Improvement in chewing activity reduces energy intake in one meal and modulates plasma gut hormone concentrations in obese and lean young Chinese men[1–3]

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ABSTRACT

Background: Mastication is the first step in ingesting food, but the effects of mastication on energy intake and gut hormones in both obese and lean subjects have not been extensively evaluated.

Objective: The current study aimed to compare the differences in chewing activities between obese and lean subjects and to examine the effects of chewing on energy intake and gut hormone concentrations in both obese and lean subjects.

Design: Sixteen lean and 14 obese young men participated in the current research. In study 1, we investigated whether the chewing factors of obese subjects were different from those of lean subjects. In study 2, we explored the effects of chewing on energy intake. A test meal consisting of 2200 kJ (68% of energy as carbohydrate, 21% of energy as fat, and 11% of energy as protein) was then consumed on 2 different sessions (15 chews and 40 chews per bite of 10 g of food) by each subject to assess the effects of chewing on plasma gut hormone concentrations.

Results: Compared with lean participants, obese participants had a higher ingestion rate and a lower number of chews per 1 g of food. However, obese participants had a bite size similar to that of lean subjects. Regardless of status, the subjects ingested 11.9% less after 40 chews than after 15 chews. Compared with 15 chews, 40 chews resulted in lower energy intake and postprandial ghrelin concentration and higher postprandial glucagon-like peptide 1 and cholecystokinin concentrations in both lean and obese subjects.

Conclusion: Interventions aimed at improving chewing activity could become a useful tool for combating obesity. This trial was registered at chictr.org as ChiCTR-OCC-10001181. *Am J Clin Nutr* 2011;94:709–16.

INTRODUCTION

Research indicates that some eating behaviors, such as eating quickly, gorging, and binge eating, have a substantial effect on being overweight (1, 2). However, as the first step in ingesting food, the effect of mastication on obesity has not been fully emphasized in these studies. The primary function of mastication is to reduce the particle size of foods before swallowing. Mastication facilitates the release of nutrients and other food constituents from the food matrix, which subsequently affects gut signaling, physical actions, and, ultimately, the digestive and absorptive processes (3). In addition, mastication stimulates salivation and enhances orosensory stimulation. Oral sensory exposure to food (4–6) and the texture of food (7–12) play important roles in food intake regulation. Some studies found that eating food faster and chewing less are associated with obesity (13, 14). However, other studies found that BMI has no effect on bite size, ingestion rate, or meal size (15, 16). Whether or not overeating among the obese can be ascribed to masticatory performance has not been well established.

Gut hormones play physiologic and pathophysiologic roles in regulating body weight and energy homeostasis and might represent potential useful targets for future obesity therapies. Ghrelin is the only known gut hormone to increase appetite through circulation. Circulating ghrelin concentrations increased after fasting and decrease after a meal. Ghrelin is considered to be involved in meal initiation (17). Moreover, oral stimulation can significantly affect circulating ghrelin concentrations (18–20). After a meal, CCK[4] (21) is released into the circulation from the small intestine and reduces food intake through cholecystokinin 1 receptors on the vagal nerve. Studies in rats suggest that the effect of CCK on satiation is enhanced by orosensory stimulation (22, 23). One study in humans reports that CCK is released after modified sham feeding (24). However, other studies have not confirmed the initial cephalic phase release of CCK (25–27). GLP-1 (28) is a potent incretin—central or peripheral administration potently stimulates insulin release. Exogenous GLP-1 strongly reduces food intake and inhibits appetite (29). Recently, one study has suggested that eating slowly increases the postprandial response of GLP-1 and peptide YY (30).

In the current study, we hypothesized the following: obese people chew less per food unit before swallowing than do lean people, improving mastication performance will reduce energy

intake in one meal, and plasma gut hormone concentrations will be modulated by chewing in obese and lean subjects.

SUBJECTS AND METHODS

Subjects

Using the criteria from the International Obesity Task Force (31) for Asians, 16 lean [BMI (in kg/m²) ≥18.5 and ≤23] and 14 obese (BMI ≥27.5) healthy young men (**Table 1**) were recruited from Heilongjiang Tourism Vocational and Technical College in Harbin City via posters on campus. All subjects were non-smokers and regular breakfast consumers. They had a full set of healthy teeth, had low dietary restraint (Three-Factor Eating Questionnaire restraint score ≤ 13) (32), had no allergies to any food, and had no endocrine or eating disorders. Their weights were stable (<3 kg change over the past 3 mo), and they were not taking medications likely to confound study outcomes. The subjects were not informed about the true purpose of the current study. Instead, they were told that the relation between masti-cation and blood triglyceride concentration was to be investigated. All participants gave their informed consent. All procedures were compliant with the Declaration of Helsinki, and the pro-tocol was approved by the ethical committee of Harbin Medical University.

Anthropometric measurements

We measured the subjects' height while wearing no footwear and weight while wearing light clothing before their breakfast and calculated their body mass index [BMI: weight (kg)/height squared (m)]. Waist and hip circumferences were measured to a precision of 0.1 cm, and the waist-to-hip ratio was calculated [waist (cm)/hip (cm)]. Percentage body fat was estimated by bioelectrical impedance analysis (OMRON HBF-306; Omron).

Study 1: differences in chewing activity between obese and lean subjects

The subjects arrived at the clinical research facility between 0700 and 0730 after a 12-h overnight fast and a 24-h period without exercise. The test food was pork pie, which was the subjects' usual breakfast food. The macronutrient composition of the test food consisted of 68% of energy from carbohydrate, 21% of energy from fat, and 11% of energy from protein. Every subject was presented with 300 g test food in a plastic dish. They were told that they could eat as much as they wanted and could

drink water ad libitum. If someone consumed >300 g, they could ask for more food. The subjects were asked to complete the breakfast between 0800 and 0830. The total weight of the con-sumed test food was then recorded.

A digital camera (HDR-CX550E; Sony) was positioned 3 m from the subject (face-on) to record their chewing activity. The video was viewed independently by 2 experimenters according to the same criterion. The average of the results obtained by these 2 experimenters was then calculated. The video-collected indica-tors were the pause duration between 2 bites (including water intake and excluding pause durations <2 s), the time per bite, and the number of bites, pauses, and chews. From these varia-bles, the following chewing kinematic variables were calculated: total MD from the start of the first bite to the end of the last bite; average bite size, which was determined by using the ratio of meal weight to bite number; average bite rate, which was ob-tained by dividing the bite number by the MD; chewing fre-quency, which was calculated as the ratio of chews to MD; and chews per gram food, which was obtained by using the ratio of chews to meal weight. The interobserver reliability was tested by using an ICC (33). The results showed that the average MD, bite size, bite rate, chewing frequency, and chews per gram food of the 2 experimenters were highly reliable (MD, ICC = 1.000; bite size, ICC = 0.954; bite rate, ICC = 0.960; chewing fre-quency, ICC = 0.959; chews per gram food, ICC = 0.927).

In another 2-d period, these participants were asked to chew as much as possible or as little as possible per bite. The results showed that the fewest number of chewing times per bite was ~15, and the largest number of chewing times per bite was ~40 for lean and obese subjects (**Table 2**). These data were used in study 2.

Study 2: effects of different chewing activities on energy intake and gut hormone concentrations in obese and lean subjects

The subjects came to the clinical research facility for 2 study sessions with 3 consecutive experimental days each. The effects of 15 chews and 40 chews per bite (these 2 conditions were chosen based on the data obtained in study 1; Table 2) on energy intake and gut hormones were researched in session 1 and session 2, respectively. There was a 1-wk washout between these 2 sessions.

To avoid the confounding effects of previous food consumption on gut hormones, the subjects were given a standard meal (2800 kJ; 60% of energy as carbohydrate, 22% of energy as fat, and 18% of energy as protein) to consume on the evening before each study

TABLE 1
Anthropometric measurements of the lean and obese subjects[1]

	Lean (n = 16)	Obese (n = 14)
Age (y)	20.8 ± 0.8	20.4 ± 0.7
Height (cm)	174.8 ± 5.2	176.5 ± 17.8
Weight (kg)	62.8 ± 8.0	94.1 ± 9.2*
BMI (kg/m²)	20.1 ± 2.0	30.1 ± 3.0*
Waist circumference (cm)	73.7 ± 5.6	99.6 ± 9.3*
Hip circumference (cm)	89.7 ± 4.4	107.1 ± 5.9*
Waist-to-hip ratio	0.82 ± 0.04	0.93 ± 0.04*
Body fat (%)	14.0 ± 4.4	27.9 ± 4.4*

[1] All values are means ± SDs. *Significantly different from lean sub-jects, P < 0.001.

TABLE 2
Eating behavior characteristics of the lean and obese subjects[1]

	Lean (n = 16)	Obese (n = 14)
Meal duration (s)	436 ± 131	526 ± 151
Bite size (g/bite)	9.3 ± 2.0	9.6 ± 1.9
Bite rate (bites/min)	3.8 ± 1.2	4.8 ± 0.7*
Chewing frequency (chews/min)	83.4 ± 14.7	87.7 ± 14.0
Chews (chews/g food)	2.3 ± 0.8	1.8 ± 0.4*
Energy intake in study 1 (kJ)	2822 ± 791	4212 ± 1083**
Fewest chews per bite	14.1 ± 4.0	13.6 ± 4.8
Largest chews per bite	44.7 ± 9.2	43.3 ± 7.8

[1] All values are means ± SDs. *,**Significantly different from lean subjects: *P < 0.05, **P < 0.001.

day at 1900 (34). The participants then fasted and did not consume anything else aside from water. On the following morning, the subjects arrived at the clinical research facility between 0700 and 0730 after a 12-h fast.

On day 1 of session 1, the preliminary experiment was carried out to acclimatize the subjects to the study protocol. Every subject was presented with 300 g test food in a plastic dish. The test food was divided into 10-g pieces (bite size of both obese and lean subjects was ~10 g in study 1; Table 2) with different shapes, one of which was consumed in one bite. All the subjects were asked to practice chewing 15 times per bite and to rate their hunger and satiety sensations using a VAS questionnaire described by Hill and Blundell (35). Each VAS was 100 mm in length, and the labels "not at all" and "extremely" were anchored at each end. The VASs were completed, and blood samples were then collected from the cubital vein before the breakfast and at intervals of 30, 60, 90, 120, and 180 min afterward. The subjects could eat as much as they wanted and could drink ad libitum. If someone consumed >300 g, they could ask for more food. They were asked to complete their breakfast within 30 min. The total weight of consumed test food was recorded. A digital camera was positioned in front of the subject to record their chewing activity. We then checked every subject's chewing activity by videotape after the experiment. If their compliance was satisfactory, they would win a 100-yuan reward. None of the data from this preliminary experiment were used in the statistical analysis.

On day 2 of session 1, the effect of 15 chews per bite on energy intake in one meal was examined. Every subject was presented with 300 g test food in 10-g pieces in a plastic dish. The subjects chewed the test food in 10-g pieces 15 times before swallowing and drank water ad libitum. They could eat as much as they wanted. If someone consumed >300 g, they could ask for more food. The breakfast was to be completed within 30 min. The total weight of consumed test food was then recorded. Video recording was used to confirm that they made the right number of chews per bite. The data for subjects with poor compliance were deleted.

On day 3 of session 1, the effect of 15 chews per bite on gut hormone concentrations was investigated. To avoid the confounding effects of different energy intakes on gut hormone concentrations, all the subjects were given 2200 kJ test food at 0 min. They were asked to eat one piece per bite, chew 15 times per bite, drink water ad libitum, and consume all given food within 30 min. The subjects could not ask for more food. VAS was measured and blood samples were collected at 0, 30, 60, 90, 120, and 180 min. Video recording was used to evaluate whether the subjects ate the test food according to the requirement. The data for subjects with poor compliance were deleted.

The study protocol of session 2 was the same as session 1, except for chewing times per bite. On day 1 of session 2, the preliminary experiment was carried out to acclimatize the subjects to chewing 40 times per bite. The effects of 40 chews per bite on energy intake and gut hormones were researched on days 2 and 3, respectively.

Preparation of plasma samples

Blood samples were collected into chilled tubes containing sodium EDTA2 and aprotinin (60 μL, 0.6 trypsin-inhibiting units/mLblood), which were then gently shaken several times. All blood samples were chilled in an ice bath until centrifugation at 1600 g for 15 min at 4°C. Plasma was collected and stored at −80°C until assayed.

Glucose and hormone assays

Plasma glucose concentrations were detected with the oxidase-peroxidase method (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). Plasma insulin, total ghrelin, CCK (26–33), and GLP-1(7–36) concentrations were measured by using an ELISA kit (Phoenix Pharmaceuticals). The minimum detectable concentrations of these assays were 0.78 μIU insulin/mL, 0.02 ng ghrelin/mL, 0.05 ng CCK/mL (26–33), and 0.14 ng GLP-1(7–36)/mL, respectively. The intraassay variation was 5–10%, and the interassay variation was 15% for all hormone assays.

Statistical analysis

All data were presented as means ± SDs. All statistical analyses were performed by using SPSS 16.0 (SPSS Inc), with $α = 0.05$. Chewing kinematic variables and the baseline data were analyzed by using Student's unpaired t test. Two-factor ANOVA [status (lean/obesity) × chewing (15 chews/40 chews)] was used to analyze energy intake data generated from day 2 of study 2. The AUC was calculated by using the trapezoidal rule to quantify overall response to chewing, which reflected both the amount and duration of the response. The time course of VAS and each postprandial hormone response was analyzed by 2-factor repeated-measures ANCOVA, with status and chewing as main effects and baseline variable as a covariate, followed by a Tukey-Kramer post hoc test. Differences in the postprandial response between chewing conditions and weight statuses were assessed via time × chewing and time × status interaction tests. The AUC was analyzed by 2-factor ANOVA (status × chewing). HOMA-IR was calculated according to the following equation: fasting insulin (μIU/mL) × fasting glucose (mmol/L)/22.5 (36).

RESULTS

Baseline assessment data

As shown in **Table 3**, fasting baseline appetite VAS, glucose, and hormone measures were equivalent across both study visits. Obese subjects had significantly higher fasting glucose and insulin concentrations and greater insulin resistance as assessed by HOMA-IR. Fasting ghrelin and GLP-1 concentrations were significantly lower in the obese than in the lean participants.

Differences in chewing activity between obese and lean subjects

Obese and lean subjects had similar bite sizes (≈10 g food per bite) and chewing frequency. On the basis of these data, the participants were presented the pie in 10-g portions in study 2. The number of chews per gram food was significantly less for obese than for lean subjects ($P = 0.031$). Correspondingly, the obese participants had a higher ingestion rate ($P = 0.035$) and energy intake ($P < 0.001$) than did the lean participants (Table 2). Moreover, the ingestion rate was negatively correlated with the number of chews per gram food in both lean ($r = 0.871$, $P < 0.001$) and obese ($r = 0.701$, $P = 0.001$) participants.

TABLE 3
Fasting appetite visual analog scale (VAS) and glucose and hormone concentrations across 2 study visits in the lean and obese subjects[1]

	15 Chews		40 Chews	
	Lean (n = 16)	Obese (n = 14)	Lean (n = 16)	Obese (n = 14)
Hunger VAS (mm)	66.5 ± 18.4	69.8 ± 17.4	64.7 ± 26.8	67.8 ± 17.0
Satiety VAS (mm)	31.8 ± 21.6	30.3 ± 18.9	31.6 ± 19.6	32.8 ± 19.2
Glucose (mmol/L)	5.3 ± 0.4	6.1 ± 0.7**	5.4 ± 0.4	6.1 ± 0.4**
Insulin (uIU/mL)	1.2 ± 0.3	5.0 ± 2.0**	1.1 ± 0.3	4.9 ± 2.3**
HOMA-IR	0.27 ± 0.16	1.21 ± 0.70**	0.24 ± 0.16	1.23 ± 0.74**
Ghrelin (pg/mL)	445.9 ± 137.2	304.3 ± 77.2*	450.2 ± 146.8	314.5 ± 90.8*
CCK (pg/mL)	256.3 ± 80.0	278.4 ± 87.7	263.1 ± 74.4	257.6 ± 71.2
GLP-1 (pg/mL)	1599.9 ± 254.0	1278.6 ± 219.3**	1678.9 ± 281.4	1231.6 ± 205.6**

[1]All values are means ± SDs. CCK, cholecystokinin; GLP-1, glucagon-like peptide 1; HOMA-IR, homeostasis model assessment of insulin resistance. There was no significant interaction between status and chewing on fasting appetite VAS or on glucose and hormone concentrations. *,**Significantly different from lean subjects: *$P < 0.05$, **$P < 0.01$.

Effect of chewing on energy intake in one meal

Main effects of status (P = 0.008) and chewing (P = 0.021) were observed, but no significant interaction between status and chewing (P = 0.451) on energy intake in one meal was observed. Regardless of status, the subjects' energy intake was 11.9% lower after 40 chews than after 15 chews (mean ± SD: 2614.7 ± 511.6 compared with 2304.4 ± 490.4 kJ; P = 0.034; **Figure 1**).

Effects of chewing on appetite and on glucose and hormone concentrations

Appetite ratings

No significant effects of status and chewing on the subjective hunger and satiety VAS appetite measures were found (**Figure 2**, A and B).

Glucose concentration

Among all participants, glucose concentrations increased shortly after ingestion of a test meal and then decreased. Significant main effects of status on plasma glucose concentrations were found (P = 0.001). However, neither lean nor obese participants differed significantly in the glucose concentrations between 15 chews and 40 chews at each time point (**Figure 3**A).

Plasma insulin concentration

Plasma insulin concentrations showed a postprandial increase and decrease. Significant main effects of status on plasma insulin concentrations were found ($P < 0.001$). However, neither lean nor obese participants differed significantly in plasma insulin concentrations between 15 chews and 40 chews at each time point (Figure 3B).

Plasma ghrelin concentration

Regardless of status and chewing, postprandial ghrelin concentrations decreased shortly after ingestion of a test meal and increased thereafter. Plasma ghrelin concentrations were significantly lower after 40 chews than after 15 chews at 60 (P = 0.037) and 90 (P = 0.038) min in lean subjects and at 90 (P = 0.041) min in obese subjects (**Figure 4**A). Significant effects of status ($P < 0.001$) and chewing (P = 0.034) and a significant interaction (P = 0.027) between status and chewing on the AUC for plasma ghrelin concentrations were found. The AUC was lower after 40 chews than after 15 chews in lean (mean ± SD: 53580.8 ± 10895.6 compared with 60285.1 ± 12204.3 pg/mL · min; P = 0.027) and obese (mean ± SD: 41627.9 ± 7753.9 compared with 43600.1 ± 8962.1 pg/mL · min; P = 0.122) participants (Figure 4B).

Plasma GLP-1 concentration

Among all participants, plasma GLP-1 concentrations increased shortly after ingestion of a test meal, reaching a peak at 30 min, and then decreased. Plasma GLP-1 concentrations were higher after 40 chews than after 15 chews at 30 (P = 0.013), 60 (P = 0.012), 90 (P = 0.008), and 120 (P = 0.005) min in lean participants and at 60 (P = 0.025) and 90 (P = 0.037) min in obese subjects (**Figure 5**A). Effects of status ($P < 0.001$) and chewing (P = 0.010), but no significant interaction (P = 0.422) between status and chewing, on the AUC for plasma GLP-1 concentrations was found. Regardless of status, the incremental AUC of GLP-1 was higher after 40 chews than after 15 chews (mean ± SD: 345,041.5 ± 60,485.1 compared with 293,718.4 ± 47,035.2 pg/mL · min; P = 0.014) (Figure 5B).
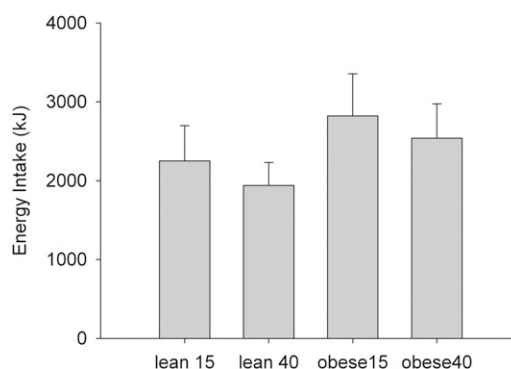

FIGURE 1. Mean (±SD) energy intake after 15 and 40 chews in lean (n = 16) and obese (n = 14) subjects. A 2-factor ANOVA showed significant main effects of status (P = 0.008) and chewing (P = 0.021) but no significant status × chewing interaction (P = 0.451) on energy intake.
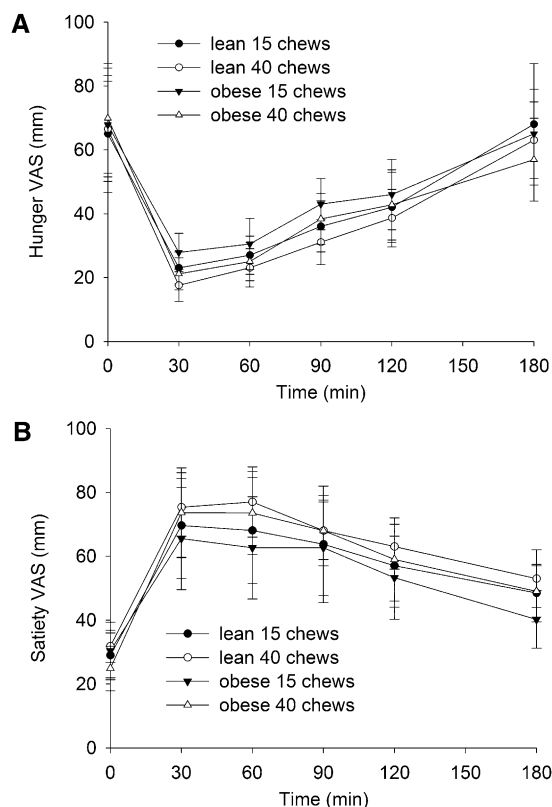
FIGURE 2. Mean (±SD) postprandial hunger (A) and satiety (B) ratings on a visual analog scale (VAS) after 15 and 40 chews in lean ($n = 16$) and obese ($n = 14$) subjects. Repeated-measures ANCOVA showed no significant main effects of status and chewing or a time × status × chewing interaction on hunger (status, $P = 0.751$; chewing, $P = 0.104$; interaction, $P = 0.680$) and satiety (status, $P = 0.702$; chewing, $P = 0.101$; interaction, $P = 0.465$).

FIGURE 3. Mean (±SD) postprandial plasma glucose (A) and insulin (B) concentrations after 15 and 40 chews in lean ($n = 16$) and obese ($n = 14$) subjects. Repeated-measures ANCOVA showed significant main effects of status but no significant chewing and time × status × chewing interaction on plasma glucose (status, $P = 0.001$; chewing, $P = 0.221$; interaction, $P = 0.160$) and insulin (status, $P < 0.001$; chewing, $P = 0.411$; interaction, $P = 0.345$) concentrations.

Plasma CCK concentration

Among all participants, plasma CCK concentrations increased shortly after ingestion of a test meal, reaching a peak at 30 min, and then decreased. Plasma CCK concentrations were higher after 40 chews than after 15 chews at 30 ($P = 0.011$) and 60 ($P = 0.021$) min in lean participants and at 30 ($P = 0.001$), 60 ($P < 0.001$), 90 ($P = 0.010$), and 180 ($P = 0.012$) min in obese participants (**Figure 6**A). A significant main effect of chewing ($P = 0.013$), but no significant effect of status ($P = 0.032$) and interaction ($P = 0.307$) between status and chewing, on the AUC for plasma CCK concentrations was found. Regardless of status, the incremental AUC of CCK was higher after 40 chews than after 15 chews (mean ± SD: 67702.5 ± 9253.1 compared with 59447.1 ± 8347.2 pg/mL · min; $P = 0.013$) (Figure 6B).

DISCUSSION

To our knowledge, this was the first study to compare the differences in chewing activity between obese and lean young Chinese subjects and then assess the effects of chewing on energy intake and gut hormone concentrations in the same participants. A few studies have compared chewing activity in obese and lean subjects (13–16), but the results are conflicting. Some studies report that obese people eat food faster and chew less than do lean subjects (13, 14). However, other studies showed that obese subjects do not chew food less, do not chew faster, and do not eat
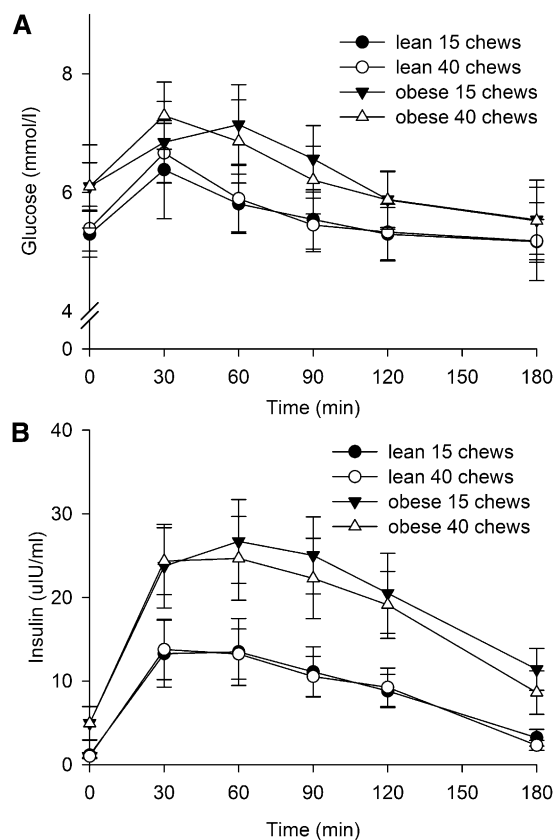
more than lean subjects (15, 16). The different results may have been affected by the palatability of the test food, which is important in dictating several aspects of chewing behavior. The preference for a food correlates positively with ingestion rate (37), and increased palatability is associated with a reduced number of chews per gram food and increased meal size (13, 38). To closely resemble the nature of mastication, the participants' usual breakfast was used as the test food to compare the differences in chewing activity between obese and lean subjects in the current study.

The results showed that obese participants chewed less and ingested more quickly than did lean participants. Moreover, the ingestion rate strongly correlated with the number of chews per gram food in obese and lean participants. However, obese and lean participants had similar bite size and chewing frequency. Common sense suggests that chewing less should result in a quicker ingestion rate, but not vice versa. Additionally, some surveys indicate that the rate of eating is positively associated with body weight (1, 2). According to the above results, mastication may underlie this association, which needs to be further confirmed by epidemiologic surveys. The current research then examined the effects of mastication on energy intake and gut hormones in obese and lean subjects.

Several studies suggest that cephalic responses play a key role in homeostasis and in preparation of the gastrointestinal tract for
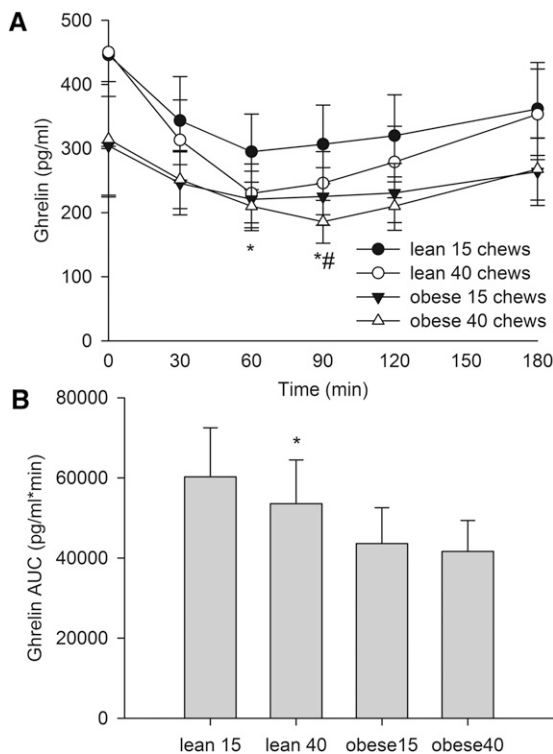
FIGURE 4. Mean ($\pm$SD) postprandial plasma ghrelin concentrations (A) and ghrelin AUC (B) over 180 min after 15 and 40 chews in lean ($n = 16$) and obese ($n = 14$) subjects. Repeated-measures ANCOVA showed significant main effects of time ($P < 0.001$), status ($P < 0.001$), and chewing ($P = 0.029$) and a time $\times$ status $\times$ chewing ($P = 0.006$) interaction on ghrelin concentrations (A). A 2-factor ANOVA showed significant main effects of status ($P < 0.001$) and chewing ($P = 0.034$) and a status $\times$ chewing interaction ($P = 0.027$) on ghrelin AUC (B). *Significantly different from corresponding 15 chews in lean subjects, $P < 0.05$. #Significantly different from corresponding 15 chews in obese subjects, $P < 0.05$.
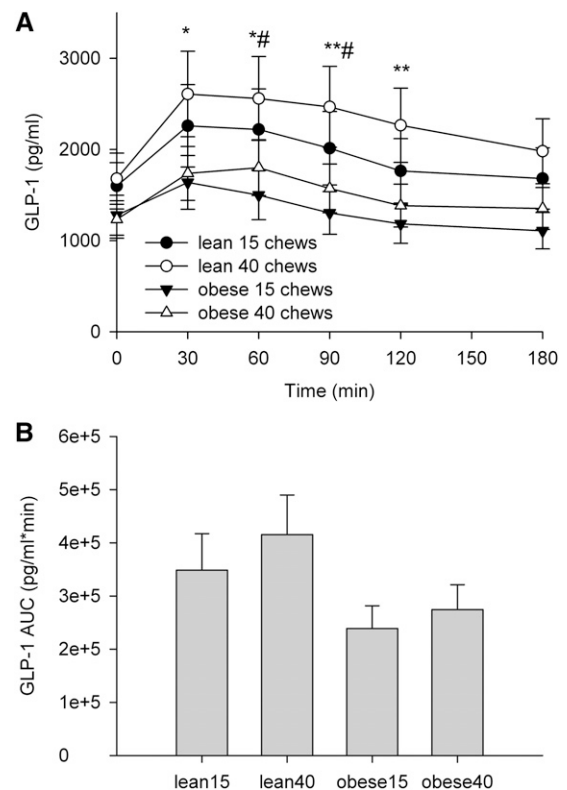

FIGURE 5. Mean ($\pm$SD) postprandial plasma glucagon-like peptide 1 (GLP-1) concentrations (A) and GLP-1 AUC (B) over 180 min after 15 and 40 chews in lean ($n = 16$) and obese ($n = 14$) subjects. Repeated-measures ANCOVA showed significant main effects of time ($P < 0.001$), status ($P < 0.001$), and chewing ($P = 0.001$) and a time $\times$ status $\times$ chewing ($P = 0.001$) interaction on GLP-1 concentrations. *,**Significantly different from corresponding 15 chews in lean subjects: *$P < 0.05$, **$P < 0.01$. #Significantly different from corresponding 15 chews in obese subjects, $P < 0.05$ (A). A 2-factor ANOVA showed significant main effects of status ($P < 0.001$) and chewing ($P = 0.010$) but no significant status $\times$ chewing interaction ($P = 0.422$) on GLP-1 AUC (B).

optimal digestion and absorption of nutrients (39). Food texture and mastication can regulate salivary secretion and affect oro-sensory stimulation. Some studies found that semisolid and solid food result in smaller bite sizes, lower intakes, and stronger appetite sensations than do matched liquids (7–9). In addition, Zijlstra et al (6) report that greater oral sensory exposure to food significantly decreases food intake. Hetherington and Boyland (4) contend that chewing gum for 15 min/h suppresses appetite, particularly for sweets, and reduces energy intake from snacks. However, Julis and Mattes (40) point out that chewing gum for 20 min has no effect on the ratings of appetite and food intake. In the current study, we found that regardless of status, participants ate less after 40 chews than after 15 chews. This finding is consistent with 2 previous studies, which indicate that decreasing bite size or increasing duration of oral processing enhances satiation and accelerate meal termination (41) and that slowing down the speed of eating and reducing the portion size contribute to weight loss (42). How does mastication affect energy intake?

Research has shown that gut hormones play key roles in energy homeostasis regulation. Chewing is an important stimulus of cephalic phase responses (43, 44), and sensory stimulation may promote the release of numerous appetitive hormones such as ghrelin, GLP-1, and CCK. Some studies suggest that sham feeding results in a decrease in plasma ghrelin concentrations (18, 20) and

exaggerates the inhibitory ghrelin response to oral fat in humans (45). However, another study reports that plasma ghrelin concentrations increase with sham feeding (19). Moreover, several studies found that effect of CCK on satiation is enhanced by orosensory stimulation in rats(22, 23) and that modified sham feeding enhances CCK release in conscious dogs (46) and humans (24). However, other studies have not confirmed the initial cephalic phase release of CCK (25–27). Recently, one study suggested that eating slowly increases the postprandial response of GLP-1 and peptide YY (30).

In the current study, postprandial plasma ghrelin concentrations were lower after 40 chews than after 15 chews in both lean and obese participants. Correspondingly, GLP-1 and CCK concentrations after 40 chews increased more than after 15 chews in both lean and obese subjects. However, the ghrelin and GLP-1 postprandial responses to changes in mastication were blunted in obese participants relative to the responses in lean participants. Mastication apparently plays a role in the gut hormone profile, which consequently influences energy intake.

Increased chewing could release nutrients from food more efficiently, which subsequently affects gut signaling, physical actions, and ultimately digestive and absorptive processes. One study indicates that chewing almonds 40 times resulted in higher
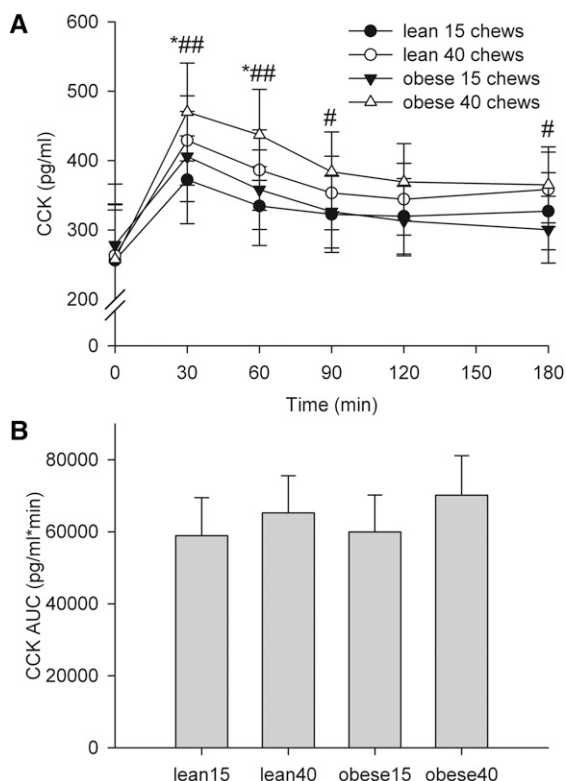
FIGURE 6. Mean (±SD) postprandial plasma cholecystokinin (CCK) concentrations (A) and CCK AUC (B) over 180 min after 15 and 40 chews in lean ($n = 16$) and obese ($n = 14$) subjects. Repeated-measures ANCOVA showed significant main effects of time ($P < 0.001$), status ($P < 0.001$), and chewing ($P < 0.001$) and a time × status × chewing ($P = 0.015$) interaction on CCK concentrations. *Significantly different from corresponding 15 chews in lean subjects, $P < 0.05$. #,##Significantly different from corresponding 15 chews in obese subjects: #$P < 0.05$, ##$P < 0.01$ (A). A 2-factor ANOVA showed a significant main effect of chewing ($P = 0.013$) and status ($P = 0.032$) but no significant status × chewing interaction ($P = 0.307$) on CCK AUC (B).

lipid bioaccessibility and postingestive GLP-1 concentrations than did chewing almonds 10 and 25 times (47). Furthermore, mastication in rats enhanced satiation by activating histamine neurons of the ventromedial hypothalamus and paraventricular nucleus (48). The vagally mediated cephalic phase, nutrient bioaccessibility, and histamine neurons in the hypothalamus seemingly played an important role in the modulation of gut hormones secretion under different chewing scenarios in the current study.

However, glucose and insulin concentrations were not influenced by chewing, which coincides with the findings of another study (30). Healthy young people are able to deal with a moderate glycemic load. The effect of mastication on glucose and insulin can be offset by other homeostatic regulatory mechanisms.

A possible limitation of the present study was that both lean and obese participants were provided with the same quantity of test meal rather than a personalized one. The appetite VAS ratings were not different between obese and lean participants, but we cannot exclude the possibility that either underfeeding in the obese or overfeeding in the lean may have influenced the outcomes of the current study to some degree. Notably, this study used a self-control design to compare the differences in glucose and hormones in both lean and obese participants after 15 and 40

chews. Thus, the confounding effects of potential differences in appetite response on the same amount of energy between obese and lean subjects were omitted by this design.

In summary, our study showed that chewing less is a risk factor for obesity. Increased chewing decreases energy intake in one meal, which is mediated partly by the modulations of plasma ghrelin, GLP-1, and CCK concentrations. Interventions for improving chewing activity could become a valuable adjunctive tool for combating obesity.

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