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## EFFECT OF CHEWING TIME OF HIGH-PROTEIN MEAL ON SUBJECTIVE SATIETY AND SERUM GHRELIN RESPONSE IN HEALTHY INDIVIDUALS

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### Abstract

Satiety and ghrelin response are influenced by the chewing rate and the macronutrient composition of food. Protein rich foods have been shown to enhance satiety and lead to a prolonged decline in post-prandial ghrelin concentrations. Chewing food slowly may help people feel fuller, however, its effect on hormones remains unclear. Therefore, aim of this study was to assess the effect of chewing time of high-protein meal on satiety and serum ghrelin. In a cross-over study, 22 healthy human participants consumed a high-protein meal with three different chewing rates i.e., normal, fast and slow. Satiety scores and serum ghrelin levels were assessed pre- and post-prandially to determine the effect of chewing time of food on their responses. Satiety scoring was done using visual analogue scale (VAS) for satiety, while serum ghrelin levels were assessed using commercially available ELISA kits. Results show that slow chewing of food significantly decreased feeling of hunger at 90 minutes ( $P = 0.02$ ) and 120 minutes ( $P = 0.02$ ). Prospective food intake decreased significantly at 90 minutes ( $P = 0.01$ ) and 120 minutes ( $P = 0.02$ ) following slow chewing. Moreover, slow chewing significantly increased feeling of fullness at 90 and 120 minutes ( $P = 0.05$ ). Similarly, satiety increased significantly after slow chewing at 60 ( $P = 0.03$ ), 90 ( $P = 0.02$ ) and 120 minutes ( $P = 0.05$ ). No effect of chewing time was seen on ghrelin response. Furthermore, ghrelin was not correlated with satiety sensations or chewing time. In conclusion, increasing the chewing time of a high-protein meal significantly increased self-reported feelings of satiety and fullness and, decreased hunger and prospective food consumption. However, chewing time was not associated with change in ghrelin concentrations. The trial is registered at [ClinicalTrials.gov](https://clinicaltrials.gov) under registration ID: NCT05822167.

**Keywords:** Chewing time, Food intake, Ghrelin, Hunger, Satiety, Mastication

## INTRODUCTION

A key strategy for maintaining normal weight is to control food intake, which is strongly influenced by appetite and satiety. Food intake is initiated by appetite, which is a psychological drive to obtain food i.e., energy, that arises from the body's need for energy (1). Whereas, the feeling of fullness that follows the food consumption is called satiety, which is a post-prandial homeostatic sensation (2). Food consumption is followed by a decrease in appetite and an increase in satiety, respectively (3).

The cycle of appetite and satiety is controlled by complex hormonal interactions between the gastrointestinal tract and the hypothalamus. These interactions either lead to food consumption or feeling of fullness (4). Ghrelin is an orexigenic peptide hormone secreted by the cells of gastric mucosa which increases appetite and food consumption. Plasma ghrelin levels show a diurnal pattern, which increases in the pre-



prandial state and declining in the post-prandial period (5-8). In fasting state, ghrelin levels increases in people with anorexia and decreases in people with obesity (8). Previous studies indicated that post-prandial ghrelin levels depend on the macronutrient composition of the meal ingested, with levels decreasing after consumption of fats and carbohydrate rich meals while levels can increase or decrease after meals high in protein (9-11). Khoury et al, reported that a high protein meal produced the most prolonged decline in plasma concentration of ghrelin (12).

To prevent obesity and its multitude comorbidities, different management and preventive strategies can be employed such as lifestyle modification, pharmacological therapies or bariatric surgeries. However, controlling dietary intake by decreasing appetite or increasing satiety can greatly reduce obesity and the recurrent weight gain among people with obesity.

The macro- and micronutrient content, chewing time, texture, presentation, taste, and odor of food all affect satiety (13). High-protein foods are known to increase satiety (14). Similarly, the duration of oral exposure enhances satiety, due to increased neurological or endocrine mechanisms brought on by chewing of food. When chewing time is increased, the chews per bite are increased whereas chewing rate, which represents chews per minute, decreases. Increased number of chews can lead to decreased food intake, while it has been shown that decreased chewing rate can increase energy expenditure per each masticatory cycle (14, 15). Therefore, increasing chewing activity can reduce food intake because longer oral processing times and more chewing are linked to enhanced satiation (2, 16, 17).

Studies that have investigated the effect of chewing time on plasma ghrelin concentrations report contradictory results. Increasing the chewing time or masticatory cycles has shown to decrease post-prandial concentrations in some studies, while others found no effect of chewing on ghrelin concentrations (17, 18). However, most of the studies that have examined the effect of chewing time on satiety and ghrelin concentrations have used high-carbohydrate and high-fat meals (19-21). Khoury et al. assessed the effect of macronutrient composition of meals on serum ghrelin and reported that a high-protein meal produces the most prolonged decline in post-prandial ghrelin concentrations (12). However, the effect of chewing time of high protein diet on ghrelin response is yet elucidated. Therefore, the aim of this study was to assess whether the increasing the chewing time of a high-protein meal would increase satiety and/or decrease serum ghrelin concentrations.

## MATERIALS AND METHODS

### STUDY DESIGN AND PROTOCOL

This was a cross-over intervention study conducted at Khyber Medical University, Peshawar, Pakistan. The sample size for the study was calculated using OpenEpi software. Twenty-two healthy individuals between the ages of 25 to 40 years with healthy Body Mass Index (BMI) ranged 18.5 to 24.9 kg/m<sup>2</sup> were recruited in this study using convenience sampling. Individuals with dental, metabolic or gastric ailments were excluded from the study sample. The ethical approval for the study was taken from Institutional Ethical Review Board under KMU/IBMS/IRBE/6<sup>th</sup>-meeting/2023/9960-B of Khyber Medical University. The trial was registered at ClinicalTrials.gov under registration ID: NCT05822167. Those who were willing to participate in the study were screened using a health screening proforma and only those who met the inclusion criteria were recruited. A written informed consent was taken from all participants prior to the start of data collection.

The participants attended three visits. On the first visit, a stopwatch was used to calculate the chewing time of the study participants. It was started as soon as the participant picked up his/her utensils to initiate food intake. Video recording using a smartphone and surface electromyography (EMG) using a Biopac machine was also done during this time. For EMG, the module EMG100C and EMG100C-MRI was selected. The stopwatch and EMG recording were stopped as soon as the participant swallowed the last bolus. The total duration of this was noted as the normal chewing time. The normal chewing time was halved (fast chewing) on the second visit and doubled (slow chewing) on the third visit. On both the second and the third visit, video recording and EMG were performed in the same manner as they were on the first

visit. The three visits in the study were separated by a washout period of one-week. This was done to give time for the injection prick sites to heal and to decrease any biases that could arise due to the previous visit. On each day of the trial, participants were asked to report to the Physiology laboratory of the Khyber Medical University after a minimum fasting time of 10 to 12 hours.

Fasting blood samples were collected for analysis of serum ghrelin and appetite was measured using Visual Analogue Scale (VAS) to assess feelings of hunger, fullness, satiety and prospective food intake. After baseline data collection, the high-protein meal was served to participants. During food consumption, video recordings were made using a smartphone and surface electromyography was done using Biopac Systems, Inc MP160. This started as soon as the participants started eating their food and stopped after the last bolus was swallowed. After food consumption, blood samples were collected at time point 30, 60 and 120 minutes for measurement of post-prandial ghrelin levels, whereas satiety was measured after every 30 minutes using VAS. The time points for measurements of serum ghrelin were chosen on the basis of previous literature which demonstrated that ghrelin levels typically decline within first hour after meal intake and returns to baseline within 2-3 hours post-prandially (7, 22).

## STUDY MEAL

All the participants consumed a high-protein meal of 500 kcal at each session. The high-protein meal comprised of grilled chicken, sautéed vegetables, boiled potato and a boiled egg was served to the study participants. The meal provided 75 g of protein, 16.5 g of fats and 12.5 g of carbohydrates, which accounted for 60%, 30% and 10% of the total calories of the meal, respectively (Table I).

**Table I.** Test meal served to study participants

Food	Amount	Calories (Kcal)	Carbohydrates (g)	Fats (g)	Proteins (g)	Moisture (%)
Grilled chicken	8 oz	375	-	8	65	154
Egg	1 Egg	70	-	5	6	32
Boiled potato	25 g	45	5	-	-	32
Sautéed vegetables	½ cup	60	5	2.5	3	57
		550	10	15.5	79	
Total		500 kcal	12.5g (10%)	16.5g (30%)	75g (60%)	275

## MEASUREMENT OF SUBJECTIVE SATIETY

Subjective satiety was measured using 100 mm visual analogue scale (VAS) for satiety, fullness, hunger and prospective food intake (23). Each question had a 100 mm line underneath it with opposing statements as anchors on each side. The phrase representing absence of the feeling was at point 0 mm and the other representing the extreme of it was at point 100 mm. Hunger was assessed by asking the question "How hungry are you?" with the anchors of "not hungry at all" and "very hungry". The feeling of fullness was measured by asking the question "How full do you feel?" which had anchors of "not full at all" and "very full". To assess satiety, participants were asked "How satisfied do you feel?" which had "completely empty" and "I cannot eat more" as its anchors. The last question in the VAS was about prospective food intake for which participants were asked "How much do you think you can eat now" followed by "nothing at all" and "a lot" on either side of the 100mm line. Each of the participants marked their perception of satiety by drawing a vertical line on 100 mm line at fasting and postprandially at time point 30, 60, 90 and 120 minutes. Satiety scores were then calculated by measuring the distance of the participant's mark from point 0 using a calibrated one-foot scale. The participants were asked to refrain to discuss their ratings with each other.

## BLOOD COLLECTION FOR SERUM GHRELIN ASSAY

3ml blood was collected from the veins of the cubital fossa in the fasting state and at 30, 60 and 120 minutes post-prandially. The blood samples were transferred to gel tubes and centrifuged at 4°C at 2000

rpm for 15 minutes for separation of serum. For ghrelin analysis, commercially available Enzyme-linked Immunosorbent Assay (ELISA) kits with catalogue numbers of Cat.No.E3091Hu were ordered from Bioassay Technology Laboratory. The intra-assay co-efficient of variance (CV) of the kits was < 8%, whereas the inter-assay CV was < 10%. The optical density (OD) of the samples was read at 450 nm and the results were expressed in ng/ml.

## STATISTICAL ANALYSIS

The data was imported into IBM SPSS Statistics Version 22 and analyzed. Normality of data was assessed using Shapiro-Wilk test. The distribution of most variables i.e., age, satiety measures and ghrelin concentration was not normal, therefore the satiety scores and ghrelin levels were presented as median (IQR) and non-parametric tests were used to analyze the data. Friedman ANOVA was used to compare satiety scores and serum ghrelin levels at different time points, whereas Kruskal Wallis ANOVA with post-hoc Mann-Whitney U test was used to assess differences in satiety and serum ghrelin after normal, fast and slow chewing. Spearman correlation was used to find the association of satiety with ghrelin.  $P \leq 0.05$  was considered statistically significant.

## RESULTS

The results are expressed as median (IQR). The age of study participants was 26 (25 – 27) years. The weight of participants was 65 (54.83 – 72) kg and height was 1.69 (1.61 - 1.73), whereas their BMI was 22.75 (21.25 - 24.78) kg/m<sup>2</sup>.

## CHEWING TIME AND RELATED PARAMETERS

Chewing time, total number of chews, total number of bites and the chews per bite are shown in Table II. The chewing time was statistically significantly different on all three rates of chewing ( $P < 0.001$ ). The total number of bites was significantly lower after fast chewing while there was no difference in the number of bites during normal and slow chewing ( $P = 0.004$ ). The number of chews was highest on slow chewing and least during fast chewing. This difference was statistically significant ( $P < 0.001$ ). The chews per bite were significantly different on each visit. It was highest for slow chewing i.e. 30.23 (18.57 - 51.11), followed by 19.23 (14.75 - 24.68) for normal chewing and 14.58 (11.28 - 20.53) for fast chewing.

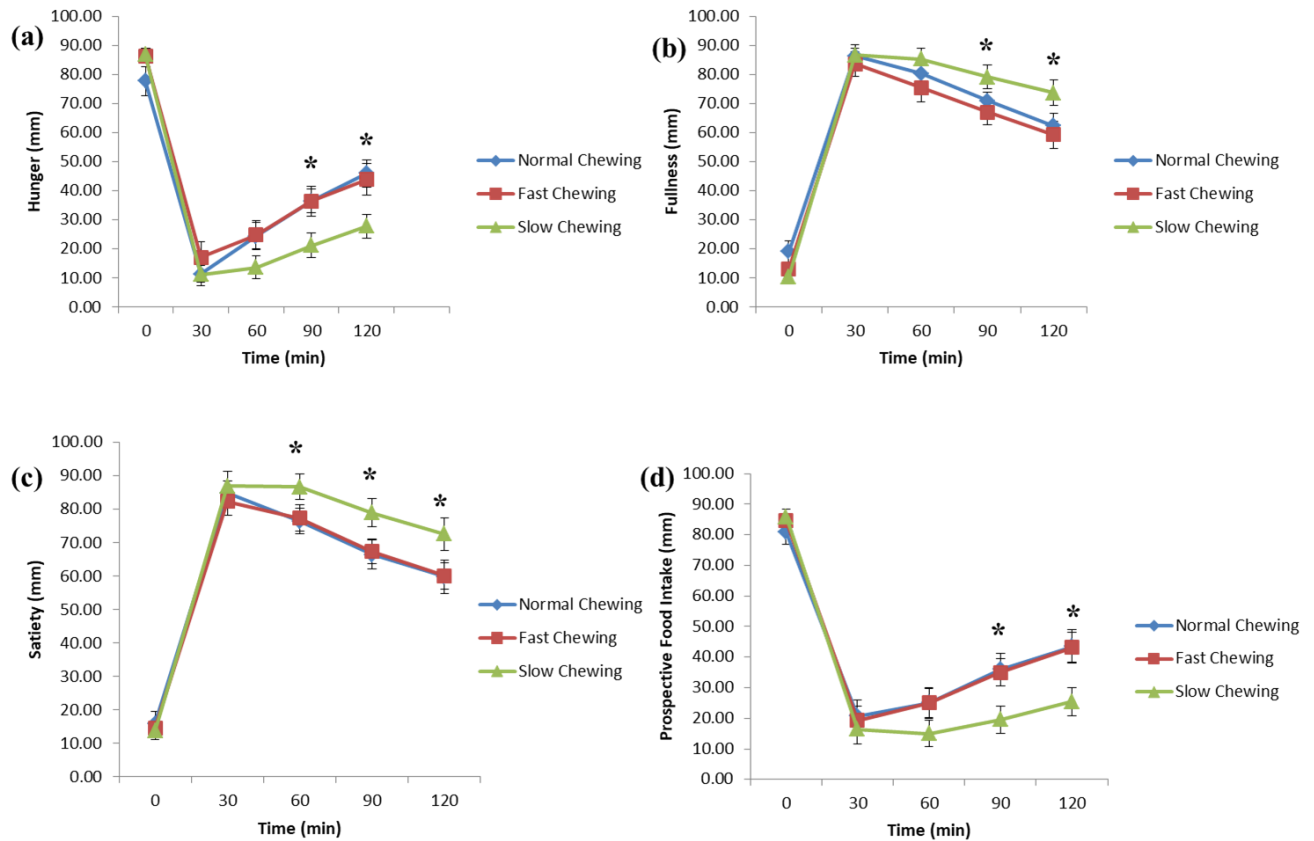
**Table II.** Comparison of chewing time and other parameters following normal, fast and slow chewing

Parameter	Normal chewing	Fast chewing	Slow chewing	P (Kruskal Wallis ANOVA)
<b>Chewing Time (seconds)</b>	701.5 (425 - 944.75) <sup>a</sup>	224 (179.25 - 390.75) <sup>b</sup>	1348 (1026.25 - 1828.5) <sup>c</sup>	<b>&lt;0.001</b>
<b>Bites (n)</b>	40 (31.75 - 46.25) <sup>a</sup>	21.5 (17.75 - 35) <sup>b</sup>	40 (32.75 - 53.25) <sup>a</sup>	<b>0.004</b>
<b>Chews (n)</b>	806.5 (596.25 - 997) <sup>a</sup>	417.5 (264.5 - 548.25) <sup>b</sup>	1530 (909.25 – 1915) <sup>c</sup>	<b>&lt;0.001</b>
<b>Chews per bite</b>	19.23 (14.75 - 24.68) <sup>a</sup>	14.58 (11.28 - 20.53) <sup>b</sup>	30.23 (18.57 - 51.11) <sup>c</sup>	<b>&lt;0.001</b>

Values are Median (IQR). N = number. Values with different superscripts along the rows are significantly different. Kruskal Wallis ANOVA:  $P < 0.05$

## SUBJECTIVE SATIETY RESPONSES

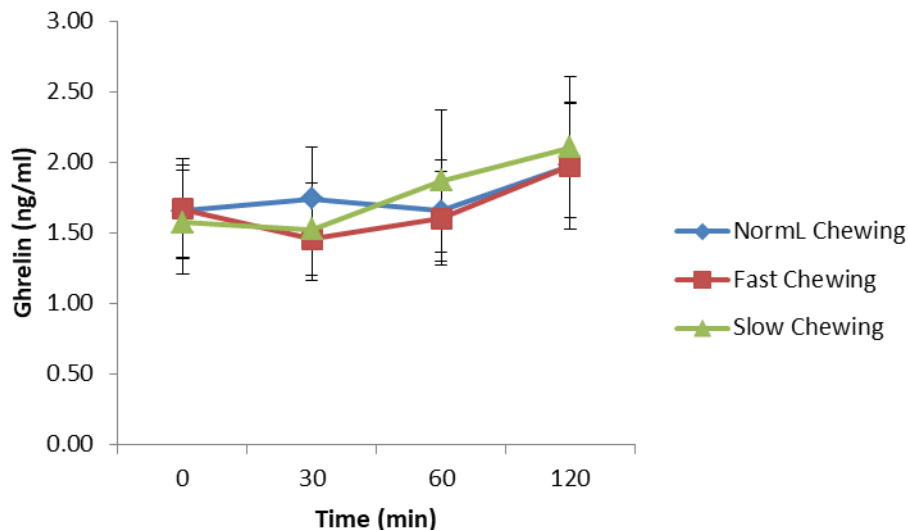
The subjective appetite responses to the different chewing times are shown in Fig. 1. Slow chewing of high protein meal led to significant decline in hunger ( $P = 0.023$ ) and prospective food intake ( $P = 0.018$ ) at time point 120 minutes, whereas, feeling of fullness ( $P = 0.046$ ) remained significantly higher after slow chewing.



**Fig. 1.** (a) Hunger (b) Fullness (c) Satiety (d) Prospective Food Intake after Normal, Fast and Slow Chewing of Food. Values are mean  $\pm$  standard error of mean. Points with asterisks (\*) are significantly different.  $P < 0.05$

### SERUM GHRELIN RESPONSE AFTER DIFFERENT CHEWING RATES

There was no significant difference in ghrelin response between normal, fast and slow chewing. Fig. 2 shows ghrelin response to different chewing conditions. Ghrelin levels declined after slow chewing at 30 minutes and then increased gradually to a peak at 120 minutes post-prandially.



**Fig. 2.** Comparison of ghrelin (mean  $\pm$  standard error) response between normal, fast and slow chewing

Correlation between hunger, fullness, satiety, prospective food intake, and ghrelin at baselines, and 30, 60 and 120 minutes showed that there was no correlation between ghrelin response and satiety at 30, 60 and 120 minutes post-prandially after normal, fast or slow chewing (Table III). These findings indicate that slow chewing made people feel fuller and less hungry, but it did not change ghrelin levels.

**Table III.** Correlation between Serum Ghrelin and Subjective measures of Satiety at Different Time Points

Ghrelin Time (min)		Normal Chewing				Fast Chewing				Slow Chewing			
		Hunger	Fullness	Satiety	Prospective Food Intake	Hunger	Fullness	Satiety	Prospective Food Intake	Hunger	Fullness	Satiety	Prospective Food Intake
0	R	-0.216	0.448*	0.345	-0.304	-0.545**	0.528*	0.404	-0.266	-0.456*	0.699**	0.525*	-0.252
	P	0.334	0.037	0.116	0.169	0.009	0.012	0.062	0.232	0.033	< 0.001	0.012	0.257
30	R	-0.071	0.105	0.139	-0.213	-0.212	0.265	0.204	-0.221	-0.078	0.124	0.137	-0.008
	P	0.755	0.642	0.537	0.34	0.343	0.233	0.363	0.323	0.73	0.581	0.544	0.971
60	R	-0.1	0.11	0.015	-0.004	-0.089	0.174	0.133	-0.167	0.029	0.13	0.016	0.007
	P	0.659	0.628	0.946	0.986	0.694	0.438	0.554	0.458	0.899	0.575	0.946	0.975
120	R	-0.299	0.152	0.167	-0.187	-0.256	0.224	0.104	-0.153	0.074	-0.036	0.028	0.017
	P	0.176	0.501	0.457	0.406	0.25	0.317	0.645	0.498	0.751	0.877	0.903	0.941

\*Correlation is significant at the 0.05 level (2-tailed); \*\*Correlation is significant at the 0.01 level (2-tailed).

## DISCUSSION

This study showed that compared to normal and fast chewing, slow chewing of food decreased feeling of hunger and prospective food intake but increased the feelings of fullness and satiety. These findings are consistent with previous studies indicating that increasing the number of chews or masticatory cycles per bite are associated with reduced post-prandial hunger and, enhanced satiety and fullness (24, 25). One plausible explanation is that slow chewing prolongs the oral processing which increased the cephalic phase response to food, thereby improving hormonal and digestive signaling. In addition, increased chewing time reduces the size of food particles in the bolus, increasing availability of nutrients in the gut. Since the release of hormones involved in the satiety cascade depends on the presence of nutrients in the gastrointestinal tract (GIT), slow chewing may contribute to enhanced satiety through improved hormonal secretion in response to foods in GIT (26-28). Moreover, slower chewing increases the duration of the meal and oro-sensory exposure, giving time to the central satiety signals to be processed, which makes it easier to recognize fullness earlier. This is consistent with research studies showing that eating slowly improves satiation by facilitating better communication between the brain's appetite-regulating regions and gastrointestinal signals (29, 30).

Despite the significant impact of chewing time of food on all measures of subjective satiety in this study, other studies have reported some inconsistencies (19, 20, 24). Zhu et al. reported reduced hunger with increased chewing time but no effect on feeling of fullness (24). Similarly, studies conducted by Li et al. and, Zhu and Hollis reported no significant effect of chewing time or number of chews of food on subjective satiety measures and Borvornparadorn et al. reported no significant difference in feelings of fullness and hunger across different chewing rates (19-21). These discrepancies may be attributed to differences in study population, ethnicity, age groups and BMI status. In addition, differences in test meals given to study participants, particularly high-carbohydrate and high-fat meals, might have influenced the outcome differently than our findings. While other studies have assessed the effect of chewing time on satiety in normal, overweight and/or obese adults, the present study only focused on healthy adults with normal BMI, which may partly explain the observed variations.

In the present study, slow chewing led to increased feelings of fullness and satiety and decreased feelings of hunger and prospective food intake but did not influence serum ghrelin response. These findings correspond with those of Cassidy et al. who also reported no significant effect of chewing time on ghrelin response (25). This suggests that eating slowly may help control food intake, even without changes in hunger hormones. One possible explanation is that increasing the chewing time may decrease the

palatability, thereby reducing hunger independently of ghrelin levels (31). Furthermore, psychological factors associated with slow chewing such as increased mindfulness may contribute to reduced hunger perception with altering ghrelin levels (32). The lack of association between serum ghrelin and subjective satiety indicate that enhancement in satiety may be driven by mechanisms other than hormones. Neural factors such as increased oro-sensory stimulation and activation of appetite-regulation pathways during prolonged chewing may facilitate earlier meal termination. In addition, behavioural factors such as slower eating rate and increased meal duration may allow sufficient time for cognitive and sensory signals of fullness to be perceived, thereby enhancing subjective satiety independently of ghrelin levels. These findings corroborate previous research indicating that eating rate and oro-sensory exposure influence satiation through neural and behavioural pathways other than endocrine responses (29, 30).

While the present study did not show changes in ghrelin response with respect to chewing rates, other studies have shown the influence of chewing time of food on gut hormones and satiety. In studies by Li et al. and Zhu et al. increasing the number of chews i.e., increasing the chewing time of a meal led to greater decline in post-prandial ghrelin levels (19, 24). These contrasting results may again reflect differences in study populations, dietary compositions, and experimental conditions.

Furthermore, no association observed between serum ghrelin levels and perception of satiety after any chewing condition in this study is consistent with previous studies (24). This may also explain the role of ghrelin primarily in initiation of food intake rather than regulation of satiety. Other GIT hormones such as peptide YY, cholecystokinin and glucagon-like peptide-1, which were not measured in this study, are known to predominantly influence satiety regulation (33). This could potentially explain lack of association between satiety and ghrelin in the present study.

As mentioned previously, ghrelin is widely known as the hunger hormone but its association with feeling of hunger is not always consistent. Monteleone et al. reported a significant association between hunger changes and ghrelin levels; however, their study included only female participants and used high-carbohydrate and high-fat meals, which are known to influence circulating ghrelin levels differently (34). In contrast, high-carbohydrate meals have been found to significantly lower ghrelin levels compared to high-fat and high-protein meals, which may further explain variations between studies (12).

The current study suggests that slow chewing food may be a simple and practical strategy to promote satiety and reduce hunger, which helps with weight management. In clinical setting, incorporation of slow eating in dietary counselling may help enhance satiety and reduce energy intake to promote weight loss. These findings also emphasize the importance of behavioural factors such as eating rate in regulation of appetite independent of hormonal changes.

The present study has several potential limitations. The sample size was relatively small ( $n = 22$ ) limiting the generalizability of the findings, so results should be tested in larger groups. In addition, food intake of the remainder of the day was not assessed which limits understanding of how alteration in chewing time at one meal affects overall energy intake. The study was conducted under laboratory conditions because of which the findings may not apply to situations in the real world. Finally, this study assessed the effect of chewing time on a single meal; therefore, long-term effects of chewing on calorie intake and eating behaviour may not be predicted by the findings of this study. Future research should explore the long-term effects of altered chewing habits and include a broader range of physiological markers to better understand the mechanisms underlying satiety regulation.

## CONCLUSION

In conclusion, chewing food slowly increases feelings of fullness and satiety but does not change ghrelin response in healthy individuals. It indicates that eating slowly may be a simple way to help manage appetite and weight loss in individuals with overweight and obesity. Further studies are required to validate these findings in interventions for reduction of obesity.

### Conflict of interests:

The authors declare that they have no competing interests.



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## Authors' contribution:

AY Conducted research; BH & MOM Conceived and designed the experiments; BH, MOM & Eh Contributed to experiments and data analysis; AY, BH KI & NN Contributed to manuscript preparation. All the authors critically reviewed the manuscript and approved the final draft.

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