

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Postprandial glucose concentration is dependent on the time of day and its concentration in the morning is lower than in the evening. However, whether it is dependent on mastication at different times of the day has not been studied before. We hypothesized that mastication affects insulin-mediated glucose metabolism differently in the morning and evening in healthy individuals. Firstly, nine healthy male volunteers (22.0 ± 0.7 SEM years, body mass index 22.0 ± 1.0 kg/m^2) performed a 75-g oral glucose tolerance test (OGTT). One week after the OGTT, they participated in a high-carbohydrate food (rice) consumption test with 10 or 40 chews per mouthful. Each experiment was conducted in the morning (0800 h) and evening (2000 h) on the same day. Blood samples were collected before and at 30-min intervals for 120 min after glucose or rice consumption. The incremental area under the curve (iAUC) for glucose in the OGTT was significantly lower in the morning than in the evening, whereas the iAUC for insulin was similar at both times. In participants who chewed 40 times, the iAUC for glucose after rice consumption was significantly lower in the morning than in the evening but was similar at both times in individuals who chewed 10 times. Chewing 40 times in the morning (but not the evening) significantly increased insulin secretion at 30 min. This suggests that morning mastication improves early-phase insulin secretion after rice consumption. This novel finding may aid in reducing the incidence of obesity and type 2 diabetes mellitus.

Keywords: circadian rhythm; early-phase insulin secretion; morning mastication; postprandial glucose metabolism; type2 diabetes
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Introduction

Epidemiological studies associate mastication and meal duration with diabetes and obesity and body composition (Takayama et al. 2002; Maruyama et al. 2008; Ohkuma et al. 2013; Yamane et al. 2014). It is generally accepted that thorough chewing of food and slow eating have the potential to decrease the risk of diabetes and obesity.

Increased number of chew and/or thorough chewing has been reported to alter the secretion of hormones related to appetite and energy metabolism. Cassady et al. (2009) demonstrated that healthy participants who chewed test food (55 g of almonds) 40 times had lower postprandial glucagon-like peptide 1 (GLP1) levels than those who chewed 10 or 25 times. Li et al. (2011) reported that both lean and obese participants who chewed 40 times had lower energy intake and postprandial ghrelin levels and higher postprandial GLP1 and cholecystokinin levels than those who chewed 15 times. Zhu et al. (2014) found that mastication improved insulin-mediated glucose metabolism in older men; older participants who chewed test food 40 times had higher levels of plasma glucose, insulin, and glucose-dependent insulinotropic peptide at meal completion than those who chewed 15 times. According to other studies, mastication also influences postprandial satiety, appetite, and diet-induced thermogenesis (Hetherington and Boyland 2007; Hetherington and Regan 2011; Mattes and Considine 2013; Zhu and Hollis 2014; Hamada et al. 2014, 2016; Komai et al. 2016).

Regarding the circadian rhythm of glucose metabolism, glucose tolerance and insulin sensitivity are higher in the morning than in the evening, as shown in a study with healthy and prediabetic participants aged 15-19 years (Jarrett and Keen 1969). Glucose concentrations are higher

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in oral glucose tolerance tests (OGTTs) conducted in the afternoon and evening than in those conducted in the morning (Jarrett et al. 1972; Carroll and Nestel 1973), whereas insulin secretions are higher and increase more rapidly in the morning (Aparicio et al. 1974; Zimmet et al. 1974; Sonnier et al. 2014). Insulin secretions tend to be lowest in the afternoon and evening, with a delayed rise and late peak response, as is typically seen in diabetes (Aparicio et al. 1974; Zimmet et al. 1974; Sonnier et al. 2014). In addition, diurnal variations in glucose and insulin responses to a mixed meal are consistent with those in OGTTs (Ahmed et al. 1976; Nuttall et al. 1985).

To our knowledge, no previous studies have evaluated the effect of time-of-day-specific mastication on postprandial glucose metabolism in healthy participants. There are two possible hypotheses that mastication could increase insulin-mediated glucose metabolism. The first is that mastication enhances so-called preabsorptive insulin response within a few minutes of ingestion (Teff 2000), which the intraoral sensory stimulation from food elicits insulin secretion through the release of acetylcholine from the vagus nerve to the pancreas (Suzuki et al. 2005). The other is that mastication may increase insulin secretion after eating, since it has been reported that the amount of dietary induced thermogenesis is positively correlated with insulin secretion after eating (Rothwell and Stock 1981; Marques-Lopes et al. 2003). Therefore, the purpose of the present study was to assess if mastication differentially improves insulin-mediated glucose metabolism between morning and evening in healthy participants.

Participants and Methods

Participants

Nine healthy male participants were enrolled in this study as paid volunteers. All participants were recruited through advertising posters and website at Hokkaido University in Japan. All participants were in good physical condition with no personal history of psychiatric, endocrine, or sleep disorders. Their jobs did not require working early in the morning, late at night, or rotational night shifts. The Morningness-Eveningness questionnaire (MEQ) (Horne and Ostberg 1976) was used to identify individual chronotypes to confirm that participants were not extreme larks or owls. The Pittsburgh Sleep Quality Index (PSQI) (Doi et al. 2000) was used to assess sleep quality.

All participants gave written informed consent before entering the study; this allowed them to withdraw the study whenever they wished. The study protocol was approved by the ethical committee of the Hokkaido University Graduate School of Education (no. 17-40), and the study was conducted in accordance with the Declaration of Helsinki.

Experimental Protocol

All participants participated in a 75-g OGTT in the morning (0800 h) and evening (2000 h) of the same day (Fig. 1). One week after the OGTT, they participated in two high-carbohydrate food (white rice) tests with a crossover design. In these tests, they consumed a fixed amount of carbohydrate-rich food (white rice) in the morning (0800 h) and evening (2000 h) on the same day (Fig. 1). In the first test, they chewed the food 10 times; in the second (conducted a week later), they chewed the food 40 times. The order of two experiments was determined by using random allocation software GraphPad Software (GraphPad Software Inc., CA, USA).

One to 2 weeks before the first OGTT, the participants were instructed to maintain a regular sleep schedule, going to sleep between 2300 h and 0000 h and waking up between 0700 h and 0800 h. On the night before each test, the participants were asked to consume a standardized meal (total energy, 261 kcal; 85% carbohydrate; 11% protein; 4% fat) before 2100 h and to fast from 2100 h until 0800 h.

On the test day, the participants arrived at the laboratory before 0800 h. They rested for 10-20 min and within the next 10 min were administered either 75 g of liquid glucose (Ay Pharma Corp, Japan) or white rice (total energy, 370 kcal; 92% carbohydrate, 6% protein, 2% fat). Approximately 3 mL of blood was drawn before consumption (0 min) and 30, 60, 90, and 120 min thereafter for measurement of blood glucose concentrations and plasma levels. Following the 120-min blood sampling (1030 h), the participants ate a standardized meal (total energy, 579 kcal; 90% carbohydrate, 8% protein, 2% fat). They did not consume any additional food or liquids other than water until the evening test (2000 h), nor did perform any physical exercise

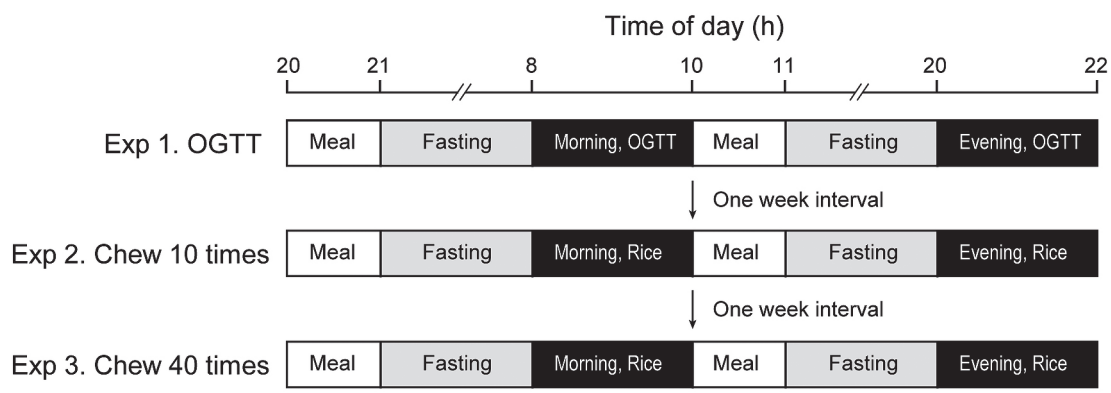

Fig. 1. Timelines for the OGTTs and high-carbohydrate food tests.
The latter had a crossover design: the participants chewed the food 10 times in the first test (Exp. 2) and 40 times in the second test (Exp. 3).
Exp., experiment; OGTT, oral glucose tolerance test.

during this interval. The evening test was performed in the same way as the morning test.

Measurements and Analytical Determinations

Approximately 3 mL of venous blood was collected through an indwelling intravenous catheter with a heparin lock placed in a forearm vein. The total blood glucose concentration was determined using a quinoprotein glucose dehydrogenase variant-based blood glucose monitoring system (ACCU-CHECK, Roche Diagnostics, Germany). The remaining blood was immediately centrifuged at 4°C (3,000 rpm, 15 min), and the plasma was frozen at −30°C for all other assays.

The plasma insulin secretion was determined using an ELISA kit (10-1113-01, Mercodia Insulin ELISA, Sweden) with a sensitivity of 6 pmol/L and intra- and inter-assay coefficients of variation of 5.9% and 2.0%, respectively. The total plasma GLP1 level was assayed using an ELISA kit (YK161, Total GLP1-HS ELISA Kit, Yanaihara Institute, Japan) with a sensitivity of 0.7 pmol/L and intra- and inter-assay coefficients of variation of 2.2% and 2.0%, respectively. All assays were performed according to the manufacturer's instructions. Plasma amylase activity was measured using a CicaLiquid-N AMY kit (Kanto Chemical, Japan); the substrate was 2-chloro-4-nitrophenyl-4-galactopyranosylmaltoside, and the measurement was performed in a commercial laboratory (SRL Sapporo, Japan).

Calculations and Statistical Analysis

The incremental areas under the curve (iAUCs) were calculated for the blood glucose concentrations and plasma insulin secretions during each 120-min test using the trapezoid method. To assess β-cell function, the insulinogenic index was calculated as follows: (insulin secretion at 30 min minus fasting insulin secretion) divided by the difference in blood glucose concentration at 30 min (Seltzer et al. 1967). All data are expressed as mean ± standard error of the mean (SEM).

To determine whether the effect of mastication on insulin-mediated glucose metabolism differed by time of day, we compared the data from the morning and evening tests. The differences between single values (iAUCs and insulinogenic indexes) in the morning and evening tests were analyzed using Student's *t* test. Repeated measures analysis of variance (ANOVA) with a post-hoc Bonferroni test was used for comparison of continuous variables. GraphPad Prism version 7 (GraphPad Software Inc., CA) was used for all statistical analyses. A p value < 0.05 was considered statistically significant.

Results

Participant Characteristics

Nine healthy men participated in the present study. All participants completed all tests. The mean age of the participants was 22.0 ± 0.7 years, and the mean body mass index was 22.0 ± 1.0 kg/m^2. The mean MEQ score across all participants was 54.6 ± 2.1, and the mean PSQI score was 2.8 ± 0.6. No participants were extreme larks (MEQ score > 70), extreme owls (MEQ score < 30) (Horne and Ostberg 1976), or poor sleepers (PSQI score > 5.5) (Doi et al. 2000).

Glucose Concentrations and Insulin Secretions in the Morning and Evening OGTTs

To assess whether or not glucose tolerance shows significant diurnal difference as reported previously (Jarret and Keen 1969; Jarret et al. 1972; Carroll and Nestel 1973), 75-g OGTTs were performed in the morning and evening. ANOVA revealed a significant time-of-day (morning vs. evening) effect on blood glucose concentration ($p < 0.001$), but not plasma insulin secretion ($p = 0.384$), in 75-g OGTTs. Mean fasting glucose concentrations (0 min) were higher (although not significantly) in the morning (87 ± 2 mg/dL) than in the evening (82 ± 2 mg/dL) (Fig. 2A). Conversely, non-fasting glucose concentrations were significantly lower in the morning than in the evening at 60 min (132 ± 8 vs. 156 ± 8 mg/dL, $p < 0.001$), 90 min (153 ± 7 vs. 125 ± 5 mg/dL, $p < 0.001$), and 120 min (130 ± 6 vs. 107 ± 3 mg/dL, $p < 0.001$).

The insulin secretion in the morning was significantly higher than that in the evening at 30 min ($p < 0.01$) (Fig. 2B). Although fasting and non-fasting blood glucose concentrations at 60, 90, and 120 min were statistically different between the morning and evening tests (Fig. 2A), no significant differences were observed between insulin secretions in the morning and evening at 60 min (70.4 ± 13.6 and 64.4 ± 17.9 mU/L), 90 min (74.8 ± 15.1 and 72.5 ± 22.3 mU/L), or 120 min (50.3 and 6.9 vs. 57.0 ± 9.8 mU/L).

The iAUC for glucose was significantly smaller in the morning than in the evening (4,285.0 ± 558.9 vs. 6,463.3 ± 611.9 mg h/dL; $p < 0.01$) (Fig. 2C). The iAUC for insulin was higher in the morning than in the evening (6,556.7 ± 1,116.0 and 5,686.7 ± 1,589.2 mg h/dL, respectively) (Fig. 2D), as was the insulinogenic index, a marker of early-phase β-cell function (1.32 ± 0.21 and 0.98 ± 0.32 mU/mg, respectively); however, neither difference was significant (both p-values = 0.25) (Fig. 2E).

Postprandial Glucose and Insulin Responses to High-carbohydrate Food Consumed in the Morning and Evening

Next, we evaluated the time-of-day effect of mastication on postprandial glucose and insulin responses to white rice. ANOVA showed significant differences between the time of day ($p < 0.05$) and glucose concentrations at various time points ($p < 0.01$) (Fig. 3A). However, the number of chews (10 vs. 40) had no effect on glucose levels, nor did the combination of time of day and number of chews ($p = 0.34$). Time of day ($p = 0.19$), number of chews ($p = 0.08$), and a combination of both variables ($p = 0.34$) did not have a significant effect on plasma insulin secretions (Fig. 3B).

The postprandial glucose levels were lower in participants who chewed 40 times in the morning vs. the evening at 60 min (106.9 ± 4.2 vs. 135.1 ± 7.3 mg/dL, $p < 0.01$), 90 min (104.1 ± 4.5 vs. 126.8 ± 6.9 mg/dL, $p < 0.05$), and 120 min (100.0 ± 3.1 vs. 118.3 ± 5.3 mg/dL, $p = 0.132$) even when the ANOVA test showed no significant differences (Fig. 3A). The iAUC for glucose was significantly lower in participants who chewed 40 times in the morning (2,548.3

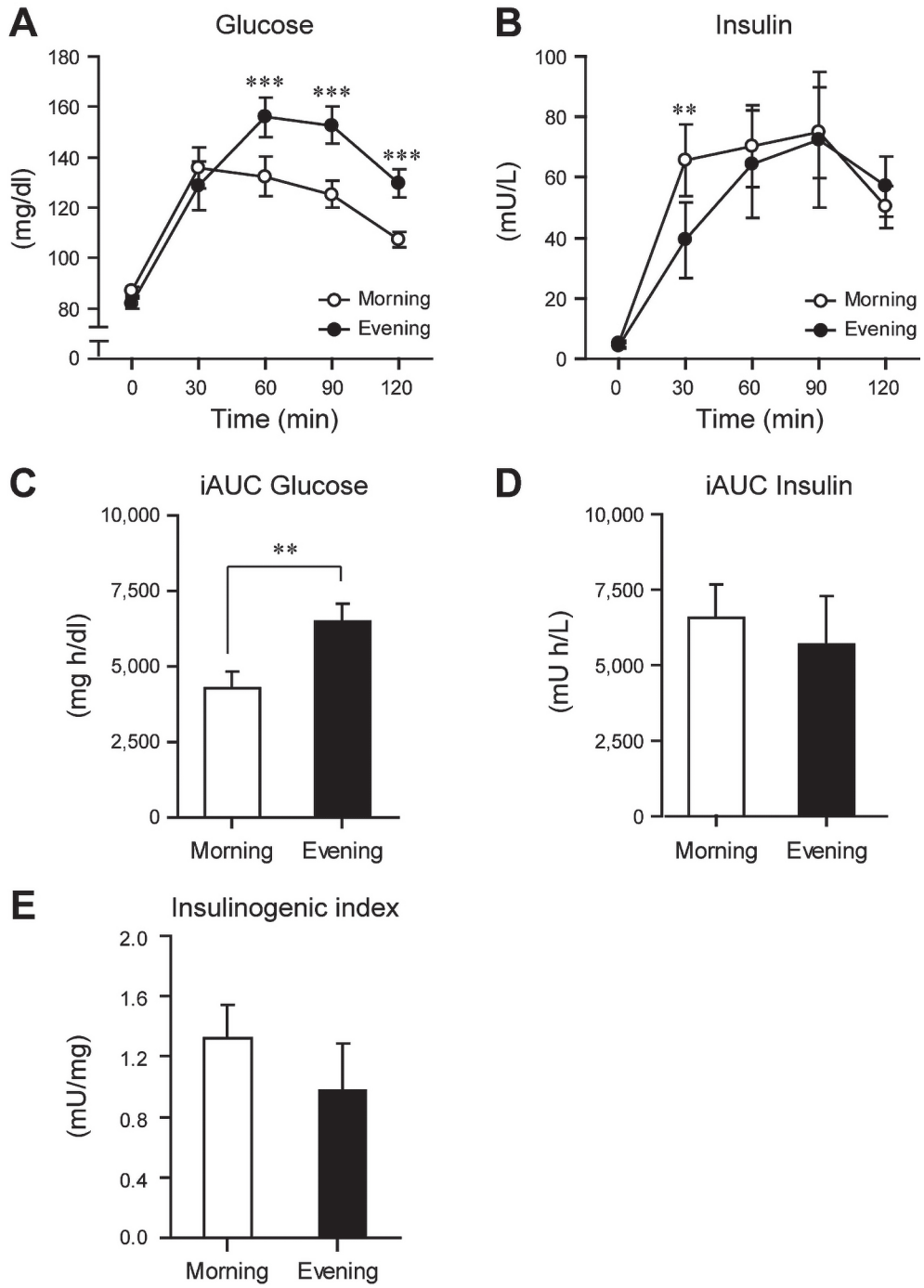

Fig. 2. Comparison of the results of the OGTTs in the morning and evening.
Glucose (A) and insulin (B), iAUCs for glucose (C) and insulin (D), and insulinogenic indices (E) are shown. The insulinogenic index [Δ insulin (0-30 min)/Δ glucose (0-30 min)] was calculated from the OGTT results in the morning and evening. Data are represented as mean ± SEM (n = 9) and were analyzed using two-way repeated measures ANOVA and post hoc Bonferroni test or the paired *t* test.
ANOVA, analysis of variance; iAUC, incremental area under the curve; OGTT, oral glucose tolerance test.
$^{**}p < 0.01$, $^{***}p < 0.001$; morning (0800 h) vs. evening (2000 h).

± 342.6 mg h/dL) than in those who chewed either 10 times (4,428.3 ± 607.2 mg h/dL; $p < 0.05$) or 40 times (4,435.0 ± 613.2 mg h/dL; $p < 0.01$) in the evening. It also tended to be lower in participants who chewed 40 times vs. 10 times in the morning (3,788.3 ± 522.5 mg h/dL) ($p = 0.06$) (Fig. 3C).

Insulin secretions at 30 min were significantly higher in participants who chewed 40 times in the morning (50.7 ± 4.0 mU/L) than in those who chewed either 40 times in the evening (34.4 ± 4.6 mU/L, $p < 0.05$) or 10 times in the

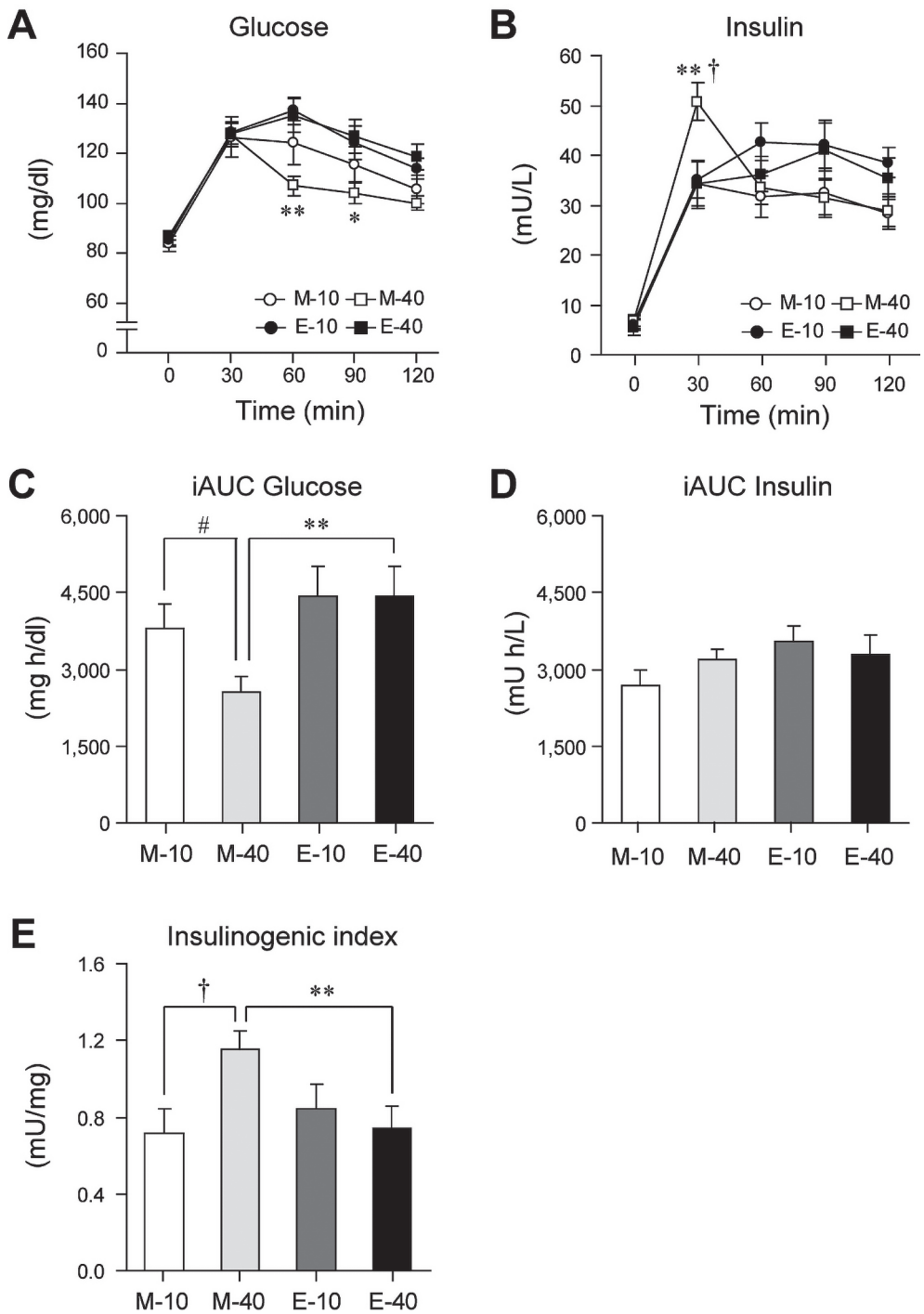

Fig. 3. Effect of mastication on glucose concentrations and insulin secretions following high-carbohydrate food consumption.

Shown are glucose (A), insulin (B), iAUCs for glucose (C) and insulin (D), and insulinogenic indices (E) in the morning and evening. The insulinogenic index [Δ insulin (0-30 min)/Δ glucose (0-30 min)] was calculated from the glucose concentrations and insulin secretions in each condition. Data are represented as mean ± SEM (n = 9) and were analyzed using two-way repeated measures ANOVA and post hoc Bonferroni test or the paired *t* test.

ANOVA, analysis of variance; E-10, 10 chews in the evening; E-40, 40 chews in the evening; iAUC, incremental area under the curve; M-10, 10 chews in the morning; M-40, 40 chews in the morning; SEM, standard error of the mean

$^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$; M-40 vs. E-40.

$^{\#}p = 0.06$, $^{\dagger}p < 0.05$; M-10 vs. M-40.

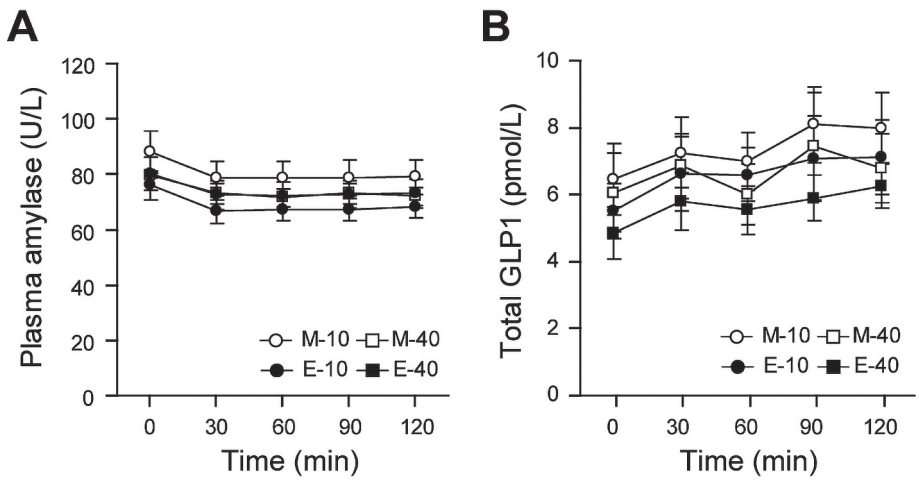

Fig. 4. Effect of mastication on total plasma amylase activity and GLP1 levels following high-carbohydrate food consumption.
Amylase activity (A) and GLP1 levels (B) levels in the morning and evening are shown. Data are represented as mean ± SEM (n = 9).
E-10, 10 chews in the evening; E-40, 40 chews in the evening; GLP1, glucagon-like peptide 1; M-10, 10 chews in the morning; M-40, 40 chews in the morning; SEM, standard error of the mean.

morning (34.2 ± 5.0 mU/L, $p < 0.05$); nevertheless, the overall ANOVA test was not significant (Fig. 3B). The iAUC for insulin did not differ among the four groups (Fig. 3D). The insulinogenic index was significantly higher in participants who chewed 40 times in the morning (1.15 ± 0.10 mU/L) than in those who chewed 10 times in the morning (0.72 ± 0.13 mU/L; $p < 0.05$) and those who chewed 40 times in the evening (0.74 ± 0.12 mU/L; $p < 0.01$) (Fig. 3E).

To determine how chewing 40 times in the morning might facilitate β-cell function, we measured plasma amylase activity (Fig. 4A) and total GLP1 levels (Fig. 4B). Mastication did not significantly affect either of these variables. Hence, the mechanism whereby morning mastication improves early β-cell function is not involved in amylase activity or GLP1 levels.

Discussion

Although previous studies (Hetherington and Boyland 2007; Cassady et al. 2009; Li et al. 2011; Hetherington and Regan 2011 ; Mattes and Considine 2013; Zhu and Hollis 2014; Zhu et al. 2014; Hamada et al. 2014, 2016; Komai et al. 2016) have described the effects of mastication on food intake, self-reported appetite (satiety), body composition, postprandial energy expenditure, and secretion of appetite-related hormones, little is known about the effects of mastication on glucose metabolism and insulin secretion at different times of day. We investigated diurnal variations in glucose metabolism by administering 75-g OGTTs in the morning and evening; the results of these tests indicated that glucose metabolism was significantly higher in the morning than in the evening (Fig. 2). This finding supports previous reports showing higher glucose tolerance in the morning than in the evening in healthy participants (Jarrett and Keen 1969; Jarrett et al. 1972; Carroll and Nestel 1973; Aparicio et al. 1974; Zimmet et al. 1974). The mechanism underlying diurnal variation in insulin sensitivity has been suggested to involve an increase in the levels of circulating free fatty acids in the evening (Yoshino et al. 2014).

Regarding glucose tolerance, previous studies of nocturnal rodents have shown that the central circadian clock in the suprachiasmatic nucleus (SCN) of the anterior hypothalamus is responsible for the 24-h rhythm in plasma glucose concentration (La Fleur et al. 1999). Interestingly, postprandial glucose concentrations and insulin secretions in rats are higher during the dark period (active phase) than during the light period (rest phase), but this distinction is lost in rats with lesions of the SCN rats (la Fleur et al. 2001). As demonstrated in these rodent studies, both the SCN circadian clock and the pancreatic clock play important roles in generating and maintaining the daily variations in glucose metabolism. In humans, the autonomous circadian clock system and desynchronization rhythm between the SCN-driven circadian rhythm (e.g., circadian rhythms of melatonin) and behavioral cycles have been reported to affect glucose metabolism (Van Cauter et al. 1991; Morgan et al. 1998; Morris et al. 2015). Therefore, the diurnal variation of glucose metabolism (e.g., glucose tolerance in OGTTs and postprandial glucose metabolism) in humans seems to be regulated by the SCN circadian clock.

Interestingly, our results show that morning mastication decreases postprandial blood glucose concentrations (Fig. 2A, C) and increases insulin secretion at 30 min (Fig. 3B) and insulinogenic index as a marker of early-phase β-cell function (Fig. 3E) following consumption of a high-carbohydrate meal. To the best of our knowledge, this represents the first evidence that the effects of mastication on early-phase insulin secretion depends on the time of day.

The results differed from our expectation that mastication could improve glucose metabolism with increasing insulin secretion both in morning and evening. It has been reported that mastication increased insulin secretion before and after food intake through an enhancement of the preabsorptive insulin response (Teff 2000) and an increase dietary induced thermogenesis (Marques-Lopes et al. 2003). Although previous studies, which examined the effect of mastication on metabolism (Zhu et al. 2014; Hamada et al. 2014, 2016) performed during in morning and afternoon not in the evening, could demonstrate that mastication increases postprandial glucose metabolism which is consistent with the present findings. However, further studies might be needed to clarify whether effects of mastication on the preabsorptive insulin response and dietary induced thermogenesis depend on time of day. In contrast to the present findings, Borvornparadorn et al. (2019) found that chewing 50 times in the morning did not affect postprandial glucose and insulin responses in healthy, lean, and overweight participants, relative to those chewing 15 times. The study cohort consisted of 21 men and 24 women, and the test meal comprised *ad libitum* ham and cheese sandwiches; these differences between that study and ours may have influenced the postprandial glucose and insulin responses (Frape et al. 1997; Robertson et al. 2002; Anderwald et al. 2011; Horie et al. 2018).

Mastication is the first step of mechanical digestion and promotes the mixing of food with salivary amylase. Previous studies showed that increased mastication raises the saliva flow rate, which positively correlates with salivary alpha-amylase activity (Mackie and Pangorn 1990). Mastication also increases the secretion of GLP1, a protein known to enhance glucose-stimulated insulin secretion (MacDonald et al. 2002). In the present study, mastication did not significantly affect either plasma amylase activity (Fig. 4A) or total GLP1 concentration (Fig. 4B); therefore, these factors are less likely to be the precise mechanism involved in morning mastication-enhanced early-phase insulin secretion.

As determined via the MEQ, all participants in our study had an intermediate chronotype. Further studies are needed to determine whether the effect of morning mastication on postprandial glucose metabolism is chronotype-dependent.

The present study is limited by its small cohort of participants and its restriction to non-obese and non-diabetic men. To fully understand its clinical implications, further studies are needed to determine whether morning mastication improves the poor early-phase insulin secretion in obese and/or diabetic patients (Matsumoto et al. 1997; Gerich 2002; Mizuno et al. 2007). In addition, we did not measure the participants' energy expenditure or levels of appetite-related hormones such as ghrelin, leptin, cholecystokinin, or peptide YY, which may have influenced our results. Future studies should be conducted to clarify the association between mastication at different times of day and enhanced early-phase insulin secretion.

In conclusion, the present study demonstrated that morning mastication decreases postprandial glucose concentrations following a high-carbohydrate meal and is associated with enhanced early-phase insulin secretion (increase the insulinogenic index). The effect of mastication on postprandial glucose metabolism was dependent on the time of day. Clinically, impairment of early-phase insulin secretion (decrease the insulinogenic index) is considered an early marker of β-cell dysfunction and the development of type 2 diabetes (Matsumoto et al. 1997; Gerich 2002; Mizuno et al. 2007); the present findings suggest that interventions to increase morning mastication may improve insulin-mediated glucose metabolism, reducing the incidence of obesity and type 2 diabetes.

Author Contributions

Y.Y. designed research; A.S., Y.Y., and Y.O. conducted research; A.S. and Y.Y. analyzed data; Y.Y. wrote paper. A.S., Y.Y., Y.O. had primary responsibility for final content.

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Conflict and Interest

The authors declare no conflict of interest.

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