

## ORIGINAL ARTICLE

## EFFECTS OF CHEWING TIME OF HIGH PROTEIN MEAL ON SATIETY AND GLP-1 HORMONE

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**Background:** Chewing or mastication process affects satiety as well as satiety regulating hormone Glucagon-like Peptide-1 (GLP-1). Proteins have the highest satiating effects among the macronutrients. This study explored the association of chewing with satiety and GLP-1 in participants consuming high-protein meal. **Methods:** Fifteen healthy volunteers, aged 25–35 years were enrolled through random sampling. Effects of chewing on postprandial satiety and plasma GLP-1 was examined through quasi-experimental study conducted in Khyber Medical University, Peshawar, from Jan to Mar 2023. Three visits were designed with one week wash-out period. Satiety and GLP-1 were compared among normal, fast, and slow chewing conditions, while providing high protein meal. Subjective satiety was assessed via visual analogue scale (VAS) and labelled magnitude scale (LMS), while serum GLP-1 levels were analysed through ELISA at baseline, 30, 120 and 240 min in each visit. **Results:** The participants had a mean chewing time of 793.66±311 second at normal rate. Mean chewing time was decreased to 496.60±135.82 second during fast chewing, while significantly increased to 1459.66±400.83 second during slow chewing. A comparison of satiety at different chewing rates revealed that slow chewing significantly reduced hunger and enhanced fullness ( $p<0.05$ ), relative to normal and fast chewing, at 240 minutes ( $p<0.05$ ). However, no significant difference was observed in plasma GLP-1 levels at any time intervals ( $p>0.05$ ). **Conclusion:** Satiety is significantly influenced by chewing and number of chews per bite of a high protein meal, whereas postprandial plasma GLP-1 levels show no significant differences across different chewing rates.

**Keywords:** Glucagon-like Peptide-1, GLP-1, Chewing, Satiety, High protein meal, VAS, LMS

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## INTRODUCTION

Obesity, a complex health issue is driven by excess body fat.<sup>1</sup> Obesity rates have significantly increased since the 1980s, with over 600 million adults globally being obese in 2015.<sup>2</sup> By year 2030, over 2 billion will be overweight, and 1.12 billion will be obese, posing significant health risks.<sup>3</sup> The aetiology of obesity involves the control of caloric intake, hunger, and physical exercise.<sup>4</sup> Physiological mechanism in development of obesity is due to an imbalance in energy between calories consumed and spent that causes extra fat to accumulate in the body.<sup>5</sup> Food consumption is significantly influenced by appetite, which also affects hunger and, consequently, caloric intake.<sup>6</sup> Due to the increased prevalence of overweight and obesity in the present time, high caloric food intake is particularly important. It is well documented that satiety and satiation regulate the amount of food intake.<sup>7</sup>

Satiety is defined as the post-ingestive process that takes place after meal consumption and inhibits further food intake. It includes hunger suppression and sensation of fullness throughout the inter-meal period.<sup>8</sup> Variety of factors are involved in affecting satiety. Eating behaviour and macronutrient composition of food is one of them.<sup>9,10</sup> Understanding the relationships between dietary structure, structural breakdown of food

during chewing, and sensory perception of food texture is crucial for determining how satiation and satiety are affected.<sup>11</sup> Chewing has shown profound effects on the food intake and satiety.<sup>12</sup> Increasing the masticatory process before swallowing of bolus can augment the cephalic responses<sup>13</sup> of satiety regulating hormones like insulin, GLP-1, CCK, and PYY<sup>14,15</sup>.

It is very well established that prolong mastication time and higher mastication decrease appetite and food intake.<sup>16</sup> Chewing pizza 40 times resulted in diminished hunger, and eating desire, compared to chewing 15 times before swallowing.<sup>17</sup> As the global prevalence of overweight and obesity continues to rise, the potential effect of chewing on food intake is becoming more relevant. However, little is known on the effect of chewing time of high protein meal on the satiety score and GLP-1 response. The current study is designed to investigate the effect of chewing time of high protein meal on satiety score and GLP-1 response.

## MATERIAL AND METHODS

Approval was obtained from the Institutional Research Ethical Board of Khyber Medical University, (Reference No. 9606-13), before the recruitment of participants for the study. The declaration of Helsinki's ethical criteria was followed in the conduct of this study.

Fifteen healthy volunteers were recruited after ethical approval and informed consent. Eligibility was established through completion of screening questionnaires eliciting health and demographic information. Healthy participants aged 25–35 years, with a BMI of 19 Kg/m<sup>2</sup> to 25 Kg/m<sup>2</sup>, healthy dentition with no missing or diseased teeth, weight stability (<3 Kg change over the past 3 months), no pregnancy or lactation, low dietary restraint (3-factor eating questionnaire restraint score ≤13), no endocrine or eating disorders, and no use of medication were the inclusion criteria.

A high-protein meal consisted of 500 kcal with a macronutrient composition of 75% protein, 7.3% carbohydrate, and 17.5% fat. The meal included 240 g of grilled chicken, 120 g of sautéed vegetables, and one medium-sized boiled egg.

The study involved three visits, one week apart, where participants visited the Physiology Skill Lab of Institute of Basic Medical Sciences, Khyber Medical University after an overnight fast. They followed specific dietary instructions, limiting their last meal to 500 kilocalories with protein intake no more than 50%. Dietary recall was assessed through 24-hour dietary recall questionnaire, and calorie intake was measured using Win diet software at each visit. During the visits, baseline blood samples and subjective satiety scores were taken before the test meal was consumed. Blood samples, satiety scores and electromyography readings were recorded at different time points after participants consumed their test meal. At first visit (V1), participants were instructed to eat their test food in their usual manner and at their usual rate. Blood samples were taken at 30, 120 and 240 min after finishing the meal. Subjective satiety ratings were recorded at 30, 60, 90, 120, 180 and 240 min through VAS and labelled magnitude scale (LMS).

During the second visit (V2), after a one-week washout period, participants returned to the lab in a fasting state for the same initial evaluations. They were instructed to chew their food quickly, reducing the duration by half compared to their normal habitual chewing time. Video cameras and electromyography were used to calculate the same variables as in the first visit. Blood sampling and subjective satiety assessments were conducted at designated time points, mirroring the procedures of the first visit.

For the third visit (V3), the same procedures were followed with a slight modification in chewing instructions. Participants were directed to chew the meal

for a longer duration, double their usual chewing time. Similar measurements were recorded before and after meal consumption, maintaining consistency with the previous visits.

During each visit, 12 mL of blood was collected at 4 time points using gel tubes. The blood was centrifuged at 4 °C and 2,000 RPM for 15 minutes. Separated serum was transferred to Eppendorf tubes, stored at -80 °C, and analyzed for GLP-1 levels using a BT Lab Biomedical ELISA kit.

Data were analysed on SPSS-26. The presentation of all data was in the form of Mean and Standard Deviation (SD). One-way analysis of variance (ANOVA) was conducted to evaluate the differences in satiety and plasma GLP-1 levels. To assess the alterations in satiety and GLP-1 levels across different time points, repeated-measures ANOVA was carried out, which was followed by a Tukey post hoc analysis to identify specific group comparisons if ANOVA was significant.

## RESULTS

The current study was performed on a group of 15 healthy participants; 4 (26.67%) females and 11 (73.33%) males, and having average BMI 22.09±2.09 Kg/m<sup>2</sup> (range=18.5–24.9). The mean age of the participants was 25.41±1.68 years, with a range of 25–31 years. Fasting glucose level of the participants was 84.27±8.11 mg/dL, (range=71–97 mg/dL. (Table-1).

During the first visit (V1), the participants had a mean chewing time of 793.66±311 seconds and a recorded number of chews were 932.20±317.25. The mean chewing time significantly increased to 1459.66±400.83 seconds in the third visit (V3) with a corresponding rise in total number of chews (*p*<0.001). The number of chews per bite was 22.22±8 during normal chewing time, while doubling the chews per bite from a mean of 22.22±7.98 to 48.27±15.19 resulted in an increased total number of chews to 1700.47±445.83 and hence an increase in chewing time (*p*<0.001), (Table-2).

The Tukey post-hoc test was performed for analysis of significant variables, as summarized in Table-3.

**Table-1: Demographics of the participants**

Variables	Mean±SD
Age (year)	25.41±1.68
Height (meter)	1.53±0.16
Weight (Kg)	63.10±10.27
BMI (Kg/m <sup>2</sup> )	22.09±2.09

**Table-2: Chewing conditions on different visits with No. of chews and No. of bites (Mean±SD)**

Considerations	Normal Chewing (V1)	Fast Chewing (V2)	Slow Chewing (V3)	<i>p</i>
Total No. of Chews	932.20±317.25	523.00 ±139.76	1700.47±445.83	<0.001
Total No. of bites	42.40±6.65	33.33±9.25	35.47±6.86	0.006
Chewing time (Sec)	793.66±311.38	496.60±135.82	1459.66±400.83	<0.001
Chews/bite	22.22±7.98	16.68±6.67	48.27±15.19	<0.001
Chews/second	1.08±0.17	1.06±0.17	1.17±0.17	0.189

**Table-3: Tukey Post-Hoc analysis**

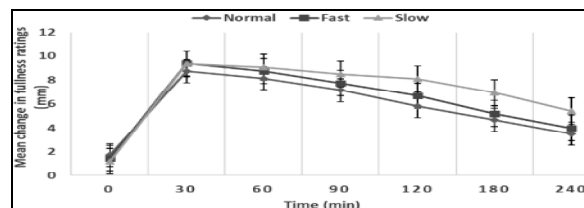
	Normal vs Fast Chewing	Normal vs Slow Chewing	Fast vs Slow Chewing
Total No. of Chews	0.003*	<0.001*	0.001*
Total No. of bites	0.006*	0.037	0.738
Chewing time (Sec)	0.029*	0.001*	0.001*
Chews/bite	0.327	0.002*	0.002*
Chews/second	0.953	0.328	0.309

\*Significant

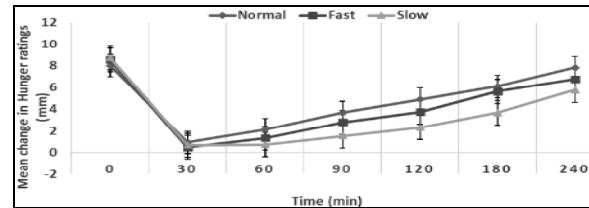
Satiety was assessed using VAS and LMS scales to measure participants' appetite perception. Pre-prandial and 30 min postprandial appetite ratings were not significant after normal, fast and slow chewing ( $p>0.05$ ). However, hunger was consistently increased from 30 to 240 min post-meal ( $p<0.05$ ), regardless of chewing conditions (Figure-1a). Fullness remained significantly elevated for slow chewing compared to normal and fast chewing until 240 min ( $p<0.05$ ), indicating delayed fullness with slow chewing (Figure-1b). Satisfaction was consistently higher in slow eating than normal and fast eating, even at 240 min post-meal (Figure-1c). Prospective consumption levels gradually increased from 30 to 240 minutes ( $p<0.05$ ), with slow eaters showing delayed responses compared to fast eaters (Figure-1d).

VAS values at 240 min showed significantly increased fullness and satisfaction, while decreased hunger and prospective consumption after slow chewing compared to normal and fast chewing, as summarized in Figure-2.

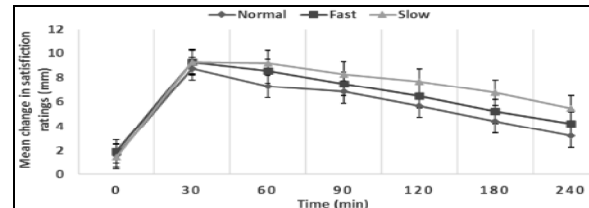
The LMS scale demonstrated a significant decline in satiety intensity rating from 30 minutes to 240 min post-meal (Figure-3), with significant differences at various time points ( $p<0.05$ ). No significant effects on plasma GLP-1 concentrations were observed after meal consumption in three visits at different time points ( $p>0.05$ ). Despite this, normal and slow chewing conditions resulted in a rapid increase in plasma GLP-1 from baseline to 30 minutes, while fast chewing exhibited a non-significant decline in GLP-1 concentration ( $p>0.05$ ). In normal chewing conditions, plasma GLP-1 gradually decreased from 30 to 120 minutes, while fast and slow chewing conditions showed an increase without significant differences ( $p>0.05$ ). From 120 to 240 minutes, plasma GLP-1 concentrations increased for all chewing conditions, with a more pronounced rise in slow chewing compared to normal chewing. (Figure-4).


**Figure-1a: VAS-1 (hunger)**

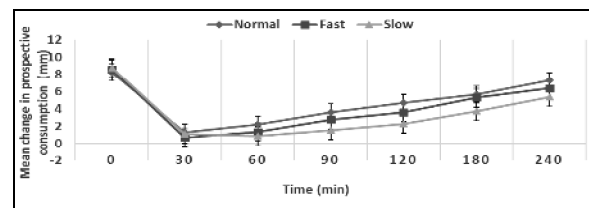
Line graph illustrates hunger comparison in different chewing conditions from baseline to 240 minutes


**Figure-1b: VAS-2 (Fullness)**

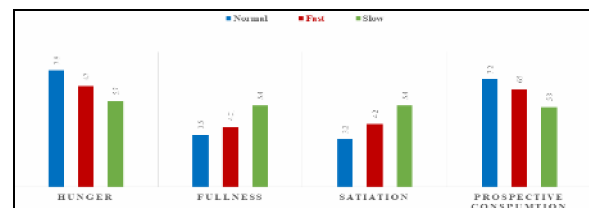
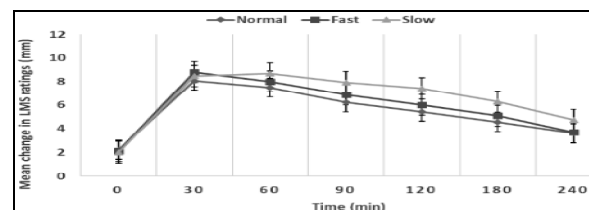
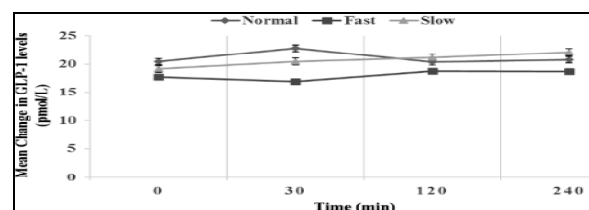
Line graph illustrates fullness comparison in different chewing conditions from baseline to 240 minutes


**Figure-1c: VAS-3 (Satisfaction)**

Line graph illustrates satisfaction comparison in different chewing conditions from baseline to 240 minutes


**Figure-1d: VAS-4 (Prospective Consumption)**

Line graph illustrates prospective consumption comparison in different chewing conditions from baseline to 240 minutes


**Figure-2: VAS values at 240 min in different chewing conditions**

**Figure-3: Line graph of LMS illustrates satiety in chewing conditions from baseline to 240 min**

**Figure-4: GLP-1 levels at different time points on normal, fast and slow chewing**

## DISCUSSION

This study revealed that slower mastication correlated with higher satiety scores, indicating prolonged chewing contributes to increased fullness and satisfaction. The number of chews per bite positively associated with chewing rate, suggesting slower eaters chewed more thoroughly. However, no significant differences in GLP-1 levels between slow and fast eaters were observed, suggesting that additional factors may exert a more notable influence on the modulation of GLP-1 secretion. Overall, the study highlights the importance of chewing time to chew food thoroughly, as it may have beneficial effects on feelings of fullness and satisfaction after meal consumption. A study conducted by Goh *et al*<sup>18</sup> revealed that there is a relationship between chewing time and satiety and found that slow eaters had higher satiety scores than fast eaters. However, in the present study both VAS and LMS scale to measure satiety ratings were employed which may provide a more comprehensive evaluation of the subjective experience of satiety. These findings suggest the significance of mastication in regulating fullness and appetite, with implications for promoting healthy eating behaviours and weight management.

Zhu *et al*<sup>17</sup> found that the number of chews per bite of a pizza slice significantly impacted satiety and eating desire, with 40 chews resulting in significant changes in gastrointestinal hormones. This aligns with another research suggesting that prolonged chewing and increased oral mastication time lead to higher satiety and decreased food intake.<sup>19</sup>

The current study investigated the impact of mastication on plasma GLP-1 concentrations that is released by the intestines in response to nutrient ingestion and is responsible for regulating glucose homeostasis and food intake. The findings indicated that chewing had no significant effect on GLP-1 concentrations in the bloodstream. These results are consistent with the study conducted by Alsalim and Ahrén<sup>20</sup> who also reported no significant changes in postprandial GLP-1 levels during both slow and fast chewing rates, suggesting that the rate of chewing did not impact GLP-1 secretion. Sonoki *et al*<sup>21</sup> conducted a study that examined the effects of fast eating compared to usual normal eating rate on plasma GLP-1 concentration, and they concluded that there was no significant change in GLP-1 concentration during quick eating, which further supports the present findings.

The study conducted by Cassidy *et al*<sup>22</sup> explored the influence of different chewing rates on post-ingestive plasma GLP-1 levels. They observed a significant reduction in GLP-1 levels when participants chewed a fixed quantity of almonds 25 times compared to when the same almonds were chewed 40 times.

Kokkinos *et al*<sup>23</sup> conducted a crossover study with 17 healthy volunteers, investigating the impact of mastication rate on plasma GLP-1 response. Participants consumed ice cream containing 675 kilocalories slowly over 30 minutes or quickly within 5 minutes. The results showed that slow consumption increased postprandial plasma GLP-1 response compared to quick consumption. The potent stimulators of GLP-1 secretion in meal components are glucose and triacylglycerol, which are major macronutrients.<sup>24</sup> Carbohydrates, in particular, have been shown to significantly stimulate GLP-1 secretion compared to fat.<sup>25</sup> In the present study, the lack of significant difference in plasma GLP-1 concentrations observed between different chewing conditions (fast and slow) may be attributed to the high protein content of the meal. The meal contained a minimal quantity of carbohydrates, whereas the aforementioned studies used carbohydrates as the test meal, which are potent stimulators of GLP-1 secretion.

## CONCLUSION

The duration of chewing process significantly impacts the satiety score of individuals. However, the rate of chewing may not be significantly associated with the postprandial increase in plasma GLP-1 levels in all chewing conditions (fast and slow).

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## REFERENCES

1. Lin X, Li H. Obesity: epidemiology, pathophysiology, and therapeutics. *Front Endocrinol (Lausanne)* 2021;12:585887.
2. GBD 2015 Obesity Collaborators: Afshin A, Forouzanfar MH, Reitsma MB, Sur P, Estep K, Lee A, *et al*. Health effects of overweight and obesity in 195 countries over 25 years. *N Engl J Med* 2017;377(1):13–27.
3. Kolahi AA, Moghisi A, Ekhtiari YS. Socio-demographic determinants of obesity indexes in Iran: findings from a nationwide STEPS survey. *Health Promot Perspect* 2018;8(3):187–94.
4. Yasmin I, Khan WA, Naz S, Iqbal MW, Awuchi CG, Egbuna C, *et al*. Etiology of Obesity, Cancer, and Diabetes. In: Egbuna C, Hassan S, (Eds). *Dietary Phytochemicals*. Cham: Springer; 2021. p. 1–27.
5. Yoo S. Syndrome M. Dynamic energy balance and obesity prevention. *J Obes Metab Syndr* 2018;27(4):203–12.
6. Gibbons C, Finlayson G, Dalton M, Caudwell P, Blundell JE. Metabolic phenotyping guidelines: studying eating behaviour in humans. *J Endocrinol* 2014;222(2):G1–12.
7. Cifuentes L, Acosta A. Homeostatic regulation of food intake. *Clin Res Hepatol Gastroenterol* 2022;46(2):101794.
8. Tack J, Verbeure W, Mori H, Schol J, Van den Houte K, Huang IH, *et al*. The gastrointestinal tract in hunger and satiety signalling. *United European Gastroenterol J* 2021;9(6):727–34.
9. Forde C, Van Kuijk N, Thaler T, de Graaf C, Martin N. Oral processing characteristics of solid savoury meal components, and relationship with food composition, sensory attributes and expected satiation. *Appetite* 2013;60:208–19.

10. Forde C, Van Kuijk N, Thaler T, De Graaf C, Martin N. Texture and savoury taste influences on food intake in a realistic hot lunch time meal. *Appetite* 2013;60:180–6.
11. Bolhuis DP, Forde CG, Cheng Y, Xu H, Martin N, de Graaf CJPo. Slow food: sustained impact of harder foods on the reduction in energy intake over the course of the day. *PLoS One* 2014;9(4):e93370.
12. Campbell CL, Wagoner TB, Foegeding EA JFS. Designing foods for satiety: The roles of food structure and oral processing in satiation and satiety. *Food Structure* 2017;13:1–12.
13. Pullicin AJ, Glendinning JI, Lim J. Cephalic phase insulin release: A review of its mechanistic basis and variability in humans. *Physiol Behav* 2021;239:113514.
14. Venegas C, Farfan Beltrán N, Bucchi C, Martínez Gomis J, Fuentes RJ. Effect of chewing behavior modification on food intake, appetite and satiety-related hormones: A Systematic Review. *Rev Chil Nutr* 2022;49(6):760–74.
15. Slyper A. Oral processing, satiation and obesity: overview and hypotheses. *Diabetes Metab Syndr Obes* 2021;3399–415.
16. Argyrakopoulou G, Simati S, Dimitriadis G, Kokkinos AJN. How important is eating rate in the physiological response to food intake, control of body weight, and glycemia? *Nutrients* 2020;12(6):1734.
17. Zhu Y, Hsu WH, Hollis JH. Increasing the number of masticatory cycles is associated with reduced appetite and altered postprandial plasma concentrations of gut hormones, insulin and glucose. *Br J Nutr* 2013;110(2):384–90.
18. Goh AT, Choy JYM, Chua XH, Ponnalagu S, Khoo CM, Whittom C, *et al.* Increased oral processing and a slower eating rate increase glycaemic, insulin and satiety responses to a mixed meal tolerance test. *Eur J Nutr* 2021;60(5):2719–33.
19. Simon SL, Pan Z, Marden T, Zhou W, Ghosh T, Hossain D, *et al.* Body mass index and variability in meal duration and association with rate of eating. *Front Nutr* 2022;9:941001.
20. Alsalmim W, Ahrén B. Insulin and incretin hormone responses to rapid versus slow ingestion of a standardized solid breakfast in healthy subjects. *Endocrinol Diabetes Metab* 2019;2(2):e00056.
21. Sonoki K, Iwase M, Takata Y, Nakamoto T, Masaki C, Hosokawa R, *et al.* Effects of thirty-times chewing per bite on secretion of glucagon-like peptide-1 in healthy volunteers and type 2 diabetic patients. *Endocr J* 2013;60(3):311–9.
22. Cassady BA, Hollis JH, Fulford AD, Considine RV, Mattes RD. Mastication of almonds: effects of lipid bioaccessibility, appetite, and hormone response. *Am J Clin Nutr* 2009;89(3):794–800.
23. Kokkinos A, le Roux CW, Alexiadou K, Tentolouris N, Vincent RP, Kyriaki D, *et al.* Eating slowly increases the postprandial response of the anorexigenic gut hormones, peptide YY and glucagon-like peptide-1. *J Clin Endocrinol Metab* 2010;95:333–7.
24. Nauck MA, Vardarli I, Deacon CF, Holst JJ, Meier JJ. Secretion of glucagon-like peptide-1 (GLP-1) in type 2 diabetes: what is up, what is down? *Diabetologia* 2011;54(1):10–8.
25. Rijkkelijkhuizen JM, McQuarrie K, Girman CJ, Stein PP, Mari A, Holst JJ, *et al.* Effects of meal size and composition on incretin,  $\alpha$ -cell, and  $\beta$ -cell responses. *Metabolism* 2010;59(4):502–11.

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**IS:** Conception, design and drafting of work, data interpretation, final approval for publication

**OM:** Conception, design and drafting of work, data interpretation, critical review, final approval for publication

**RM:** Conception, design and drafting of work, data interpretation, critical review, final approval for publication

**AA:** Data collection, statistical analysis, data interpretation, writing assistance, proofreading

**OQ:** Conception, design and drafting of work, data collection, statistical analysis, data interpretation, critical review, final approval for publication, accountable for all aspects of the work

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