Effect of ingestive behavior on appetite in young and older adults

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Effect of ingestive behavior on appetite in young and older adults

by

Yong Zhu

A dissertation submitted to the graduate faculty
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

Major: Nutritional Sciences

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Iowa State University
Ames, Iowa

2012

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- Non-physiological Factors that Influence Body Weight and Appetite
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<td>AgRP</td>
<td>Agouti-related protein</td>
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<tr>
<td>ANOVA</td>
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<tr>
<td>ARC</td>
<td>Arcuate nucleus</td>
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<tr>
<td>BMI</td>
<td>Body mass index</td>
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<tr>
<td>CART</td>
<td>Cocaine- and amphetamine-regulated transcript</td>
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<tr>
<td>CCK</td>
<td>Cholecystokinin</td>
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<tr>
<td>CPR</td>
<td>Cephalic phase response</td>
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<tr>
<td>CRH</td>
<td>Corticotropin-releasing hormone</td>
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<tr>
<td>CV</td>
<td>Coefficient of variation</td>
</tr>
<tr>
<td>EDTA</td>
<td>Ethylenediaminetetraacetic acid</td>
</tr>
<tr>
<td>ELISA</td>
<td>Enzyme-linked immunosorbent assay</td>
</tr>
<tr>
<td>EMG</td>
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<td>fMRI</td>
<td>Functional magnetic resonance imaging</td>
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<tr>
<td>GI</td>
<td>Gastrointestinal</td>
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<tr>
<td>GIP</td>
<td>Glucose-dependent insulinotropic peptide</td>
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<tr>
<td>GLP-1</td>
<td>Glucagon-like peptide-1</td>
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<td>LHA</td>
<td>Lateral hypothalamic area</td>
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<tr>
<td>MCH</td>
<td>Melanin-concentrating hormone</td>
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<td>NHANES</td>
<td>National Health and Nutrition Examination Survey</td>
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<tr>
<td>NPY</td>
<td>Neuropeptide Y</td>
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<tr>
<td>NTS</td>
<td>Nucleus tractus solitarius</td>
</tr>
<tr>
<td>PET</td>
<td>Positron emission tomography</td>
</tr>
<tr>
<td>PFA</td>
<td>Perifornical area</td>
</tr>
<tr>
<td>POMC</td>
<td>Pro-opiomelanocortin</td>
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<tr>
<td>PVN</td>
<td>Paraventricular nucleus</td>
</tr>
<tr>
<td>PYY</td>
<td>Peptide YY</td>
</tr>
<tr>
<td>RIA</td>
<td>Radioimmunoassay</td>
</tr>
<tr>
<td>SEM</td>
<td>Standard error of the mean</td>
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<tr>
<td>SSS</td>
<td>Sensory specific satiety</td>
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<tr>
<td>TRH</td>
<td>Thyrotropin-releasing hormone</td>
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<tr>
<td>VAS</td>
<td>Visual analogue scale</td>
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<td>VMN</td>
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APPETITE is the motivation to eat. A better understanding of the factors that influence appetite may aid the development of new or improved strategies for body weight management. Accumulating evidence suggests that there is a correlation between certain ingestive behaviors and obesity. However, whether mastication, a key aspect of ingestive behavior, is associated with body weight is not known.

There is a considerable inter-individual variation in human masticatory performance. Moreover, food characteristics also influence masticatory performance. Despite having a major role in ingestion it is not clear if mastication influences appetite.

In this dissertation, we hypothesize that there is a negative correlation between body weight and the number of chews at the swallowing threshold for a given food. We further hypothesize that increasing the number of chews made before swallowing reduces meal size and promotes postprandial satiety. As there are aging-related changes in both appetite and mastication, we are also interested in the response in both young and older adults so that information gained from our research can be applied in both populations.

A series of experiments have been conducted. In the first study, we collected habitual mastication data from 64 young adults using pizza rolls as the test food. Regression analysis revealed a significant negative association between body mass index (BMI) and the number of chews (P=0.020). Similar results were found for BMI and chewing duration (P=0.005). To further investigate the association between body weight and mastication performance, we recruited 11 young (age 18-40) and 11 older (age ≥65) adults and measured their
microstructure of mastication by using an electromyographic recording device. It was found that aging and food hardness had a significant impact on mastication and controlling for age and food characteristics, the correlation between BMI and the number of chews was significant (P=0.010). Similarly, a significant negative correlation was found between BMI and other mastication parameters such as maximal bite force (P=0.002), mean bite force (P<0.001), muscle activity (P<0.001) and chewing rate (P=0.025).

While those results show a negative association between body weight and mastication, it is still not known what physiological mechanism explains these results. We then conducted a study to investigate the influence of masticatory cycles on meal size by asking participants to chew pizza rolls either 100%, 150% or 200% of their baseline number of chews. 47 young adults participated in the study and it was found the ad libitum food intake in the 150% and 200% sessions was reduced by 9.5% (P=0.023) and 14.8% (P=0.001) respectively, compared to the 100% session. A similar intervention was conducted in 18 older adults but there was no difference in the food intake across different test sessions. In both studies, eating rate was significantly reduced when the number of chews was increased.

To investigate the influence of mastication on postprandial satiety in both young and older participants, we conducted studies using a fixed-portion meal, by asking participants to chew each portion of the food either 15 or 40 times before swallowing. For young adults, 40 chews resulted in lower hunger (P=0.009), preoccupation with food (P=0.005) and desire to eat (P=0.002). Meanwhile, plasma concentrations of glucose (P=0.024), insulin (P<0.001) and glucose-dependent insulinotropic peptide (GIP) (P<0.001) were higher following the 40 chews meal. Chewing 40 times before swallowing also resulted in a higher plasma
cholecystokinin concentration (P=0.045) and a trend toward a lower ghrelin concentration (P=0.051) but there was no difference in food intake at a subsequent meal (P=0.851). Similar results on subjective appetite in older adults were found. However, the effect of masticatory cycles on plasma concentrations of hormones and glucose were different: although higher levels of insulin, GIP and glucose were observed in 40 chews immediately after eating (P<0.05), they became significantly lower after two or three hours (P<0.05). In addition, no difference on cholecystokinin and ghrelin was found (P>0.05). Moreover, there was a trend toward significance that older adults ate more at the subsequent meal in the 40 chews condition (P=0.066). Those results suggest increasing the number of chews before swallowing suppresses subjective appetite and facilitates glucose absorption in both young and older adults, but the satiating effect was different, probably due to aging-related impairment in appetite response in older adults.

In conclusion, the studies involved in the dissertation suggest body weight is a variable explaining for the inter-individual variation in habitual masticatory performance. The ingestive behavior, characterized by eating slowly and chewing thoroughly, suppresses appetite and influences glycemic response in both young and older adults. Information gained from this dissertation is useful as it provides potential dietary and behavioral strategies for body weight management through increased mastication activity, i.e., choosing hard food that requires more mastication activity and/or eating slowly by increasing the number of chews before swallowing.
CHAPTER 1. GENERAL INTRODUCTION

Introduction

The mean body mass index (BMI) of adults has increased globally and in 2008 it was estimated that 1.46 billion adults were overweight (25.0 ≤ BMI ≤ 29.9 kg/m²) or obese (BMI ≥ 30.0 kg/m²)\(^1\). The United States is among those countries with the highest overweight and obesity prevalence, which is still increasing\(^2\)-\(^4\). A recent analysis of the 2009-2010 National Health and Nutrition Examination Survey (NHANES) has revealed that the prevalence of overweight was 33.3% and the prevalence of obesity was 35.9% in US adults\(^5\). For those whose age ≥ 60, the prevalence of overweight was 39.9% in men and 31.2% in women, whereas the prevalence of obesity was 36.6% in men and 42.3% in women\(^5\). It is estimated by 2030, 86.3% of the US adults will be overweight or obese and 51.1% of US adults will be obese if the trend continues\(^6\).

Obesity is associated with an increased risk of several co-morbidities, including type-2 diabetes, cardiovascular diseases and several types of cancer\(^7\)-\(^9\). Obesity in the elderly further increases risk of mortality\(^10\) and the risk of cognitive diseases such as Alzheimer’s disease\(^11\). Besides the impact on health status, obesity also imposes an economic burden on the family and the society\(^12\). On average, each obese person incurs higher annual medical costs by $2741, and it is estimated that 20.6% of US national health expenditures is spent treating obesity-related illness\(^13\). In view of the obesity prevalence and its consequences, research on obesity prevention is urgent and of great significance.
A fundamental principle behind gaining body weight is that energy intake exceeds energy expenditure. The components of energy balance provide a potential powerful tool for body weight management\textsuperscript{14-15}. As foods and beverages are the sole sources of energy intake, research on regulation of appetite and food intake, may provide dietary or behavioral strategies and therapeutic targets for obesity intervention\textsuperscript{15-21}.

Appetite is the motivation to eat. It is often divided into three components: hunger, satiation and satiety\textsuperscript{22}. Integration of the definitions given by Blundell \textit{et al.}\textsuperscript{23} and Mattes \textit{et al.}\textsuperscript{22} suggest hunger is the sensation that drives to eat and promotes food consumption; satiation, known as intra-meal satiety, refers to the sensation or process related to meal termination, which controls meal size and meal duration; satiety, known as inter-meal satiety, is termed as the sensation or process after a meal which leads to a period of abstinence from eating\textsuperscript{22-23}. Various factors, including physiological\textsuperscript{24}, environmental\textsuperscript{25} and cognitive\textsuperscript{26} factors, as well as their interactions\textsuperscript{27} are involved in the mechanisms that regulate appetite and food intake.

Food characteristics, such as macronutrient composition\textsuperscript{28-30}, physical properties\textsuperscript{31-33}, and ingestive behaviors, such as eating rate (amount of food consumed per unit of time)\textsuperscript{34-35} and bite size\textsuperscript{36}, have been found to influence appetite and food intake. The effect of mastication, which is another important part of microstructures of ingestive behavior, however, has gained little attention.

It has been reported that the hardness of habitual diet is negatively associated with the waist circumference in Japanese young women\textsuperscript{37}. Moreover, a recent study has shown there is an association between masticatory performance on body weight in Brazilian children: normal weight children had a smaller median bolus size (a theoretical sieve size which 50% of
chewed bolus can pass through) compared with overweight and obese children\textsuperscript{38}. In addition, results from animal study by Oka \textit{et al.} also suggest diet hardness is associated with body weight\textsuperscript{39}. In their study, rats were fed with either standard or soft pellets with the same nutrient and water components but softened by increasing air content, it was found rats fed with soft pellets had shown greater adiposity\textsuperscript{39}.

The results from the above studies suggest a potential relationship between mastication and body weight. However, the mechanism to explain such a relationship is not known at this stage. Moreover, due to the extremely limited number of studies available, more studies are needed to confirm the relationship between mastication performance and body weight.

It has been demonstrated that older adults have impaired regulation of appetite, characterized by a lower feeling of hunger, higher circulating concentrations of satiety hormones and reduced food intake\textsuperscript{40-47}. Meanwhile, it is widely known that older adults have impaired mastication performance due to age-related change in dental function, characterized by a longer chewing time and an increase in the number of chews made before swallowing than young adults\textsuperscript{48-51}. It is not known, however, whether such a mastication pattern in older adults have contributed to the characteristics of appetite in this population. If increased mastication does suppress appetite and energy intake, it provides a reasonable explanation for the relationship between mastication and body weight observed in previous studies\textsuperscript{37-39}.

By now, three studies have been conducted to elucidate the effect of mastication on appetite; the results suggest increased mastication activity promotes both satiation\textsuperscript{52-53} and satiety\textsuperscript{53-54}. A detailed review of these studies\textsuperscript{52-54} will be presented in the next chapter of the dissertation. Due to the fact that only three studies are available, but there is a huge inter-individual
variation in mastication performance \textsuperscript{55-57} whereas mastication is influenced by food characteristics \textsuperscript{58-61}, further studies using different populations and different test foods are required.

The objectives of the studies involved in the dissertation are to elucidate the relationship between mastication and appetite, with the focus on how does increasing the number of masticatory cycles (i.e., the number of chews or chewing cycles) influence satiation and satiety in young and older adults. Although the habitual number of chews made before swallowing varies among people \textsuperscript{55-57}, the variation in human chewing rate seems to be relatively small \textsuperscript{62-63}. Consequently, a change in the masticatory cycles per mouthful during ingestion will also prolong oral processing time and reduce eating rate, makes it difficult to isolate the masticatory cycle as the sole treatment factor. For this reason, the term “ingestive behavior”, characterized by chewing thoroughly and eating slowly, is used in the title of the dissertation, rather than “mastication” or “masticatory cycles”.

**Dissertation Organization**

The dissertation begins with an overall introduction, followed by in-depth review on the topics related to appetite and ingestive behaviors. The next five chapters consist of five manuscripts that have summarized five independent projects during my PhD study: the association of body weight and mastication performance, the effect of masticatory cycles on satiation in young adults, the effect of masticatory cycles on satiation in older adults, the effect of masticatory cycles on postprandial satiety in young men, the effect of masticatory cycles on postprandial satiety in older men. The second and the fourth manuscripts have been submitted to peer-reviewed journals and the rest will be submitted soon. The completed
manuscripts include inputs from co-authors, who have contributed to experimental design, data collection and analysis, as well as manuscript preparation. After the five manuscripts, a general conclusion chapter is presented to summarize the overall findings in those research projects, followed by recommendations for future research.

References


CHAPTER 2. LITERATURE REVIEW

This chapter starts with an overview of physiological regulation of body weight and appetite, followed by the discussion of non-physiological factors that influence body weight and appetite. Ingestive behavior is then reviewed, with a focus on mastication and appetite. In addition, aging-related changes in appetite and the methodology for appetite measurement are discussed. The hypothesis and objectives of the dissertation are presented at the end of this chapter.

Physiological regulation of body weight and appetite

The prevalence of obesity in both young and older adults in the United States has increased dramatically over the past 50 years. The consequences of obesity include an adverse effect on chronic disease risk and the quality of life for obese individuals and families, as well as a negative impact on the economy. A better understanding of how body weight or energy homeostasis is regulated would potentially help the discovery of new or more effective strategies to prevent obesity.

Over 60 years ago, Kennedy proposed that body weight, or more specifically body fat, is physiologically regulated. He proposed that the adipose tissue produces a signal which is sensed by the brain and compared to a target level of adiposity; any deviations from the target level of adiposity would be corrected for by changes in food intake or energy expenditure. The concept of body weight regulation has since been refined resulting in the set-point hypothesis which proposes that the amount of body fat is signaled to the hypothalamus by the hormone leptin and deviations from the set point are corrected by
modifying appetite and food intake\textsuperscript{6-12}. There are several lines of evidence that support the set-point hypothesis. First, studies show that when body weight is altered by a period of over- or under-feeding, the perturbation is corrected when conditions permit and body weight returns to its pre-intervention level\textsuperscript{13-17}. Second, body weight remains remarkably constant over a number of years in adults and increases, on average, by 0.5 kg a year in western societies\textsuperscript{18}. This remarkable precision requires a very small error in the precision to match energy intake with energy expenditure, which could only be achieved by physiological regulation\textsuperscript{19-20}.

A considerable body of research has identified many aspects of a body weight regulatory system although there are still gaps to be filled in. In brief, the adipose tissue secretes the hormone leptin in direct proportion to its mass\textsuperscript{21-22}. The amount of circulating leptin is sensed by the hypothalamus and compared to the body fat set point. If there are any deviations from the set point, corrective action is taken by up-regulating or down-regulating appetite to correct for the perturbation. This change in appetite will alter the sensitivity to satiety signals that are induced by food intake so that meal size or eating frequency is changed and the deviation from the set-point corrected.

\textit{Central neural circuits related to energy homeostasis and appetite}

Early brain lesioning and stimulation experiments suggested that the ventromedial nucleus (VMN) is the “satiety center” and the lateral hypothalamic area (LHA) is the “hunger center”\textsuperscript{23-24}. However, this view has been superceded by recent research that indicates the hypothalamus is the brain region which integrates information about body fat storage and
appetite; meanwhile, it is the integrative neural circuits, rather than discrete nuclei modulate appetite\textsuperscript{6, 25-26}.

The arcuate nucleus (ARC) in the hypothalamus is the primary region that integrates peripheral adipose and satiety signals as it has receptors for these hormones, or receives projections from nerve fibers that have those receptors\textsuperscript{27-29}. The ARC has two types of interconnected neurons: one releases orexigenic molecules neuropeptide Y (NPY) and agouti-related peptide (AgRP), and the other releases anorexigenic molecules pro-opiomelanocortin (POMC) and cocaine- and amphetamine-regulated transcript (CART)\textsuperscript{25}. In a positive energy balance condition, NPY/AgRP is inhibited whereas POMC/CART is activated and vice versa. These neurons project to hypothalamic areas including the paraventricular nucleus (PVN), perifornical area (PFA) and LHA\textsuperscript{30}. Activation of PVN stimulates the secretion of anorexigenic substances such as thyrotropin-releasing hormone (TRH), corticotropin-releasing hormone (CRH) and oxytocin, which inhibit food intake; by contrast, activation of PFA and LHA results in release of orexigenic molecules such as melanin-concentrating hormone (MCH) and orexin, which stimulate appetite\textsuperscript{6, 31}.

\textit{Adipose signals}

To be considered as an adiposity signal several criteria need to be satisfied. First, the molecule needs to be secreted into the plasma in proportion to the body fat stores. Second, it needs to interact with well established hypothalamic centers that regulate energy homeostasis and body weight. Third, the administration of the metabolite leads to predictable changes in body fat. It has been proposed that the hormones leptin and insulin satisfy these three criteria and can be termed adiposity signals\textsuperscript{9, 32}. 
Leptin is secreted largely by adipose tissue and to a lesser extent, by the stomach\textsuperscript{33-35}. Studies using humans and animal models demonstrate that leptin is secreted in direct proportions to the amount of body fat\textsuperscript{22, 36}. Moreover, changes in body fat are reflected by changes in circulating leptin\textsuperscript{21-22, 36-40}. Leptin interacts with its receptors in several parts of the hypothalamus, such as ARC, VMN and LHA, which are key areas associated with energy homeostasis\textsuperscript{11, 30}. In addition, animals with a mutation in the leptin gene are characterized by hyperphagia and extreme obesity, and these symptoms can be reversed by administering leptin\textsuperscript{35, 41-42}.

Insulin is secreted by the pancreas and studies have shown that the basal level of plasma insulin and the elevated level of insulin in response to increased blood glucose are related to the body fat mass\textsuperscript{43-45}. Moreover, insulin receptors are expressed in the central nervous system including the hypothalamus\textsuperscript{46-47}. Insulin-deficient animals are hyperphagic and the symptom can be eliminated by local injection of insulin into brain\textsuperscript{48}. In addition, chronic infusion of insulin into cerebrospinal fluid in primates resulted in predictable decrease in food intake and body weight\textsuperscript{49} whereas injection of insulin antibody into VMN in rats increases food intake and weight gain\textsuperscript{50}. However, recently whether insulin senses adiposity is questioned\textsuperscript{51} as it was found although insulin level increases during forced weight gain, it returns to a normal level on the first day of recovery\textsuperscript{14}. Similar results showing the basal insulin level decreases dramatically within few days after termination of chronic overfeeding have been reported\textsuperscript{15}. If insulin senses adiposity, a gradual decrease in the basal insulin level parallel to the chronic weight loss is expected, rather than an acute decrease in the basal insulin level.
Satiety signals in response to eating

Ingestion of food activates several gastrointestinal (GI) responses, including increased secretion of hormones such as cholecystokinin (CCK), glucagon-like peptide-1 (GLP-1), peptide YY (PYY), and a decrease in the secretion of ghrelin. It has been suggested that these hormones act as satiety signals.

CCK is secreted from the duodenal mucosa in response to nutrients in the GI tract, especially fat and protein. It activates the vagus nerve that projects to the ARC in the hypothalamus and activates POMC neurons, resulting in reduced appetite. Intravenous infusion of CCK significantly reduces food intake in humans, although this effect may be due to feelings of nausea rather than an effect on satiety. Results from studies using CCK receptor antagonists also support the appetite-suppressing effect of CCK. Moreover, this effect is enhanced when the stomach is distended. Nonetheless, the appetite-suppressing effect of CCK is short-lived and when CCK is infused more than 30 min before a meal, there is no significant impact on food intake. Continuous infusion of CCK does not influence food intake over 24 hours as the appetite-suppressing effect is compensated by increased meal frequency, resulting in unchanged energy intake.

GLP-1 is a hormone secreted from the ileum in response to ingestion of food. It is involved in the “ileal brake” mechanism that slows gastric emptying to reduce the flow of nutrients from the stomach to the small intestine. It also enhances insulin secretion and executes an anorexigenic effect through activation of sensory afferent neurons, which in turn, activates POMC/CART neurons in ARC. GLP-1 infusion reduces energy intake in a dose-dependent manner in human. Results from studies using a GLP-1 receptor antagonist or agonist.
further support the short-term appetite-suppressing effect of GLP-1. However, chronic
intraventricular infusion of GLP-1 does not influence body weight in both lean and obese rats\textsuperscript{75}.

PYY is secreted from the ileum and colon in response to the amount of calories ingested\textsuperscript{76}. It is hypothesized that it acts a signal to reduce appetite through vagal pathway afferent, which is mediated by inhibition of NPY neurons in ARC through Y\textsubscript{2} receptor\textsuperscript{77-78}. Consistent with the results from animal models\textsuperscript{74, 79}, several studies in humans have shown that peripheral infusion of PYY reduces food intake\textsuperscript{80-82}. Attenuated response of PYY has been found in obese people, which may account for their reduced satiety\textsuperscript{83}. However, based on the results from animal studies\textsuperscript{84-85}, the effect of PYY on body weight is inconclusive at this stage.

Ghrelin is the only known gut hormone that is orexigenic. It is mainly secreted by the fundus of the stomach\textsuperscript{86}. The plasma concentration of ghrelin decreases after a meal, then increases before initiation of the next meal\textsuperscript{87}. It stimulates synthesis of NPY and AgRP in ARC whereas inhibits POMC neurons, leading to increased appetite\textsuperscript{88}. Animal studies have shown that infusion of ghrelin increases food intake and adiposity\textsuperscript{89-90} whereas infusion of anti-ghrelin immunoglobulin inhibits normal feeding response after fasting\textsuperscript{91}. In healthy human participants, it has been shown intravenous ghrelin infusion results in an increase in food consumption in a free-choice buffet and enhances subjective appetite\textsuperscript{92}.

*Physiology of meal initiation and termination*

Several theories have been proposed to understand the physiology of meal initiation. The glucostatic mechanism, proposed by Mayer 60 years ago, suggests the role of blood glucose
on regulation of food intake\textsuperscript{93}. The theory suggests that the hypothalamus “glucoreceptor” senses the fluctuation in blood glucose to regulate meal initiation; it was found the difference in arterial and venous blood sugars is closely correlated with caloric intake and feeling of hunger\textsuperscript{93}. Campfield \textit{et al.} further expanded the role of blood glucose and proposed that meal initiation is dependent on blood glucose, such that a transient decline in blood glucose is sensed by the central nervous system to trigger food consumption\textsuperscript{94,95}. Several studies have found there is a brief transient decline in blood glucose, followed by a spontaneous meal request in time-blinded participants\textsuperscript{96-98}. Nonetheless, these studies require continuous blood withdrawal which can be invasive and have restricted participants’ behavior. Moreover, as blood glucose is regulated by various metabolic pathways involved in glucose production and utilization, it remains unknown what have caused the transient decline in the blood glucose. In addition, whether blood glucose is associated with appetite remains controversial\textsuperscript{99-101} and such a transient decline in blood glucose does not predict meal size. A recent study has shown intestinal glucose, rather than blood glucose predicts energy intake\textsuperscript{102}.

Feeding induced satiety response such as CCK secretion controls meal size by activating vagal afferent fibers that are projected to hypothalamus\textsuperscript{52, 103}. In addition, gastric distention during meal ingestion also activates vagal afferents, which send signals from the stomach to the brain and contribute to meal termination\textsuperscript{104-105}. Earlier studies on gastric distention and food intake suggest the correlation of stomach capacity and \textit{ad libitum} food intake was modest ($r=0.44$-$0.53$)\textsuperscript{106-107}. Moreover, various studies have shown that the weight or volume of food ingested was not different when foods with different macronutrient compositions were consumed\textsuperscript{108-110}.
Non-physiological factors that influence body weight and appetite

While a considerable body of evidence supports the presence of a body-fat set-point, it can also be seen that it does not adequately explain human body weight dynamics. For instance, it does not adequately explain why there has been a rapid increase in the number of obese individuals worldwide over the past 30 years. It does not explain why low socioeconomic groups are more prone to obesity in developed countries whereas individuals in higher socioeconomic groups are more prone to obesity in developing countries. It does not explain why watching television is associated with weight gain. It does not explain why getting married is associated with weight gain in adults. Consequently, it is argued that body weight is not physiologically regulated and its level is determined by a myriad of environmental factors that influence meal frequency or meal size.

It has been proposed that the increase in body weight over the past 30 years is driven by the increase in the total energy intake. Mounting evidence supports an association between obesity and certain types of eating behavior and food choices, such as consumption of energy-dense food, sugar-sweetened beverages, food in large portion sizes. A better understanding of how these non-physiological factors influence appetite and energy intake will provide dietary and behavioral strategies for body weight management.

While eating may be initiated in response to a decline in plasma glucose, it is unlikely that plasma glucose falls to the levels required to initiate a meal except for extreme metabolic emergencies. In a study that participants were asked to record food consumption and the reason of meal initiation, it was found nearly half of the participants ate “because it was mealtime” or “because of regular lifestyle”, whereas only 20.9% of them initiated a meal
“because I was hungry”, with the rest of them ate because of various reasons such as “I was reading/watching TV”, “I fancied food”\textsuperscript{134}. These data suggest meal initiation is more frequently driven by environmental and psychological clues rather than physiological needs. When the participants were asked the reason for meal cessation, over half of them indicated that “I had eaten enough” or “my stomach became full”, with 24.3\% of them stopped eating because “no food left” or “no drink left” or “no time to eat more” or “other people were watching my eating” or “I finished reading/watching TV”\textsuperscript{134}. Although meal termination tends to be a physiological controlled process, the results from this study suggest environmental and psychological factors can also influence the amount eaten. Indeed, people may continue to eat if there are more food or more time to eat\textsuperscript{135}. Moreover, simply seeing a food or it is convenient to get the food can initiate consumption even if the one is satiated\textsuperscript{136}. For example, people ate 2.2 more candies each day if the candy is put in a clear jar than a opaque jar and people ate 1.8 more candies when it is put on their desk than it is put 2 meters away\textsuperscript{137}. Other factors, such as the number of eating companions\textsuperscript{138-139} can also influence food intake.

Although these studies have shown environmental factors influence food intake, however, the mechanism is not clearly known. Wansink \textit{et al.}\textsuperscript{140} proposed that consumption norms help to determine how much we consume and this can be further influenced by other norms or cues in the environment, whereas consumption monitoring helps to reduce discrepancies between perceived and actual consumption levels and this can be biased by environmental factors. For example, people usually eat until plate or food package is empty. When self-refilling bowls are used, participants consume 73\% more than using a normal bowl but they
do not believe they have consumed more\textsuperscript{141}. By increasing the portion size it is observed food intake is significantly increased\textsuperscript{142-144}. People underestimate their consumption by at least 20\% when food in a larger package is consumed but they think the amount consumed is not affected\textsuperscript{140, 145}.

Sensory specific satiety (SSS) refers to the phenomenon that during food consumption, the feeling of pleasantness of the food being eaten decreases but other foods remain pleasant\textsuperscript{146}. Hetherington\textsuperscript{147} proposed SSS has contributed to meal termination. In his study, participants first had an \textit{ad libitum} access to a type of food; the same procedure was then repeated after 60 min for a second eating course, but participants were allowed to choose either the same food, a different food or no food\textsuperscript{147}. It was found 40\% of the participants stopped eating in the first eating course because “got tired of food” and for those who chose to eat at the second eating course, 78\% of them chose a different food and only 18.5\% stopped due to “got tired of food” in the second eating course\textsuperscript{147}. The results of the study suggest meal termination is influenced by the repeated exposure to the same food, which decreases the enjoyment and pleasantness of the food. It also explains for the studies that have shown increased food variety promotes food intake\textsuperscript{148-149}. In addition, the time-course of SSS has also been evaluated and it is found the pleasantness of food declines rapidly within 2 min after consumption and can be suppressed over a period of 60 min, but the change in pleasantness of uneaten foods is minimal\textsuperscript{150}. As shown by Hetherington\textsuperscript{147}, meal initiation for the same food during the postprandial period is less likely to occur as people tend to choose a different food. Similar results that people seek for flavor-based variety rather than brand-based variety have been reported\textsuperscript{151}. 

Ingestive behavior and appetite

It has been shown that many ingestive behaviors are associated with the obesity epidemic, including skipping breakfast\textsuperscript{152-153}, snacking\textsuperscript{154-155}, eating away from home\textsuperscript{156-157}, eating foods in large portion sizes\textsuperscript{127, 158}, eating fast food\textsuperscript{159-160}. While those studies deal with the broadly defined behaviors related to eating, the microstructures of ingestive behavior, such as bite size, mastication and eating rate, however, have received much less attention.

The hardness of habitual diet has been shown to be related with body weight in both human and animal studies\textsuperscript{161-162}, suggesting a possible effect of mastication on energy homeostasis. In addition, accumulating evidence suggests there is a positive relationship between eating rate and body mass index\textsuperscript{163-171}. Although explanations for the association between the microstructures of ingestive behavior and body weight have yet to be proposed, effects on appetite or food intake could be the possible mediator. In this section, the effect of eating rate, bite size and mastication on appetite and food intake will be reviewed respectively.

Eating rate

Several laboratory studies on eating rate and satiation have been conducted. Thirty years ago, it was found the eating rate between obese and non-obese people were different: the cumulative intake curves of non-obese people were negatively accelerated, indicating their eating rate gradually slowed down during ingestion; whereas obese people had an approximately linear curve with a relatively constant eating rate\textsuperscript{172}. In that study it was also found more food was ingested under the fast eating rate condition, regardless of body weight\textsuperscript{172}. A recent study has further explored the relationship between eating rate and food
intake and shown that linear eaters ate more food when eating rate was increased\textsuperscript{173}. In addition, in obese subjects it was found the initial eating rate was positively correlated with amount eaten\textsuperscript{174}. These results from laboratory studies suggest eating rate differs between lean and obese people, and increasing eating rate can enhance food intake. The effect of eating rate on energy intake could explain for the association between eating rate and body mass index in those epidemiological studies\textsuperscript{163-171}.

Andrade \textit{et al.}\textsuperscript{175} compared the satiation effect under slow and quick eating rate conditions and found that \textit{ad libitum} food intake was reduced and the satiating efficiency index, calculated by satiety rating/energy intake, was higher when eating slowly. In this study, participants were given a small spoon and instructed to take a smaller bite, make frequent intra-meal pauses and chew thoroughly in the slow eating rate condition\textsuperscript{175}. Similar results were reported by another study, which used a similar intervention and found participants achieved satiation quicker with comparable amount of food intake when eating slowly\textsuperscript{176}. While these studies support the claim that eating slowly promotes satiation, Yeomans \textit{et al.}\textsuperscript{177} found eating slowly by introducing within-meal pauses resulted in a significant increase in food intake. Several factors may account for these inconsistent results. First, methods for manipulating eating rate were not consistent. Controlling bite size, increasing the number of chews, introducing intra-meal pauses, or combination of these methods were used. Currently it is not known whether the method used to slow down eating rate matters. Second, the “definition” of slow and fast eating rate was not consistent in these studies, as it was hard to pre-specify the eating rate for participants. Third, the sample size in these studies was relatively small and characteristics of subjects (gender, BMI, age, etc) were different, which
may have contributed to the inconsistent results. For example, gender could have influenced the results; it was found eating slowly reduced food intake in women\textsuperscript{175} whereas another study had reported that it reduced food intake in men but not women\textsuperscript{178}.

Few studies have examined the effect of eating rate on post-prandial satiety but conflicting results have been reported\textsuperscript{179-181}. Karl \textit{et al.} conducted a study using a fixed portion of beef hash as the test food and manipulated the eating rate by a portable monitor; it was found there was no main effect of eating rate on any of the subjective appetite ratings, plasma concentration of appetite-regulating hormones and blood glucose over three hours\textsuperscript{179}. By contrast, the other two studies\textsuperscript{180-181} have suggested eating rate could influence some appetite-regulating hormones, unfortunately, the results were contradictory. The study by Kokkinos \textit{et al.}\textsuperscript{180} which introduced within-meal pauses to slow down eating rate had shown increased postprandial plasma concentrations of PYY and GLP-1 with no effect on ghrelin, insulin and glucose\textsuperscript{180}. The study by Sobki \textit{et al.}, however, had found a slower eating rate resulted in a significant increase in plasma concentration of ghrelin\textsuperscript{181}. Since PYY and GLP-1 are anorexigenic hormones whereas ghrelin is an orexigenic hormone, the later study indicates eating slowly was less satiating, while the study by Kokkinos \textit{et al.} suggests eating slowly was more satiating. Although the limited number of studies may prevent from drawing a conclusion, a closer examination reveals several differences in the treatment factors in those studies. The study by Kokkinos \textit{et al.} used a high fat ice cream as the test food and had a fixed meal duration of either 5 or 30 min\textsuperscript{180}, by contrast, the breakfast used in the study by Sobki \textit{et al.} had a typical balanced macronutrients composition with the average meal duration of 10 and 22 min in fast and slow eating rate conditions\textsuperscript{181}. Moreover, the
difference in meal durations and the different types of food among those studies make it difficult to compare them directly. It has been shown that eating rate of commonly consumed food varies a lot and it is inversely associated with energy density but positively associated with water content\(^ {182} \), therefore, there is a potential interaction effect of meal duration and food type.

Currently the mechanism to explain for the relationship between eating rate and appetite is unknown. However, several possible reasons could be considered. First, eating slowly allows more time for the physiological satiation signals to be developed before the excessive amount of food is consumed. Second, the strategies used to slow down eating rate may also have contributed to the appetite-suppressing effect. For example, eating slowly by taking a smaller bite size and chewing thoroughly would result in smaller particle sizes of the food bolus and alter bolus consistency. Animal studies had shown eating rate can be modulated by food consistency through brain histamine\(^ {183} \), which mediates the activation of the satiety center in rodents\(^ {184-185} \). Moreover, when the smaller food particles with increased total surface areas enter the GI tract, it would promote bio-accessibility of the nutrients, which could further influence the secretion of several satiety hormones\(^ {186} \). Third, the effect of eating rate on appetite could be mediated by sensory exposure. As the perceived sensation of sensory stimulus is a function of intensity and time course\(^ {187} \), when a meal is consumed quickly, less sensory exposure from each unit of food is perceived, requiring a larger amount of food to achieve a similar degree of satiation\(^ {188-189} \).

Other components of the microstructures of ingestive behavior can influence eating rate. For example, a smaller bite size or an increased mastication activity reduces eating rate\(^ {190-191} \).
Thus eating rate may not be viewed as an isolated treatment factor. Further studies with better strategies to control other factors related to eating rate, as well as studies to compare different methods to reduce eating rate, are required.

**Bite size**

A limited number of studies have been conducted to investigate the effect of bite size on food intake. By using a peristaltic pump to serve chocolate custard to healthy participants, Zijlstra et al. found participants consumed significantly more food when the bite size was larger\(^2\). In this study the way that food is delivered is atypical, but it gives a precise amount of food each mouthful for the participants. Similar results were reported by Weijzen et al., who found the *ad libitum* intake of orangeade was lower when consumed at a smaller sip size\(^3\). By contrast, Spiegel et al. found bite size does not influence the total amount of food consumed, as the meal duration decreased when bite size increased\(^1\). In this study nine lean and nine obese participants were recruited, but a comparison between two groups under five treatment conditions were made\(^1\). It is likely to be underpowered which resulted in a negative result.

The effect of bite size on food intake is further supported by a field study\(^4\). The investigators manipulated bite size using large and small forks in a local restaurant; after adjusting for confounding factors such as initial food weight, food price, and types of food consumed, it was found more food was consumed for consumers who used large forks\(^4\). Unlike the traditional laboratory studies, this study has better external validity and provides practical applications for both diners and restaurant owners.
The effect of bite size on food intake and perceived satiation could be mediated by oro-sensory or retro-nasal sensory perception. It has been shown that making multiple smaller bites resulted in significantly higher cumulative release of retro-nasal aroma, compared with the condition where the same amount of food was consumed in a manner with fewer but larger bite size\textsuperscript{195}. An increased sensory stimulation when a smaller bite size is made contributes to the development of sensory specific satiety, which is a key determinant of meal size\textsuperscript{147}.

*Mastication*

As all projects involved in this dissertation are related to mastication, a brief overview of mastication, assessment of mastication performance, factors that affect mastication performance and how it influences appetite will be discussed here.

Mastication is the first step involved in ingestion of solid food, to breakdown food particles and to prepare a bolus suitable for swallowing. The masticatory system includes teeth, temporomandibular joints, muscles involved in mastication (masseter, temporal muscle and tongue), and nervous and vascular systems associated with these muscles\textsuperscript{196}. When food enters the oral cavity, it is transported from the front of the mouth to the occlusal surfaces of the post-canine teeth, followed by a series of masticatory cycles until a suitable bolus is formed before it is swallowed\textsuperscript{197-198}. Action of saliva also contributes to the bolus formation\textsuperscript{199}. The size of the food bolus and the degree of lubrication determine the swallowing threshold\textsuperscript{200}.
Assessment of mastication performance can be achieved through recording of jaw movements and measurement of the particle size of the chewed bolus. Several quantitative methods for recording jaw movements have been used, such as electromyography (EMG)\textsuperscript{201-204} and video recording\textsuperscript{205}. The EMG method is more frequently used in assessing the microstructure of mastication. In such studies, a computer is connected to the electromyographic recording device, and electrodes from the device are attached to temporalus and masseter muscles of the participants. Participants are then instructed to chew the test food and the muscle activity will be recorded. Unlike video recording, the EMG methods provide information including not only chewing duration, number of chews and chewing rate, but also other microstructure of mastication, such as bite force and muscle activity\textsuperscript{206}.

Particle size of the chewed bolus can also be used as an indicator of mastication performance. In such studies, chewed bolus is expectorated and collected. Several methods can be used to quantify particle size, including sieving\textsuperscript{207}, optical scanning and image analysis\textsuperscript{208-209}, and laser diffraction\textsuperscript{210}. Among these methods, sieving is the most widely used, probably because it is more feasible as the equipments required are simple compared with other methods. Moreover, comparison of the sieving method with other methods suggests a consistent result can be obtained\textsuperscript{209,211}. Nonetheless, the variation in recovery rate is huge\textsuperscript{207,212-213} because it is not possible to collect every food particle from the oral cavity by spitting. Therefore, particle size data is usually expressed as the percentage of bolus weight for each particle size range. In some studies, the median particle size, which is the theoretical sieve through which 50\% of the particle weight can pass, is used\textsuperscript{214-216}. 
Several factors can affect mastication performance. Food characteristics including food hardness\textsuperscript{217-218}, food size\textsuperscript{219} and food type\textsuperscript{220} have a significant impact on mastication performance. Generally, harder and larger food requires increased mastication activity. Moreover, given a certain type of food, there is also a considerable inter-individual variation in masticatory performance, for example, the number of chews required for carrots ranged from 9 to 65 and it was 14 to 44 for Brazil nuts\textsuperscript{221}. Therefore, internal factors (factors related to the characteristics of subjects) must have contributed to the variation in mastication performance.

Internal factors such as age and gender, as well as characteristics of the oral systems, including the number of teeth, bite force and salivary flow rate could influence masticatory performance\textsuperscript{197, 222}. Among these factors, age-related change in mastication has been extensively studied. Older adults require more number of chews and longer chewing time to form a bolus before swallowing than young adults\textsuperscript{201, 213, 223-225}. Meanwhile, they have a lower bite force\textsuperscript{201, 222, 225}, and they have difficulties in adapting mastication to the change in food texture in mouth\textsuperscript{201, 224, 226}. However, the particle size of the bolus before swallowing does not differ between young and older adults\textsuperscript{213}, and there is no difference in the amount of saliva incorporated in the bolus\textsuperscript{224}. It is possibly because older adults compensate their reduced chewing ability by increasing the number of chews; this would prolong chewing duration and enhance saliva production, until a suitable bolus is formed. Age-related teeth loss and deteriorated muscle strength could have accounted for their reduced chewing ability\textsuperscript{201, 225, 227-228}. Although nowadays missing teeth are often replaced by prosthodontic
apparatus, it has been shown that denture wearers still experience impaired masticatory function compared with people with full dentition\textsuperscript{213, 229}.

An internal factor that has gained little attention is body weight. A limited number of studies have been conducted to investigate the difference in habitual chewing behavior between lean and obese people. Using almonds as the test food, Frecka \textit{et al.} found no statistically significant effect of BMI on mastication performance, measured by both EMG and particle size of the bolus\textsuperscript{207}. By contrast, Li \textit{et al.} found obese subjects required less number of chews before swallowing than lean subjects\textsuperscript{230}. However, the study by Smit \textit{et al.} failed to show a significant difference in the habitual number of chews between lean and obese participants\textsuperscript{190}. While the limited number of studies with a relatively small sample size has contributed to the controversy, results from epidemiological studies related to body weight and mastication may provide further clues.

It has been found the hardness of habitual diet is negatively associated with the waist circumference in Japanese women\textsuperscript{161}. In addition, a recent study\textsuperscript{231} has investigated the association between body weight and mastication performance in children; it was found overweight and obese children presented a larger median particle size at the swallowing threshold than normal-weight children\textsuperscript{231}. Results from those epidemiological studies suggest there may be a potential effect of mastication on body weight, the mechanisms, although not fully understood at this stage, could be medicated by an effect on appetite and energy intake, as animal studies reveal mastication activates satiety center through histaminergic pathway\textsuperscript{184, 232} and leads to a higher adiposity\textsuperscript{162}. 
Currently there are two published studies that have evaluated the effect of mastication on satiation. Smit et al. conducted a study using 11 participants, who were required to attend two test sessions to consume the *ad libitum* pasta meal in a different manner\(^{190}\). The results suggest increasing the number of chews from 10 to 35 chews per mouthful resulted in 12% reduction in food intake\(^{190}\). Similar results have been reported by Li et al.\(^{230}\). In that study, 16 lean and 14 obese Chinese consumed pork pie for breakfast until comfortably full; it was found after adjusting for body weight, the energy intake was 11.9% lower in the 40 chews condition than the 15 chews condition\(^{230}\). The same participants in this study also attended another two test sessions, in which a fixed portion of pork pie was chewed either 15 or 40 times before swallowing each mouthful, and markers of post-prandial satiety were measured for three hours\(^{230}\). It was found although there was no effect of chewing on subjective appetite, a lower plasma concentration of ghrelin and higher plasma concentrations of CCK and GLP-1 were found in the 40 chews session\(^{230}\). Cassady et al. had also investigated the effect of mastication on postprandial satiety\(^{233}\). It was found hunger was acutely suppressed and fullness was elevated for a longer period, with a higher GLP-1 level after 40 chews than after 25 chews\(^{233}\).

Those results suggest mastication promotes both satiation and satiety and could explain for the relationship between dietary hardness and body weight observed in previous studies\(^{161-162}\). Several factors could have contributed to the effect of mastication on appetite. First, increasing the number of chews results in a reduction of the eating rate\(^{190}\) as well as a further reduction of the particle size of the bolus. Meanwhile, mastication is a key stimulus for the
cephalic phase response which affects appetite\textsuperscript{234-235}. In addition, animal studies have shown mastication activates the satiety center via histamine neurons\textsuperscript{185, 236}.

\textbf{Aging and appetite}

The absolute number and the percentage of older adults are increasing throughout the world as the average life expectancy is increasing\textsuperscript{237-239}. Therefore, it is important to understand how aging changes appetite and regulation of energy homeostasis, in order to provide better strategies to promote nutrition and health status for older adults.

The disturbance in energy homeostasis in older adults is characterized by anorexia of aging leading to decreased body mass\textsuperscript{240-241}. Although conflicting results have been reported\textsuperscript{242}, it has been shown that aging is associated with dysregulation of appetite, which may account for their reduced energy intake and weight loss\textsuperscript{58, 243-248}.

Several factors may have contributed to the impaired appetite response in older adults, including psychological factors, environmental factors such as age-associated change in lifestyles, and medical conditions\textsuperscript{241, 249-250}. Moreover, physiological factors, including reduced feeling of hunger\textsuperscript{240, 243}, change in the response of appetite-regulating hormones\textsuperscript{251}, alteration of regulators of food intake in the central nervous system\textsuperscript{252}, slower gastric emptying\textsuperscript{243}, alteration in glucose homeostasis\textsuperscript{253}, impaired taste and smell sensation\textsuperscript{254}, and reduced dietary variety\textsuperscript{255}, may account for the dysregulation of food intake in the older adults. These physiological factors will be further discussed in this section.

It is found that older adults have a significantly lower baseline rating of hunger after overnight fasting\textsuperscript{248, 256}. In addition, lower postprandial hunger and desire to eat are also
associated with aging. A recent study has revealed that the average and peak hunger and desire to eat over 24 hours are significantly lower in older adults. These results suggest the reduced food intake and weight loss in older adults could be partly due to a lower feeling of hunger and a lower desire to eat.

Satiety signals involved in appetite are also altered due to aging. It has been reported that the baseline plasma level of CCK was higher in older participants although others found no difference in baseline CCK plasma level between young and older adults. The elevation in postprandial CCK was also higher in older adults, which is probably due to its delayed clearance. In addition, the satiety property of CCK appears to be more potent in older adults as infusion of CCK resulted in a suppression of energy intake, which was twice that in young adults. Comparable results on the orexigenic hormone ghrelin have been found. For example, aging is associated with a lower baseline plasma level of ghrelin. In addition, an impaired ghrelin response has been reported in this population. Currently the data with regard to aging and other satiety hormones, such as GLP-1 and PYY are very limited and inconclusive. However, it has been shown that there is a significant inverse relationship between plasma level of leptin and age. Moreover, age-related impairment of leptin action, including reduced responsiveness and impaired signal transduction, leading to diminished decrease in food intake, was revealed by animal studies. All these findings suggest the patterns of appetite-regulating hormones are altered in older adults.

Animal studies suggest an age-associated change in the expression of orexigenic neuropeptides such as opioid peptides, NPY, and anorexigenic neuropeptides CART and POMC. Moreover, administration of those neuropeptides elicits different responses in
young and old animals. For example, intracerebroventricular injection of NPY stimulated food intake in young rats but did not affect food intake in old rats\textsuperscript{251}. Intravenous infusion of naloxone, an opioid antagonist in human resulted in a greater reduction in food intake in young participants, although the reduction was not significantly different from that in older participants\textsuperscript{274}. These results, although not conclusive at this stage, suggest a potential impairment of central regulators of food intake due to aging.

A slower gastric emptying rate presumably prolongs gastric distention, which activates satiety circuitry in brain\textsuperscript{104}. It has been shown that the gastric emptying for solid and liquid food was prolonged in older subjects compared with young subjects\textsuperscript{243, 275-276}. A decrease in smooth muscle relaxation resulted from failure of adequate production of nitric oxide due to aging\textsuperscript{277} would partly account for the mechanism. While antral distention is positively related to both satiation and satiety\textsuperscript{256}, a slower gastric emptying rate may contribute to longer feeling of fullness\textsuperscript{278}.

Aging is associated with a progressive elevation in the glucose tolerance curve\textsuperscript{253}. It has been reported that following consumption of 2092 and 4148 kJ meal, older individuals showed an exaggerated response and a delayed return to the basal levels of glucose and insulin\textsuperscript{279}. This could be partly due to delayed gastric emptying rate in older adults. Some other studies have also shown reduced insulin sensitivity as their post-prandial insulin response is higher than young adults\textsuperscript{260, 279}. The elevated postprandial blood glucose and reduced insulin sensitivity could potentially contribute to an attenuated return of hunger\textsuperscript{240}. Moreover, the high level of circulating insulin has a central satiety effect, which activates POMC neurons and inhibits NPY/AgRP neurons, leading to reduced appetite and anorexia\textsuperscript{6}. 
Reduced chemosensory perceptions occur in older adults\textsuperscript{254, 280-281}. The thresholds for taste and smell sensation are higher and the attenuated brain regions where taste and smell sensations are processed contribute to the lower sensitivity of taste and smell in this population\textsuperscript{254}. Taste and smell are vital stimuli for cephalic phase response, which elicits initial increase in gastric and pancreatic secretions to prepare for digestion, and partly contributes to the development of satiation signals\textsuperscript{234-235}. In addition, taste and smell are the key sensations for the judgment on the food palatability, which has an impact on appetite and hedonic response\textsuperscript{282}. Therefore, it is likely that the impairment of taste and smell sensations partly mediates the dysregulated food intake in the older adults.

Some studies\textsuperscript{255, 283-284} but not all\textsuperscript{285-286} reported that older adults have less dietary variety. It is reported that lower socioeconomic status may account for their lower dietary variety\textsuperscript{287}. Another possible reason is age-associated change in dental function may limit their food choices\textsuperscript{288}. Moreover, older adults, unlike young adults, failed to develop sensory-specific satiety\textsuperscript{289}, suggesting it is possible that lacking of sensory-specific satiety may be accompanied by a failure to respond to the enhancing effects of variety in the diet\textsuperscript{290}.

**Measurement of appetite**

Appetite is an abstract concept therefore it is not possible to be measured directly. Currently, three types of indirect assessments are widely used: food intake as an index of appetite\textsuperscript{291}, questionnaires for assessment of subjective feeling of appetite\textsuperscript{292-293} and biomarkers of appetite\textsuperscript{186}. In this section, the methodology for each of those measurements will be discussed.
Food intake

In free-living conditions, food intake is usually estimated by self-reported energy intake. However, the food intake data collected under free-living conditions is not accurate due to under-reporting\textsuperscript{294-295}. Methods such as doubly labeled water technique can be adopted to determine the reliability of dietary report in free-living conditions\textsuperscript{296}. Recently, technology-based methods for dietary assessment such as digital photography have gained more attention, although more studies should be conducted to evaluate their validity and reliability\textsuperscript{297-298}.

An advantage of free-living studies is the higher external validity. By contrast, laboratory studies have higher internal validity due to tightly controlled experimental conditions. In laboratory conditions, food intake can be directly measured. Nonetheless, various factors can influence the amount eaten in laboratory conditions. For example, in studies with a preload meal provided, participants may be asked to consume food at a subsequent meal as a measurement of satiety. It has been found the interval between two meals can influence the amount eaten\textsuperscript{299}. Moreover, the palatability of food\textsuperscript{300-301}, environmental clues\textsuperscript{139}, and consumption norm\textsuperscript{140} can also influence the amount of food consumed. Therefore, it is vital to control these factors in designing experiments that measure food intake in laboratory as an indicator of appetite.

One should be aware that food intake and subjective appetite may not always be tightly coupled. Studies have found no significant association exists between hunger and eating or thirst and drinking in free-living conditions\textsuperscript{302-303}. This is not surprising as human ingestive behavior is also influenced by several environmental and cognitive factors. Lack of availability of food or social constraints may refrain one from eating when hungry\textsuperscript{291},
whereas stress could induce eating even if the one is not hungry\textsuperscript{304}. In fact, the correlation between appetite and food intake was only moderate, for example, the correlation coefficient was 0.32 for pre-meal hunger and food intake, and it was -0.43 for pre-meal fullness and food intake\textsuperscript{305}.

\textbf{Questionnaires}

Questionnaires have been widely used for assessment of subjective feeling of appetite. A typical questionnaire consists of a set of questions with regard to appetite, such as “how hungry do you feel right now” with a quantitative scale or a categorical scale to capture a response to the question. The most widely used scale is the visual analogue scale (VAS). Under each question, there is a 100 mm or 150 mm line, which is anchored with opposing statement in each end, such as “not hungry at all” and “as hungry as I have ever felt”. Participants are instructed to draw a mark on the line as the response to the question. In questionnaires where categorical scales are used, participants are instructed to circle the category which reflects their feeling of subjective appetite. A comparison of VAS and categorical scales has shown little difference in the outcome measures\textsuperscript{306}. Another type of scale, although not commonly used, is the labeled magnitude scale with a quasilogarithmic spacing between labels\textsuperscript{307}. This scale has multiple labels between the most positive and the most negative statement at each end, with the space between labels being non-linear. It is more frequently used in sensory studies as it provides comparable results for measurement of “broadly defined” sensations such as taste\textsuperscript{308-309}. However, whether the perceptual sensation for the strongest imaginable hunger and fullness from participants is the same as the
reference used for scaling is in doubt; this might have limited its application in appetite studies.

Recently electronic appetite rating systems such as Apple Newton and Palm Pilot systems have been used in appetite studies\textsuperscript{310-311} and they have been found to produce comparable results to the traditional paper questionnaires\textsuperscript{312-313}. The advantage of using electronic questionnaires is that records entered by participants are marked with time and date information, providing an additional way for the investigator to verify protocol compliance when used under free-living conditions.

A flaw from theoretical basis for assessing subjective appetite is that the mathematical difference measured by VAS should not be assumed to be equal to the perceptual difference in appetite sensations. For example, hunger rating at 100 mm does not mean the feeling of hunger is twice as that when hunger rating is 50 mm; the change on rating from 10 to 20 mm does not necessarily equal to the change on rating from 80 to 90 mm. This actually violates the assumption of statistical analysis. Nonetheless, due to the difficulty in quantifying psychological terms, VAS is still widely used in research in various disciplines to measure subjective phenomena\textsuperscript{314}. Moreover, the reliability and validity of VAS in appetite studies have been tested and the results suggested it is a reliable and valid measurement\textsuperscript{292, 305, 312, 315-318}.

**Biomarkers**

The most commonly used biomarkers are appetite-related hormones such as CCK, GLP-1, PYY, ghrelin, insulin and metabolites such as glucose. Feeding-induced changes in hormones
are usually measured by taking blood samples, the plasma concentrations of these hormones are then quantified by enzyme-linked immunosorbent assay (ELISA) or radioimmunoassay (RIA). However, whether those biomarkers are associated with appetite is still inconclusive. For example, it has been shown that there are statistically significant correlations \((r=0.5-0.7)\) between subjective appetite ratings and plasma concentrations of gut hormones\(^{319-320}\), whereas some other studies did not show a correlation\(^{321-322}\). The relationship between blood glucose and appetite is also not confirmed: some studies have shown food intake and subjective appetite are negatively associated with blood glucose\(^{101, 323}\) whereas others do not\(^{99-100}\). These inconclusive results are partly due to various populations and test foods studied. Moreover, the concentration of hormones measured from blood samples may not necessarily reflect its concentration at the local sites of secretion and action.

Gastric distention can be viewed as another biomarker as there is clear evidence showing the role of stomach distension on appetite and food intake\(^{104, 106-107}\). It can be measured by magnetic resonance imaging\(^{324}\) but it has not been widely used, probably due to the cost, especially for studies with a large sample size. As a slower gastric emptying rate would presumably prolong the gastric distention, measurement of gastric emptying rate, either by acetaminophen absorption test\(^{325}\) or carbon-labeled octanoic acid breath test\(^{326}\), is more frequently conducted in appetite studies to evaluate effects of physical properties of food\(^{327-328}\). Significant correlations between gastric-emptying rate and sensation of satiety and hunger have been reported\(^{329-331}\).

Functional neuro-imaging techniques such as positron emission tomography (PET) and functional magnetic resonance imaging (fMRI) can be used to measure brain activity in
response to food stimuli. As satiation and satiety signals are integrated in the brain, it is possible that measuring brain responses can serve as markers of appetite. However, as these techniques require expensive equipment and they have not been widely used in appetite studies. Moreover, fMRI and PET measure associated changes in blood flow rather than direct measurement of brain neural activity, therefore, the results measured by these techniques may not necessarily be due to a causal effect.

**Hypothesis and objectives**

The long-term objective of the research is to explore the relationship among mastication, appetite and body weight, in order to provide effective dietary and behavioral strategies for body weight management. The central hypothesis is that body weight is related to habitual mastication performance whereas appetite is the bridge that links them together.

The working hypotheses in this research are:

1. Body weight and age are variables that contribute to habitual mastication performance in adults.
2. Increasing the number of chews promotes satiation in an *ad libitum* meal in young and older adults.
3. Increasing the number of chews during a fixed-amount meal promotes postprandial satiety in young and older adults.

To test the working hypotheses a series of experiments were conducted, in order to investigate the correlation between body weight and mastication performance, and to test the effect of ingestive behavior (increasing the number of chews) on appetite. As aging-
associated changes in both mastication performance and appetite have been widely
demonstrated, another objective of the current research is to provide individualized
information for both young and older adults.

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CHAPTER 3. RELATIONSHIP BETWEEN BODY MASS INDEX AND MASTICATION PERFORMANCE: THE EFFECT OF AGING AND FOOD HARDNESS

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Abstract

It has been clearly shown that mastication performance is affected by both food characteristics and subject characteristics such as age and gender. It is not known, however, if there is a relationship between mastication performance and body weight, although accumulating laboratory studies suggest mastication may play a role in the regulation of energy balance. To investigate the relationship between mastication and body weight, we conducted two studies. We first conducted a study to explore the possible relationship between habitual masticatory parameters and body variables in young adults, followed by a second study in which both young and older participants chewed carrots sample with different hardness until their normal swallowing threshold, with the microstructure of mastication being measured by electromyographic recording. We also assessed the particle size distribution of food bolus before swallowing. A significant negative correlation between the habitual number of chews and BMI was found in both studies. The electromyographic
recording data suggest older adults had a reduced masticatory efficiency compared with young adults, whereas food with higher hardness also resulted in higher masticatory efficiency. However, the particle size distribution was less affected by age and food hardness and there is no association between particle size and BMI. In summary, these results indicate body weight, aging and food hardness affect the microstructure of mastication. Moreover, the results suggest a potential direction in research to elucidate the mechanisms for the relationship between mastication and body weight.

**Keywords:** mastication, body weight, aging, hardness

**Introduction**

Mastication is the first step involved in the ingestion of solid food; it reduces particle size of food to form a bolus suitable for swallowing with the action of saliva [1-2]. It is generally known that mastication affects nutritional status in human [3-5]. For example, people with impaired mastication may alter their food choices and this could result in an unbalanced diet with potential nutrient deficiency [6-7].

Mastication performance is influenced by food characters such as food hardness and food type [8]. It is also determined by internal characters of the subject, such as dental status, age and gender [9]. For example, aging is associated with an impaired masticatory performance [10-11]. It has been reported that the elderly require a greater number of chews before swallowing threshold and a longer chewing duration [12].

Body weight is another internal character that may be associated with mastication and there is a considerable variation in body weight among population. Accumulating evidence from
recent research suggests mastication may play a role in the regulation of energy balance [13-22]. Studies have shown increasing the number of chews per mouthful before swallowing suppresses post-prandial satiety [15, 17] and reduces food intake in an *ad libitum* meal [15-16]. It has been reported that masticatory efficiency influences nutrient bioaccessibility and absorption [17] as well as post-prandial protein metabolism [18]. In addition, the degree of particle size breakdown during mastication affects glycemic responses [14, 19]. Animal studies, on the other hand, have shown mastication contributes to the activation of the satiety center in hypothalamus through histamine neurons [13, 20]. Meanwhile, rodents fed with hard chows have shown a lower level of adiposity [21] and enhanced glucose metabolism [22]. While all these results indicate the influence of mastication on several factors involved in regulation of energy balance, currently less is known about the relationship between masticatory performance and body weight.

The aim of the research was to explore the possible relationship between mastication performance and body mass index (BMI). We conducted a study in young adults to investigate the association between habitual chewing parameters and body variables, followed by another study in young and older adults to investigate the effect of aging on masticatory performance in response to foods with different hardness.

**Methods**

**Study 1**

Adults aged from 18 to 40, with a full set of natural teeth and self-reported good health, were invited to participate in the study. During the study session, their body weight and height
were measured. Five pieces of Totino’s cheese pizza rolls (General Mills Inc., Minneapolis, MN, USA) were provided as the test food. Nutrient label from the manufacturer reported every six rolls provided 837 kJ (200 kcal) energy and had 7 g protein, 26 g carbohydrate and 8 g fat. The weight for each roll was approximately 14 g.

Participants were instructed to put one piece of pizza roll in mouth at a time and consume it in their usual manner. The number of chews they made before swallowing (a complete cycle of jaw movement) was counted by research personnel and the chewing duration was recorded. The same process was repeated for five times. The habitual masticatory parameters, including the number of chews, chewing duration and chewing rate (the number of chews per second) were obtained by averaging the results from the five replicates. Linear regression analysis was then performed by SPSS (v17.0, SPSS Inc., Chicago, IL, USA), using each of the masticatory parameters as the dependent variable, gender, age and BMI as the independent variables. Gender was coded as a categorical variable, using 0 for males and 1 for females.

Study 2

Participants

The study was advertised by a mass e-mail sent to students and retired faculty, and by fliers distributed throughout the local community. Individuals interested in the study were invited to attend a screening session during which a detailed description of the study protocol was explained and a screening questionnaire was completed. Inclusion criteria for the study were: age 18-40 or ≥ 65, BMI between 20.0 and 29.9, a full set of natural teeth or well-fitted
dentures and a willingness to eat the test foods. Participants were excluded from the study if they: were using tobacco products, had presence or history of gastrointestinal disease, had presence of acute diseases, were using medication that influences ingestive behavior or appetite, were restrained eaters (>13 on the restraint section of the three-factor eating questionnaire [23]), had an allergy or intolerance to the test foods or rated palatability of any of the test foods less than 6 on a 9-point scale.

Test food

Dole® mini cut carrots (Dole Fresh Vegetables, Inc., Monterey, CA, USA) were used as the test food in the study. Nutrient labeling reported by the manufacturer reported each serving size (85 g) provided 147 kJ (35 kcal) energy and had 8 g carbohydrate and 1 g protein. Carrots with diameter of 1 cm and length of 4 cm were selected; the weight for each carrot sample was approximately 8.6 g.

Four treatments were involved in the study: raw whole carrot (RWC), raw chopped carrot (RCC), cooked whole carrot (CWC) and cooked chopped carrot (CCC). To prepare CWC, RWC was cooked for 15 minutes in boiling water. To prepare RCC and CCC, RWC and CWC were chopped into four portions respectively; each portion had the diameter of 1 cm with the length of 1 cm.

The hardness of carrots was measured by Instron Universal Testing Machine (Model 5566, Norwood, MA) with the Warner Bratzler shear attachment. A speed at 200 mm/min for the cross-head was applied during measurements and the maximal compressive load for each sample was recorded.
General procedure

On the test day, participants were required to report to the laboratory at 7:30am after an overnight fast. Each participant was taken to an isolated room and asked to chew a small piece of raw carrot to determine the dominant chewing side of their mouth. A cup of distilled water was then used to rinse their mouth prior to the electromyographic recording.

BioPac MP36 (BioPac Systems, Inc., Goleta, CA, USA) was used to assess the microstructure of mastication. The temporalus and masseter on the dominant chewing side of the participant were identified by palpation, and a bipolar surface electrode was placed on each muscle, apart by approximately 3 cm. A third electrode was placed on wrist on the same side. A 5-minute acclimatization period was given, followed by presentation of a series of four carrot varieties in a random order, each in duplicate. For each carrot sample, participant put the whole sample in mouth at a time and chewed it until they were about to swallow. Until the swallowing threshold, they expectorated the bolus into a container and rinsed their mouth with 20 ml distilled water for three times. Each rinse was expectorated into the same container. The same procedure was repeated for all the eight samples.

Masticatory performance

Signals from electromyographic recording were translated into microstructures of mastication, including number of chews (the number of peaks), chewing duration (signal duration for each carrot sample), chewing rate (calculated as dividing the number of peaks by chewing duration), maximal bite force (maximal amplitude of the electric potential), mean
bite force (mean amplitude of the electric potential) and muscle activity (the total integral over time) [11, 24-25].

Particle size analysis of the food bolus

Each expectorated sample was transferred to a clean dish and dried at 54 °C for 6 hours in a food dehydrator (Nesco FD-75PR, The Metal Ware Corporation, Two Rivers, WI, USA). The dried sample was weighed and separated through a set of five sieves (WS Tyler, Mentor, OH, USA) yielding the following particle size ranges: >4 mm, 3.35-4 mm, 2-3.35 mm, 1-2 mm, 0.5-1 mm, <0.5 mm. Weight from each fraction was expressed as the percentage of the total weight.

Statistical analysis

All data are presented as mean ± standard error. SPSS (v17.0, SPSS Inc., Chicago, IL, USA) was used to perform the statistical analysis. A general linear model with fixed factors of age (young or older) and treatment (carrot varieties), using BMI as a covariate, was applied on each of the mastication parameters as well as data from each category of the particle sizes. If a significant effect was found, least square means were computed followed by comparison with Bonferroni correction when necessary. Pearson correlation test was performed for the mastication parameters. To explore the possible association between BMI and mastication performance, partial correlation analysis was performed by controlling for age and treatment. The statistical significance was set at P<0.05, two-tailed.

Both studies were approved by the Iowa State University Institutional Review Board and all subjects signed an informed consent form before being included in the study.
Results

Study 1

Sixty-four adults (25 males and 39 females, BMI ranges from 19.5 to 44.8) participated in the study. Statistics for the regression models were summarized in table 1. The regression analysis revealed that there was a significant negative association of BMI and the number of chews (P=0.020). Similar results were found for BMI and chewing duration (P=0.005) but not for BMI and chewing rate (P=0.493). In addition, increase in age was associated with increase in the number of chews (P=0.048) but it was not related to chewing rate or chewing duration. Moreover, males have a shorter chewing duration (P=0.049) with a faster chewing rate (P=0.042) compared with females.

Study 2

Participants

Eleven young adults (7 males and 4 females) and eleven older adults (6 males and 5 females) completed the study. Table 2 shows the results of the anthropometric measurement, score from the three-factor eating questionnaire and their palatability ratings on each of the test foods.

Maximal compressive load of test foods

The RWC required the highest maximal compressive load (40.6±0.8 N), followed by RCC (30.1±0.9 N), CWC (3.6±0.4 N) and CCC (2.0±0.2 N).
Masticatory performance

Figure 1 shows the effect of age and carrot varieties on masticatory performance. Adjusting for BMI, a significant effect of age on the number of chews (P<0.001), chewing duration (P<0.001) and muscle activity (P=0.004) was found. Young adults had less number of chews, shorter chewing duration and lower muscle activity. However, there was no difference on maximal or mean bite force, as well as chewing rate between young and older adults (P>0.05).

Treatment effects were significant on the number of chews (P<0.001), chewing duration (P<0.001), maximal bite force (P=0.020), mean bite force (P=0.041), muscle activity (P<0.001) and chewing rate (P<0.001). Post-hoc comparison revealed that both RWC and RCC resulted in greater number of chews, longer chewing duration and greater muscle activity than both CWC and CCC (P<0.05). However there was neither difference between RWC and RCC, nor between CWC and CCC on these variables (P>0.05). CCC resulted in a faster chewing rate than RCC (P=0.011), CWC resulted in a faster chewing rate compared with RWC and RCC (P<0.05). No significant treatment by age interaction was found on any of the mastication parameters.

Significant correlations between most pairs of variables of mastication performance were found (Table 3).

Particle size of food bolus

The effect of aging was significant on the particle size from 0.5-1 mm (P=0.018) and there was a trend toward significance on the particle size from 1-2 mm (P=0.057), indicating older
adults had a greater percentage of particles in these two particle size ranges. The effect of treatment was not significant, however, a significant age by treatment interaction on some of the particle sizes were found (P<0.05) (Table 4).

*Correlation between BMI and mastication*

Controlling for treatment and age, the correlation between BMI and number of chews was significant (r=-0.194, P=0.010). Significant correlations were also found between BMI and maximal bite force (r=-0.235, P=0.002), BMI and mean bite force (r=-0.311, P<0.001), BMI and muscle activity (r=-0.323, P<0.001), BMI and chewing rate (r=-0.170, P=0.025). However, the correlation between BMI and chewing duration was not significant (r=-0.111, P=0.143).

The correlation between BMI and each particle size range was week (correlation coefficient ranges from -0.10 ~ 0.05) and none of them were significant (P>0.05).

**Discussion**

In these two studies we found a significant negative association between the number of chews made before the swallowing threshold and BMI. In addition, aging and food hardness have significant effects on mastication performance as assessed by electromyographic recording but particle size distribution was less affected.

It must be emphasized that the association between the number of chews and BMI found in the research does not necessarily implicate that the less number of chews made before swallowing contributes to the development of obesity as the association does not mean a
causal relationship. Additional laboratory studies should be conducted to provide a reasonable explanation for such an association. As mentioned previously, recent studies suggest the potential role of mastication on the regulation of energy balance, however, the mechanism is poorly understood at this stage.

In this study, it was found the number of chews had a significantly strong correlation with chewing duration (r=0.861). Theoretically, an increase in the number of chews with longer meal duration would contribute to a slower eating rate. Positive association between eating rate and body weight has been reported in several epidemiological studies [26-28]. Laboratory studies, on the other hand, also suggest obese people eat faster compared with subjects with normal body weight [29-30]. Moreover, recently eating rate has been suggested as a factor that influences satiation [31] and satiety [32], whereas behavioral modification to slow down eating rate significantly reduced body weight [33]. In view of those results, it is possible that eating rate acts as mediator connecting mastication performance and body mass index.

It has been reported that given the same food, the intra-individual variation on mastication was small; however, the inter-individual variation was much larger [34-35]. In the first study, the test food used was consistent for all participants and only participants with full set of natural teeth were invited, it is noticed that from the regression models established, the variables BMI, age and gender only accounted for 10-20% variation in the habitual chewing parameters, suggesting other internal factors also contribute to the variation in parameters involved in habitual chewing behaviors. Currently there are not much clue and clear evidences for such factors, but it is probably because of different perceptions of food texture
and the variation in perception of food portion size among different people [37]. In addition, inter-individual variation in saliva flow rate and composition [38], oral physiology [39] and muscle strength [40] may also play a potential role.

Compared with young adults, the older adults exhibited reduced mastication efficiency in this study. Similar results have been reported by others using either a single test food or the same food with different textures [11, 41]. Nonetheless, different results have been reported by others [42-43]. The study by Kohyama et al., using various types of food had shown cheese, bread, apple and peanut resulted in difference between young and older subjects, but no difference was found when rice was used [42]. The other study had shown no difference in mastication performance between young and older subjects except that the maximal bite force was lower in older subjects using six different types of food [43]. Further studies should be conducted to investigate the effect of aging on mastication efficiency using different types of food, especially foods with dramatic difference in texture and hardness. It is possible that the difference in mastication efficiency between young and older adults may gradually diminish as the test food becomes less hard.

It was found that food hardness affects mastication performance in the second study. Similar results had been reported by using almond as the test food [44]. A recent study has shown histamine release in brain is modulated by food hardness [45], while anti-obesity actions of mastication was driven by histamine neurons [13], in view of the results from long-term animal study [21] and short-term appetite studies in human [15-16], it is possible that people may consider choosing food with higher hardness as a means to increase the number of chews during ingestion to curb appetite or aid body weight management.
In this second study it was found the effect of aging and food hardness on the particle size distribution of food bolus at swallowing threshold was very limited. This is probably because mastication function was adapted to aging and food characteristics. For example, an increase the number of chews until a suitable bolus is formed may be used for harder food and for people with impaired mastication. In fact, the particle size of food at the swallowing threshold was very similar among subjects [46]. Peyron et al. had analyzed the particle size distribution of boluses using six different foods and found no inter-individual variability in particle size distribution [47].

Tureli et al. had studied the association of mastication performance and body variables in children [48]. It was found overweight and obese children had larger median particle size (a theoretical sieving size that 50% of chews bolus can pass) than normal-weight children [48]. In their study they did not measure the microstructure of mastication, however it is possible that obese and overweight children chewed less times before swallowing, therefore their bolus was larger. Gaviao et al. had also studied the correlation in masticatory efficiency and body variables such as body weight and height, but they failed to observe any significant correlations [49]. This is probably because in their study the dependent variable chosen was the particle size [49] but not the microstructure of mastication such as the number of chews at the swallowing threshold, as we has also shown no significant association between particle size and BMI in this study.

In conclusion, a significant correlation of habitual masticatory parameters and body mass index was found in the present studies. The masticatory efficiency, evaluated by electromyographic recording, was influenced by aging and food hardness. People have the
ability to adapt mastication so that a suitable and similar size of bolus is formed. Further studies should be conducted to elucidate if there is a causal relationship between the habitual chewing behavior and body weight, to provide possible approach for obesity prevention and intervention.

References


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### Table 1. Statistics for the linear regression models in study 1

<table>
<thead>
<tr>
<th>Dependent variable</th>
<th>R²</th>
<th>BMI</th>
<th></th>
<th></th>
<th>Age</th>
<th></th>
<th></th>
<th>Gender</th>
<th></th>
<th></th>
<th>Intercept</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of chews</td>
<td>0.133</td>
<td>-0.522</td>
<td>0.217</td>
<td><strong>0.020</strong></td>
<td>0.455</td>
<td>0.048</td>
<td>2.134</td>
<td>0.255</td>
<td>0.048</td>
<td>2.539</td>
<td>0.404</td>
<td>28.99</td>
<td>6.92 &lt;0.001</td>
</tr>
<tr>
<td>Chewing duration</td>
<td>0.207</td>
<td>-0.514</td>
<td>0.175</td>
<td><strong>0.005</strong></td>
<td>0.299</td>
<td>0.106</td>
<td>4.121</td>
<td>0.182</td>
<td>0.048</td>
<td>2.048</td>
<td><strong>0.049</strong></td>
<td>27.96</td>
<td>5.58 &lt;0.001</td>
</tr>
<tr>
<td>Chewing rate</td>
<td>0.101</td>
<td>0.003</td>
<td>0.004</td>
<td>0.493</td>
<td>0.004</td>
<td>0.342</td>
<td>-0.099</td>
<td>0.047</td>
<td>1.04</td>
<td>0.13</td>
<td><strong>0.042</strong></td>
<td>1.04</td>
<td>0.13 &lt;0.001</td>
</tr>
</tbody>
</table>

P-values below the significant level (P<0.05) were shown in bold.

### Table 2. Characteristics of the participants in study 2

<table>
<thead>
<tr>
<th></th>
<th>Young (n=11)</th>
<th>Older (n=11)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>24.5±1.3 a</td>
<td>74.0±1.6 b</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>23.1±0.7 a</td>
<td>26.5±0.9 b</td>
</tr>
<tr>
<td>3-factor eating questionnaire score</td>
<td>8.5±0.7 a</td>
<td>11.5±0.5 b</td>
</tr>
<tr>
<td>Palatability of RWC</td>
<td>7.2±0.4 a</td>
<td>8.5±0.3 b</td>
</tr>
<tr>
<td>Palatability of RCC</td>
<td>7.3±0.3 a</td>
<td>8.4±0.3 b</td>
</tr>
<tr>
<td>Palatability of CWC</td>
<td>7.5±0.3 a</td>
<td>7.5±0.5 a</td>
</tr>
<tr>
<td>Palatability of CCC</td>
<td>7.2±0.4 a</td>
<td>7.5±0.5 a</td>
</tr>
</tbody>
</table>

All values are mean±SEM.
Different letters indicate a significant difference between the young and older participants (P<0.05).
### Table 3. Correlation between variables of mastication parameters

<table>
<thead>
<tr>
<th></th>
<th>Number of chews</th>
<th>Chewing duration</th>
<th>Maximal bite force</th>
<th>Mean bite force</th>
<th>Muscle activity</th>
<th>Chewing rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of chews</td>
<td>r=1</td>
<td>r=0.861**</td>
<td>r=0.160*</td>
<td>r=0.157*</td>
<td>r=0.604**</td>
<td>r=-0.080</td>
</tr>
<tr>
<td>Chewing duration</td>
<td>r=0.861**</td>
<td>r=1</td>
<td>r=0.142</td>
<td>r=0.026</td>
<td>r=0.552**</td>
<td>r=-0.508**</td>
</tr>
<tr>
<td>Maximal bite force</td>
<td>r=0.160*</td>
<td>r=0.142</td>
<td>r=1</td>
<td>r=0.837**</td>
<td>r=0.705**</td>
<td>r=0.028</td>
</tr>
<tr>
<td>Mean bite force</td>
<td>r=0.157*</td>
<td>r=0.026</td>
<td>r=0.837**</td>
<td>r=1</td>
<td>r=0.738**</td>
<td>r=0.231**</td>
</tr>
<tr>
<td>Muscle activity</td>
<td>r=0.604**</td>
<td>r=0.552**</td>
<td>r=0.705**</td>
<td>r=0.738**</td>
<td>r=1</td>
<td>r=-0.116</td>
</tr>
<tr>
<td>Chewing rate</td>
<td>r=-0.080</td>
<td>r=-0.508**</td>
<td>r=-0.028</td>
<td>r=0.231**</td>
<td>r=-0.116</td>
<td>r=1</td>
</tr>
</tbody>
</table>

Partial correlation after controlling for treatment and age.
* Correlation is significant at the 0.05 level.
** Correlation is significant at the 0.01 level.

### Table 4. Particle size distribution of the food bolus by the percentage of dry weight

<table>
<thead>
<tr>
<th></th>
<th>&gt;4 mm</th>
<th>3.35-4 mm</th>
<th>2-3.35 mm</th>
<th>1-2 mm</th>
<th>0.5-1 mm</th>
<th>&lt;0.5 mm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Young RWC</td>
<td>78.9±2.5a</td>
<td>8.8±1.0a</td>
<td>8.8±1.4ab</td>
<td>2.4±0.4b</td>
<td>0.8±0.1a</td>
<td>0.3±0.07a</td>
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<td>8.4±0.8a</td>
<td>7.4±0.9b</td>
<td>3.2±0.4b</td>
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<td>11.7±1.1a</td>
<td>12.7±0.8a</td>
<td>4.5±0.3ab</td>
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<td>0.2±0.03a</td>
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<td>10.7±1.4a</td>
<td>11.1±1.3ab</td>
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</tr>
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<td>4.1±0.4ab</td>
<td>0.9±0.1a</td>
<td>0.2±0.04a</td>
</tr>
</tbody>
</table>

All values are mean±SEM.
Different letters indicate a significant difference within the same column (P<0.05).
Figure 1. The number of chews (A), Chewing duration (B), Maximal bite force (C), Mean bite force (D), Muscle activity (E) and Chewing rate (F) of raw whole carrot (RWC), raw chopped carrot (RCC), cooked whole carrot (CWC) and cooked chopped carrot (CCC) for young (N=11) and older (N=11) adults. Effect of aging, treatment and their interaction on each variable were tested by a general linear model using BMI as the covariate. Different letters indicate the effect of aging was significant. Treatment effect was significant on all variables; refer to the text for details.
CHAPTER 4. INCREASING THE NUMBER OF CHEWS REDUCES MEAL SIZE IN NORMAL WEIGHT, OVERWEIGHT AND OBESE ADULTS

A paper submitted to Appetite

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Abstract

To determine the effect of increasing the number of chewing cycles before swallowing on meal size, 16 normal weight, 16 overweight and 15 obese participants participated in this study. During a preliminary session the number of chews each participant required to swallow a portion of pizza (baseline number of chews) was determined. The participants then attended three test sessions at their habitual lunch time that were separated by at least one week. After baseline appetite questionnaires were collected, participants were asked to eat pizza until comfortably full, by chewing each portion for 100%, 150% or 200% of their baseline number of chews before swallowing. Food intake in the 150% and 200% sessions was reduced by 9.5% (P=0.023) and 14.8% (P=0.001) respectively compared to the 100% session. There was no effect of body mass index status on the food intake. In addition, changing the chewing parameters had no effect on appetite on meal termination or during the immediate post-prandial period. These data indicate that increasing the
number of chews before swallowing may be a behavioral strategy to reduce food intake and potentially aid weight management.

**Keywords:** mastication, ingestive behavior, eating rate, appetite, food intake

**Introduction**

Overweight and obesity are leading public health problems throughout the world. Due to the health, social and economic consequences of excess body weight effective strategies to aid weight management are required. Accumulating evidence suggests that certain eating behaviors, such as a slower eating rate or a smaller bite size, are associated with a smaller meal size (Andrade, Greene, & Melanson, 2008; Zijlstra, de Wijk, Mars, Stafleu, & de Graaf, 2009) or lower body mass index (BMI) (Leong, Madden, Gray, Waters, & Horwath, 2011; Otsuka, et al., 2006; Sasaki, Katagiri, Tsuji, Shimoda, & Amano, 2003). Despite being a major part of ingestion, little is currently known about the effect of mastication on meal size.

The idea that chewing food more thoroughly to reduce food intake was popularized by Horace Fletcher who proposed that food should be chewed until it turns into liquid or swallows itself. To date, there have been few scientific studies conducted to evaluate his claims although recent studies suggest that making a higher number of chews before swallowing reduces meal size. For instance, in a preliminary study using 11 participants, it was found that chewing 35 times rather than 10 times before swallowing reduced meal size by 12% (Smit, Kemsley, Tapp, & Henry, 2011). A similar finding was reported by a study of Chinese males that found chewing 40 times rather than 15 times before
swallowing reduced meal size by 11.9% (Li, et al., 2011). In these studies the number of chews made before swallowing was pre-determined by the investigators and were not based on the participant’s normal chewing patterns. Further studies are warranted to determine if increasing the number of chews based on the participant’s normal chewing behavior reduces meal size.

In addition to the limited number of studies that have examined the influence of mastication on meal size there are several reasons to believe that increasing the number of chews will reduce meal size. First, increasing the number of chews before swallowing slows eating rate. Some (Andrade, et al., 2008; Azrin, Kellen, Brooks, Ehle, & Vinas, 2008; Zandian, Ioakimidis, Bergh, Brodin, & Sodersten, 2009) but not all (Yeomans, Gray, Mitchell, & True, 1997) studies have found that a slower eating rate reduces meal size possibly because it allows the development of satiety signals (de Graaf & Kok, 2010). Second, mastication is a key stimulus for the cephalic phase response (Mattes, 2000) and increasing the number of chews may increase the magnitude of the cephalic phase response, including hormones related to satiety such as cholecystokinin (CCK) or pancreatic polypeptide, leading to earlier satiation (de Graaf, Blom, Smeets, Stafleu, & Hendriks, 2004; Smeets, Erkner, & de Graaf, 2010; Teff, 2010; Teff, Mattes, & Engelman, 1991; Wisen, Bjorvell, Cantor, Johansson, & Theodorsson, 1992). Third, studies using rodents indicate that mastication activates satiety centers in the hypothalamus via histamine neurons resulting in smaller meals (Fujise, et al., 1998; Sakata, Yoshimatsu, & Kurokawa, 1997). Fourth, increasing the number of chews before swallowing would likely prolong sensory exposure. Accumulating evidence suggests that
sensory exposure may have a role in the development of satiation (Ruijschop, Boelrijk, Burgering, de Graaf, & Westerterp-Plantenga, 2010; Zijlstra, et al., 2009; Zijlstra, Mars, de Wijk, Westerterp-Plantenga, & de Graaf, 2008).

In this present study the effect of increasing the number of chewing cycles before swallowing on meal size was determined. Moreover, how people in different weight groups responded to the treatment was investigated. Our hypothesis was that increasing the number of chews would reduce food intake and subjective appetite.

**Methods**

**Test meal**

Tostino’s cheese pizza rolls (General Mills Inc., Minneapolis, MN, USA) were used as the test food in this study. Nutrient labeling by the manufacturer reported that a serving size of 85 g (6 pizza rolls) provided 837 kJ (200 kcal) energy with 14% from protein, 51% from carbohydrate and 35% from fat. Every six pizza rolls were microwaved on high power for 55 seconds and kept in a food warmer at 60°C before serving.

**Participants**

This study was advertised using an e-mail that was sent to Iowa State University faculty, students and staff and by fliers distributed throughout the local community. Individuals interested in taking part in the study were invited to attend a screening session to determine their eligibility. Participants’ weight and height was measured using calibrated weighing scales and a stadiometer. BMI was calculated as weight in kg divided by square
of height in meter. Inclusion criteria were: age 18-45 years, a full set of natural teeth, and a willingness to eat the test foods. Participants were excluded from the study if they: use tobacco products, are underweight (BMI<18.5), have current or history of gastrointestinal disease; have current chronic or acute diseases, are currently using medication that influences appetite, are restrained eaters (>13 on the restraint section of the three-factor eating questionnaire (Stunkard & Messick, 1985)), have an allergy or intolerance to the test foods, or rate the palatability of any of the test foods less than 6 on a 9-point scale.

During this preliminary screening session, participants were instructed to consume five pizza rolls in their usual eating manner. For each portion of pizza rolls, the number of chews made before swallowing was counted and the chewing duration were measured using a stopwatch by a researcher that was sat with the participant. The average number of chews was then calculated and used as the baseline number of chews to determine the number of chews specified for the treatment conditions for each participant (100%, 150% and 200% of their baseline number of chews).

**General procedure**

This study used a randomized cross-over design. Participants attended three test sessions for lunch in a random order and each session was separated by a 7-day washout period. To reduce potential bias, participants were told the study was “a study on effects of eating rate on hand-to-eye coordination task performance”. To maintain the ruse participants were required to complete tasks relating to hand-to-eye coordination (typing speed and accuracy) during the test session.
On each test day, participants were asked to consume the same breakfast at their habitual breakfast time and avoid strenuous exercise or activity for 24 hours prior to the test session. They were instructed not to eat or drink any food except water following breakfast until the test session. The participant was required to report to the laboratory at their habitual lunch time for the test session. Before being served, participants were asked to type a paragraph of sentences using a laptop computer. The duration of typing and the number of errors were measured. An appetite questionnaire was then completed to assess the participant’s baseline appetite ratings. The questionnaire posed four questions: How hungry do you feel right now? How full do you feel right now? How palatable do you find the food right now? What is your desire to eat right now? Responses were captured using a 100-mm visual analogue scale (VAS). The VAS was anchored with diametrically opposed statements in each end (e.g. not hungry at all, as hungry as I have ever felt). The participant was instructed to draw a vertical marker on the scale at the position they felt reflected their current strength of their appetitive feeling. A large plate of pizza rolls was then provided (t0) and the participant was required to chew each pizza roll the required number of times before swallowing. A study investigator was present while the participant was eating to confirm the participant followed the eating instructions. Additional pizza rolls were supplied whenever there were few portions left on the plate and the participant was instructed to eat until comfortably full. No beverage consumption was allowed during the test session.

Meal duration was measured using a stopwatch and appetite questionnaires were completed at t0 + 5, 10, 15, 20, 25, 30, 45, 60 min. The questionnaire was also
administrated upon meal termination and participants were then asked to type the same paragraph of sentences again. They were allowed to leave the laboratory after completing the last appetite questionnaire. Appetite questionnaires were collected for 60 minutes following meal initiation to a) collect data on the participant’s appetite sensations and b) to ensure that all participants stayed in the laboratory for the same amount of time so there was no advantage to eat less in order to leave the laboratory quicker.

The amount of food eaten was determined by weighing the plate before and after serving out of the sight of the participants. Average eating rate was calculated, by dividing the weight of food consumed by meal duration.

The study protocol was approved by the Iowa State University Institutional Review Board and all subjects signed an informed consent form before being included in the study.

Statistical analysis

All data are presented as mean ± standard error. SAS v9.2 (SAS Institute, Cary, NC, USA) was used to perform the statistical analysis. Data were categorized into three groups based on participant’s BMI (normal weight, overweight and obese). A power calculation indicated that to detect a difference of 50 kcal on food intake and 10 mm on subjective appetite, 16 participants in each group were required to maintain a power of 80% at a significance level of 0.05. The initial model using gender as a covariate found the gender effect was not significant so the data were pooled. One-way ANOVA was used to test if there were differences on the baseline mastication parameters among different weight
groups. Repeated measures ANOVA was used to test overall treatment effect (100%, 150% and 200% of baseline number of chews), BMI effect, time effect and their interactions on subjective appetite ratings using baseline value as a covariate. Food intake, meal duration, average eating rate and appetite ratings at meal termination were tested using a two-way ANOVA to assess the effect of treatment and BMI, and their interaction. Least square means were computed and compared with Bonferroni correction for post-hoc comparison.

**Results**

*Anthropometric measurements and baseline mastication parameters*

Sixteen participants for each body weight category were recruited. One obese participant did not complete the study due to personal reasons and was not included in the analysis. Anthropometry data and the participant’s baseline chewing parameters are summarized in Table 1. There was no difference in the baseline chewing parameters between the different weight groups.

*Food intake*

There was a statistically significant main effect of treatment on food intake ($P=0.003$, Figure 1A). Participants consumed $323 \pm 23$ g food in the test session when the baseline number of chews was made. Food intake during the 150% and 200% of the baseline number of chews sessions was reduced by 9.5% ($292 \pm 23$ g, $P=0.026$) and 14.8% ($275 \pm 20$ g, $P=0.001$) respectively. However, there was no difference in food intake between the 150% and 200% sessions ($P=0.213$). There was no statistically significant effect of BMI ($P=0.745$) or BMI by treatment interaction ($P=0.481$) on food intake.
Meal duration

The meal duration was 640±50s when the 100% of baseline number of chews was made. A significant increase in meal duration was observed when increased number of chews was made (P<0.001, Figure 1B). 150% of the baseline number of chews resulted in significantly longer meal duration than the 100% session (P<0.001), whereas 200% of the baseline number of chews resulted in significantly longer meal duration than both the 100% and 150% sessions (P<0.001). There was no statistically significant main effect of BMI (P=0.430) or a treatment × BMI interaction (P=0.514) on meal duration.

Average eating rate

The main effect of treatment on average eating rate was significant (P<0.001, Figure 1C). Both the 150% and 200% of baseline number of chews had shown a slower average eating rate (P<0.001). Meanwhile, the main effect of BMI was statistically significant (P=0.026). Normal weight participants had a slower eating rate than overweight (P=0.011) and obese participants (P=0.039), but there was no difference between overweight and obese participants (P=0.642). There was no statistically significant treatment × BMI interaction on average eating rate (P=0.097).

Subjective appetite at meal termination

There was no statistically significant main effect of treatment or BMI on any subjective appetite measure (hunger, fullness, palatability of the food and desire to eat) at meal termination (P>0.05, Table 2).
Subjective appetite over 60-minute period

There was a statistically significant main effect of time (P<0.001) but no main effect of treatment (P>0.05) or interaction (P>0.05) on subjective hunger, fullness, palatability of the food and desire to eat (Figure 2).

A main effect of BMI was significant on hunger, fullness and desire to eat (P<0.001). Post-hoc comparison reveals that normal weight participants had a higher level of hunger and desire to eat compared with overweight (P<0.001) and obese participants (P<0.001); obese participants showed a higher fullness rating compared with normal weight (P<0.001) and overweight participants (P<0.001). However, no significant main effect of BMI on palatability of the food was found (P=0.821).

Discussion

This present study found that increasing the number of chewing cycles before swallowing significantly reduces meal size in normal weight, overweight and obese adults. Despite these differences in meal size between sessions this present study found that subjective appetite did not differ at meal termination or during the immediate post-prandial period. BMI status had no effect on food intake or appetitive ratings at meal termination. While a reduction in food intake of this magnitude (9.5% and 14.8%) would likely result in clinically significant weight loss it is not known if this effect would persist over the long-term and further studies are required to determine the effect on outcome measures such as body weight or composition.
Our results are broadly in line with other studies that report a higher number of chewing cycles before swallowing reduces food intake by approximately 12% (Li, et al., 2011; Smit, et al., 2011). However, this present study differs from the previous studies (Li, et al., 2011; Smit, et al., 2011) as the number of chewing cycles was participant-dependent and based on their normal chewing behavior rather than being preset by the research team. Our approach has the advantage of being individualized so it was known that all participants increased their chewing activity and to what degree. However, we assumed that the number of chews made during the preliminary session reflected normal chewing behavior although factors such as an individuals’ appetitive state have been shown to influence chewing behavior (Frecka, Hollis, & Mattes, 2008).

In addition to food intake, the effect on subjective appetite was also determined. While it is of interest that appetite ratings were similar at meal termination we only continued these measures for a relatively short-time after meal termination and it is not known if differences in appetite would have appeared during the post-prandial period. For instance, it is possible that there would be a faster return of hunger resulting in increased snacking or a compensatory increase in food intake at the next meal due to eating a smaller meal. However, recent studies report that increasing the number of chewing cycles before swallowing reduces appetite and increases plasma concentration of satiety hormones for three hours after eating (Cassady, Hollis, Fulford, Considine, & Mattes, 2009; Li, et al., 2011) so it is possible that increasing chewing may reduce meal size and augment post-prandial satiety. While this outcome is plausible, results from single meal studies should not be extrapolated to predict effects on food intake or appetite over the long-term and
compensatory mechanisms could be enacted to lessen the effectiveness of this strategy (Blundell, et al., 2010).

A limitation of this study, and previous studies, is that it is not known if it was mastication, eating rate or a combination of both that explained the reduction in meal size. Consequently, it is not known if chewing contributes any advantage over just eating slower which is a key aspect of behavioral therapy interventions (Benecke, 2002; Kaplan, 1980; Spiegel, Wadden, & Foster, 1991). However, studies that have manipulated eating rate to determine the effect on meal size provide inconsistent results reporting that reducing eating rate reduces meal size (Andrade, et al., 2008), reduces meal size in men but not women (Martin, et al., 2007), has no effect on meal size (Karl, Young, & Montain, 2011) or increases meal size (Yeomans, et al., 1997). It is likely that these inconsistent results are due to differences in the methods used to slow eating rate (including smaller bite sizes, pauses in eating, slower chewing rate or a combination of these). A systematic evaluation of how methods used to reduce eating rate to reduce food intake is required.

While there are several ways to reduce eating rate advice to increase chewing may have several advantages. First, increasing the number of masticatory cycles before swallowing may also confer a beneficial effect on postprandial satiety as accumulating evidence indicates that increased chewing activity increases postprandial satiety (Cassady, et al., 2009; Li, et al., 2011). It is not clear if this effect would also be gained from slowing eating rate alone as studies of eating rate and satiety provide mixed results with Kokkinos et al. (2010) reporting that reducing eating rate increased satiety while Karl et al. (2011) failed to find an effect. A second advantage may be that advice to increase the number of
chews before swallowing is relatively straightforward and easy to implement compared to other methods of slowing eating rate. However, behavioral studies are required to confirm this.

There are also potential disadvantages to an approach based on increased chewing. We did not collect data to determine if the participants had a negative impression of increasing chewing effort before swallowing and it is possible that this approach is not feasible due to poor acceptability. While a previous study suggested that excess chewing may be unpopular with participants (Smit, et al., 2011) a systematic study to determine the acceptability of this approach, especially compared to other methods to slow eating rate, is required. Another potential drawback is that additional chewing may liberate more nutrients from the food matrix which would increase nutrient absorption (Cassady, et al., 2009; Ranawana, Henry, & Pratt, 2010), which would counteract the reduction in food intake. In addition, additional chewing has been shown to cause higher peaks in plasma glucose and insulin (Ranawana, Monro, Mishra, & Henry, 2010) and may be an inadvisable strategy to slow eating rate for some individuals. Long-term studies are required to determine how these factors interact to influence overall health status.

Our study has several limitations. First, this study was conducted under laboratory conditions and the results may not be generalizable to real-world situations. Second, the participants were not allowed to drink with the meal. This was to reduce the risk of participants drinking different amounts of water potentially confounding the results. It is not clear if this affected the results as it has been reported that drinking with a meal does not affect food intake (Rolls, Bell, & Thorwart, 1999). Third, this is a single-meal study
and the results may not reflect changes in food intake over a longer time-span. Despite these limitations, this study builds on previous studies and provides further impetus for a systematic investigation of the effects of ingestive behavior on food intake.

In conclusion, increasing the number of chews before swallowing may be an effective strategy to reduce food intake. However, further research is required to evaluate its effect on body weight and other markers of disease.

References


Table 1. Characteristics of the participants

<table>
<thead>
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<th>Normal weight (n=16)</th>
<th>Overweight (n=16)</th>
<th>Obese (n=15)</th>
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<td>Age (y)</td>
<td>22.2±1.3</td>
<td>23.1±1.7</td>
<td>25.3±1.8</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>22.2±0.5</td>
<td>27.1±0.3</td>
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<td>Baseline number of chews</td>
<td>27.9±2.8</td>
<td>22.3±2.0</td>
<td>24.6±2.1</td>
</tr>
<tr>
<td>Baseline chewing duration (s)</td>
<td>24.8±1.8</td>
<td>19.5±1.4</td>
<td>20.5±1.8</td>
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<td>Chewing rate (chew/s)</td>
<td>1.12±0.04</td>
<td>1.15±0.04</td>
<td>1.22±0.05</td>
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All values are mean±SEM. One-way ANOVA tests indicate there was no significant difference in baseline number of chews, chewing duration and chewing rate among normal weight, overweight and obese participants.

Table 2. Subjective appetite at meal termination

<table>
<thead>
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<th>100% of baseline chews (n=47)</th>
<th>150% of baseline chews (n=47)</th>
<th>200% of baseline chews (n=47)</th>
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<tbody>
<tr>
<td>Hunger (mm)</td>
<td>13.8±1.5</td>
<td>14.6±1.4</td>
<td>14.3±1.6</td>
</tr>
<tr>
<td>Fullness (mm)</td>
<td>79.4±1.7</td>
<td>79.6±1.7</td>
<td>78.3±1.8</td>
</tr>
<tr>
<td>Palatability (mm)</td>
<td>30.0±3.4</td>
<td>32.8±3.1</td>
<td>31.9±3.5</td>
</tr>
<tr>
<td>Desire to eat (mm)</td>
<td>14.0±2.0</td>
<td>16.7±2.1</td>
<td>16.5±2.1</td>
</tr>
</tbody>
</table>

All values are mean±SEM. Pooled data were presented (n=47). Two-way ANOVA tests indicate there was no significant main effect of treatment or main effect of BMI or their interactions on any of the subjective appetite ratings.
FIGURE LEGENDS

**Figure 1.** Food intake (A), meal duration (B) and average eating rate (C) for normal weight (n=16), overweight (n=16) and obese (n=15) participants in an *ad libitum* meal, when the number of chews per mouthful was 100%, 150% and 200% of their baseline number of chews. Significant main effect of treatment was indicated by different letters (P<0.05). BMI effect was significant on average eating rate; normal weight participants have a slower eating rate than overweight (P=0.011) and obese participants (P=0.039). No significant treatment by BMI interactions was found in any of these measurements.

**Figure 2.** Hunger (A), fullness (B) and palatability of food (C) and desire to eat (D) for participants (n=47) in the *ad libitum* meal, when the number of chews per mouthful was 100%, 150% and 200% of their baseline number of chews. No significant effect of treatment or treatment by BMI interactions was found. The effect of BMI was significant on hunger, fullness and desire to eat (P<0.001, data not shown on the plot). Normal weight participants had higher hunger and desire to eat compared with overweight and obese participants (P<0.001 for all comparisons), whereas obese participants had higher fullness rating compared with overweight (P<0.001) and normal weight participants (P<0.001).
Figure 1.

(A) Food intake

(B) Meal duration

(C) Average eating rate
Figure 2.

A. Hunger

B. Fullness

C. Palatability

D. Desire to eat

(Time (min))
CHAPTER 5. THE EFFECT OF MASTICATORY CYCLES ON SATIATION IN OLDER ADULTS

A paper to be submitted to The Journal of Nutrition Health and Aging

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Abstract

Studies have shown increasing the number of chews reduces meal size in young adults, however, currently it is not known the effect in older adults. In this randomized cross-over study, 18 older adults were recruited and pizza rolls were used as the test food. A preliminary session was conducted to assess their habitual mastication parameters including habitual number of chews, chewing duration and chewing rate for a single piece of pizza roll. For each test session, participants reported to the laboratory for lunch after a standardization of breakfast and inter-meal interval, once a week for three weeks. After baseline appetite measures were made they were asked to eat pizza rolls until they were comfortably full, by chewing each portion of the food at their habitual number of chews, 150% or 200% of their habitual number of chews before swallowing. Subjective appetite was measured regularly using visual analogue scale for 60 minutes and upon meal termination. Obese participants had a faster chewing rate than lean participants although there was no difference in habitual number of chews and chewing duration.
Adjusting for body mass index, increased number of chews before swallowing resulted in longer meal duration (P<0.001) but did not influence food intake (P=0.536). The number of chews did not affect hunger, fullness, desire to eat and palatability of the test food over 60 minutes. These results suggest increasing the number of chews does not affect satiation in older adults and the aging-related dysregulation of appetite may account for the results.

**Introduction**

The number of obese elderly is increasing worldwide [1]. Obesity in the elderly contributes to the risk for cardiovascular disease and several cancers, and has an association with increased mortality [2-3]. Although there is a trend showing more aged population throughout the world [4], compared with children and young adults, less attention has been focused on the body weight management in older adults. A better understanding of factors that impact appetite and food intake in older adults may provide feasible approach for prevention of obesity in this population.

Mastication is a major process involved in ingestive behavior. It can affect nutritional status by influencing sensory perception of food as well as the properties of swallowed bolus [5]. While both sensory perception and particle size of food bolus have an impact on appetite, another possibility is mastication may directly affect appetite. As a key stimulus of cephalic phase response [6], mastication activity affects the secretion of satiety hormones [7] and glycemic response [8]. While studies have shown increasing the number of chews enhances satiation and reduces meal size in young adults [7, 9], currently no similar data is available for older adults. As aging-related change includes
reduced masticatory efficiency [10] and impaired response of appetite-related hormones [11], the effect of increased mastication activity on satiation in older adults is worth investigating.

In this present study it is hypothesized that older adults will have a reduced food intake and suppressed subjective appetite in a single meal when increased number of chews is made.

Method

Test meal

Totino’s cheese pizza rolls (General Mills Inc., Minneapolis, MN, USA) were used as the test food in the study. Nutrient labeling reported by the manufacturer reported each serving size (six rolls, 85 g) provided 837 kJ (200 kcal) energy and had 7 g protein, 26 g carbohydrate and 8 g fat.

Meals were prepared by arranging 6 pizza rolls on a plate then microwaved on high for 55 seconds. A considerable large amount of rolls were put in a bowl and stood for 2 minutes before served. The bowl was covered by aluminum foil so that participants were not aware of the portion size.

Participants

Potential participants were informed about this study by a mass e-mail sent to retired faculty and staff, and by fliers distributed throughout the local community. Individuals interested in the study were invited to attend a screening session to determine their
eligibility for the study. Inclusion criteria were: age ≥ 65, full set of natural teeth or well-fitted dentures, and a willingness to eat the test foods. Participants were excluded from the study if they: were using tobacco products, were underweight (BMI < 18.5), had presence or history of gastrointestinal disease, had presence of acute diseases, were currently using medication that influences appetite, were restrained eaters (>13 on the restraint section of the three-factor eating questionnaire [12]), had an allergy or intolerance to the test foods, or rated palatability of any of the test foods less than 5 on a 9-point scale.

During a preliminary session, participants were instructed to consume five pizza rolls in their habitual eating manner. For each pizza rolls, the number of chews made before swallowing and the chewing duration were recorded. The average number of chews was calculated and used as the habitual number of chews to determine the number of chews specified for the treatments for each participant (100% of their habitual chews, 150% of their habitual chews and 200% of their habitual chews).

General procedure

The study used a randomized cross-over design. Participants attended three test sessions for lunch and sessions were separated by a 7-day washout period. To reduce potential bias, participants were told the study was “A study on effects of eating rate on hand-to-eye coordination task performance”.

On each test day, participants were required to consume the same breakfast at their habitual breakfast time at home. Following breakfast, they were instructed not to eat or
drink any food items except water until the test session. The participant was required to report to the laboratory at their habitual lunch time for the test session. Before being served lunch participants were asked to type a paragraph of sentences using a laptop computer and the duration of typing was measured. An appetite questionnaire was then given to assess their baseline appetite. The questionnaire posed four questions: How hungry do you feel right now? How full do you feel right now? How palatable do you find the food right now? What is your desire to eat right now? Responses were captured using a 100-mm visual analogue scale (VAS). The VAS was anchored with diametrically opposed statements in each end (e.g. not hungry at all, as hungry as I have ever felt). Participants were instructed to draw a vertical marker on the scale at the position they felt reflected their current strength of their appetitive feeling. Meals were provided immediately after completing the appetite questionnaire. One of the research personnel was serving the participant by taking each pizza roll from the covered bowl to the plate in front of the participant. By this way participants did not know how much food was left in the bowl, thus removing cognitive bias.

Participants were required to chew each pizza roll exactly certain times as instructed before they could swallow. They were instructed to eat until comfortably full. Depending on test sessions, the number of chews specified was 100% of their habitual chews, 150% of their habitual chews or 200% of their habitual chews and the treatment order was randomized.

Meal duration was measured and appetite questionnaire was given at 5, 10, 15, 20, 25, 30, 45, 60 min after they started eating. The questionnaire was also given upon meal
termination and participants were then asked to type the same paragraph of sentences. They were allowed to leave the laboratory after completing the last appetite questionnaire. During the test session no fluid consumption was allowed.

Each bowl of food was weighed before and after serving out the sight of the participants. Average eating rate was calculated, by dividing the weight of food consumed by meal duration.

The study protocol was approved by the Iowa State University Institutional Review Board and all subjects signed an informed consent form before being included in the study.

Statistical analysis

All data are presented as mean ± standard error. SAS v9.2 (SAS Institute, Cary, NC, USA) was used to perform the statistical analysis. The initial model with gender as a covariate found the gender effect was not significant thus data were pooled for males and females. A one-way ANOVA was used to assess the difference on habitual chewing parameters among lean, overweight and obese participants. Repeated measures ANOVA was used to test overall treatment effect, BMI effect, time effect as well as their interactions on subjective appetite ratings. Food intake, meal duration, average eating rate and appetite ratings at meal termination were tested by two-way ANOVA to assess treatment effect and BMI effect and their interaction. If significant effect was found, least square means were computed and compared with Bonferroni correction. Statistical significance was set at P<0.05.
Results

Anthropometric measurements and habitual mastication parameters

Anthropometric measurements for the participants (n=18) and their habitual mastication parameters are summarized in table 1. There was no difference in habitual number of chews as well as chewing duration among lean, overweight and obese participants. However, obese participants had a faster chewing rate compared with lean participants (P=0.025).

Food intake

There was no significant main effect of treatment on food intake (P=0.536, Figure 1A). Participants consumed 177.8±43.0 g pizza rolls in the test session when the habitual number of chews was made, and they consumed 164.2±41.7 g and 166.6±33.0 g food in the sessions when 150% and 200% of the habitual number of chews were made, respectively. No significant main effect of BMI (P=0.442) or BMI by treatment interactions (P=0.353) was found.

Meal duration

The main effect of treatment was significant (P<0.001) but no main effect of BMI (P=0.341) and treatment by BMI interaction (P=0.956) were found on meal duration (Figure 1B). Both 200% and 150% of the habitual chews resulted in a significantly longer meal duration compared with 100% of the habitual chews (732±130s vs 496±113s, P<0.001 and 613±140s vs 496±113s, P=0.042 respectively). There was a trend toward a
significant difference in the meal duration between sessions when 200% and 150% of the habitual chews were made (P=0.057).

*Average eating rate*

The main effect of treatment on average eating rate was significant (P<0.001). However there was no main effect of BMI (P=0.734) as well as treatment by BMI interaction (P=0.678) (Figure 1C). Both 200% and 150% of the habitual chews resulted in significantly slower eating rate compared with 100% of the habitual chews (0.23±0.01g/s vs 0.37±0.03g/s, P<0.001 and 0.27±0.02g/s vs 0.37±0.03g/s, P<0.001 respectively). In addition, average eating rate in the session when 200% of the habitual chews were made was also slower than that in the condition where 150% of the habitual chews were made (P=0.046).

*Subjective appetite at meal termination*

There was no significant main effect of treatment, main effect of BMI, as well as their interactions on hunger, fullness as well as desire to eat at meal terminations (P>0.05, Table 2). However, a significant main effect of treatment on the palatability of food was found (P=0.018). Participants had less rating of the food palatability as the number of chews increased.

*Subjective appetite over 60-minute period*
The main effect of time was significant (P<0.001) but there was no significant main effect of treatment (P>0.05) or any effect of interactions (P>0.05) on subjective hunger, fullness, food palatability, and desire to eat (Figure 2).

The main effect of BMI was significant on the food palatability (P<0.001). Lean participants had higher rating compared with obese participants (P<0.001). However, no significant main effect of BMI on hunger, fullness and desire to eat was found (P>0.05).

Discussion

In this present study it was found that increasing the number of chews before swallowing does not affect food intake in an ad libitum meal in older adults. This is different from previous studies in young adults which had shown 35 or 40 chews per mouthful resulted in about 12% reduction in food intake compared with 10 or 15 chews per mouthful [7, 9]. A study with the same protocol in young adults from our laboratory has shown 200% of the habitual number of chews resulted in a reduction of food intake by 14.8% whereas 150% of the habitual number of chews reduced food intake by 9.5% compared with condition when the habitual chews was made. It is not clearly known about the reason for the difference between young and older adults, but it is believed impaired appetite regulation due to aging plays an important role here.

Accumulating evidences have shown a dysregulation of appetite due to aging. Unlike young men, older men do not change energy intake in response to overfeeding or underfeeding conditions [13]. It has also been reported that compensation for energy in a preload was less precise in the older men than in the young men as they consistently
overate in the meal 30 minutes after the preload [14]. Recent studies suggest age-associated changes of appetite-regulating hormones, including enhanced sensitivity of cholecystokinin (CCK) and reduced CCK postprandial response, decreased ghrelin concentration and poorer ghrelin postprandial recuperation phase, may partly account for the dysregulation [11, 15-17].

Another possibility is the losses in chemosensory perception in older adults [18-19]. Chemosensory signals such as taste and smell play a vital role in regulation of appetite as they elicit cephalic phase responses [6, 20-21]. In addition, they are involved in food selection, initiation and termination of ingestion which partly control the meal size [22-23]. Therefore, the aging related change in chemosensory perception could contribute to the dysregulation of appetite in this population.

A reduced mastication efficiency in older adults, due to declined bite forces as well as reduction in verticular mandibular displacement and velocity [5, 10], may also contribute to the losses in chemosensory perception since a major role of mastication is to reduce food particle size which facilitates flavor perception. Generally, an increased number of chews is expected to enhance flavor perception by the participants as it provides smaller food bolus and allows longer oral stimulation. Interestingly, in this study we found participants had a significantly less rating on the palatability of the food at meal termination when the number of chews was twice of their habitual chews. This is probably due to sensory specific satiety [24-25]. In this study we found meal duration was significantly longer as the number of chews increases, as a result, participants had a declining rating on satisfaction and pleasantness of the test food. Nonetheless, the
reduction in palatability rating was not accompanied by a reduction in the amount of food ingested, which may provide an example to support aging-related dysregulation of appetite.

In this present study we found eating rate was significantly reduced as the number of chews increased but no effects on meal size or subjective appetite was observed. Recent studies suggest a slower eating rate promotes satiation and reduces meal size in young adults [26-27], however, no result has been reported in the aged population. Further studies should be conducted to investigate the effects of eating rate and appetite in older adults.

Older adults have a reduced energy intake compared with young adults [28]. A possibility for the observed result is the difference induced by increased number of chews was too minor to be detected. As previous studies suggest increased chewing activity reduces meal size by 10-15% in young adults [7, 9], a reduction by a similar percentage in food intake for the older adults, equals to a less amount of food intake compared with young adults. This requires an increase in sample size to achieve the same statistical power. In view of the number of participants involved in this study, it is likely to be underpowered, as one of the limitations in the study.

Another limitation for this study was only a single food was offered. We did not provide multiple test foods because the habitual number of chews per mouthful changes depending on test foods as hard food generally requires more chews before it can be swallowed. Also, studies using multiple test foods in a buffet styles provides will be confounded by food choices and macronutrient composition of foods. However, in a
design where single test food was used to assess the satiation property of the test food it might be affected by sensory specific satiety. While participants had a similar amount of food intake in different test sessions, it is not known how they response to additional food products.

In this study it was found obese participants have a faster habitual chewing rate than lean participants. This is different from studies in young adults which had shown no difference in chewing rate between the lean and obese groups [7, 9]. Chewing rate contributes to the overall eating rate and studies have suggested obese people ate faster than lean people [29-30]. In view of the relationship among number of chews, chewing rate and eating rate, it is worth investigating the effect of chewing rate on appetite, to see if meal size will be affected by asking participants to chew the food by the same number of chews but chew more slowly as a means to reduce the eating rate.

In summary, the study showed that increasing the number of chews does not influence food intake in an *ad libitum* meal in older adults. Further studies should be conducted to elucidate the mechanisms responsible for their dysregulated appetite and approaches should be made to aid body weight management for this population.

**References**


### Table 1. Characteristics of the participants

<table>
<thead>
<tr>
<th></th>
<th>Lean (n=8)</th>
<th>Overweight (n=5)</th>
<th>Obese (n=5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>71.3±2.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>73.8±2.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>70.2±2.0&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>BMI (kg/m&lt;sup&gt;2&lt;/sup&gt;)</td>
<td>21.7±0.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>27.4±0.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>31.4±0.5&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Habitual chews</td>
<td>28.9±1.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>39.6±4.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>43.1±10.2&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>Habitual chewing duration (s)</td>
<td>29.6±1.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>34.0±2.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>35.4±8.8&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Chewing rate (chew/s)</td>
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<td>1.16±0.05&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>1.30±0.13&lt;sup&gt;b&lt;/sup&gt;</td>
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</table>

All values are mean±SEM. One-way ANOVA tests were used to assess difference among lean, overweight and obese participants. Different letters indicate significant difference (P<0.05).

### Table 2. Subjective appetite at meal termination

<table>
<thead>
<tr>
<th></th>
<th>100% habitual chews (n=18)</th>
<th>150% habitual chews (n=18)</th>
<th>200% habitual chews (n=18)</th>
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<tr>
<td>Hunger (mm)</td>
<td>16.5±2.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>18.3±2.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>17.3±2.5&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Fullness (mm)</td>
<td>72.2±2.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>71.8±2.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>71.3±3.4&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>Palatability (mm)</td>
<td>46.4±6.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>38.8±6.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>32.9±6.3&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Desire to eat (mm)</td>
<td>19.9±2.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>22.2±3.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>18.9±3.7&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

All values are mean±SEM. Two-way ANOVA tests indicated no main effect of BMI and treatment by BMI interactions was found. Significant main effect of treatment was indicated by different letters (P<0.05).
FIGURE LEGENDS

**Figure 1.** Food intake (A), meal duration (B) and average eating rate (C) for lean (n=8), overweight (n=5) and obese (n=5) older participants in the *ad libitum* meal, when the number of chews per mouthful was 100%, 150% and 200% of their habitual number of chews. Different letters indicate a significant main effect of treatment. No significant main effect of BMI or treatment by BMI interactions was found in any of these measurements.

**Figure 2.** Hunger (A), fullness (B) and food palatability (C) and desire to eat (D) for older participants (n=18) in the *ad libitum* meal, when the number of chews per mouthful was 100%, 150% and 200% of their habitual number of chews. No significant effect of treatment or treatment by BMI interactions was found on any of these measurements (P>0.05). The effect of BMI was significant on food palatability (P<0.001), lean older adults had higher rating on the food palatability than obese older adults (P<0.001, data not shown in the plot). Main effect of BMI was not significant on hunger, fullness and desire to eat (P>0.05).
Figure 1.

A. Food intake

B. Meal duration

C. Average eating rate
Figure 2.
CHAPTER 6. INCREASING THE NUMBER OF MASTICATORY CYCLES IS ASSOCIATED WITH REDUCED APPETITE AND ALTERED POSTPRANDIAL PLASMA CONCENTRATIONS OF GUT HORMONES, INSULIN AND GLUCOSE

A paper submitted to British Journal of Nutrition

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Abstract

To determine the influence of masticatory efficiency on post-prandial satiety and glycemic response, twenty-one healthy males were recruited for this randomized cross-over trial. The participants consumed a fixed amount of pizza provided in equal sized portions by chewing each portion either 15 or 40 times before swallowing. Subjective appetite was measured by appetite questionnaires at regular intervals for three hours after meal and plasma samples were collected for measurement of selected satiety-related hormones, glucose, insulin and glucose-dependent insulino tropic peptide (GIP). An ad libitum meal was provided shortly after the last blood draw was made and the amount eaten recorded. Compared with 15 chews, chewing 40 times per portion results in lower hunger (P=0.009), preoccupation with food (P=0.005) and desire to eat (P=0.002). Meanwhile, plasma concentration of glucose (P=0.024), insulin (P<0.001) and GIP
(P<0.001) were higher following the 40 chews meal. Chewing 40 times before swallowing also resulted in higher plasma cholecystokinin concentration (P=0.045) and a trend toward a lower ghrelin concentration (P=0.051). However, food intake at the subsequent test meal did not differ (P=0.851). The results suggest higher number of masticatory cycles before swallowing may provide beneficial effects on satiety and facilitate glucose absorption.

Introduction

Due to the high prevalence of overweight and obesity new strategies to aid weight management are required. This would be aided by a better understanding of the factors that influence satiety so that this information can be used to identify individuals at increased risk of weight gain or for the development of improved therapeutic diets\(^{(1,2)}\). It has been reported that a fast eating rate, a larger bite size, or shorter oral processing time can promote overeating\(^{(3-8)}\) and is associated with elevated body weight or risk of weight gain\(^{(9-11)}\). A key influence on eating rate is masticatory efficiency (i.e., the number of masticatory cycles required before swallowing); however, its influence on satiety has gained little attention.

The primary purpose of mastication is to reduce the particle size of a food to form a bolus for swallowing. There is substantial inter-individual variation in the number of masticatory cycles required to form a bolus and it has been reported that the number of masticatory cycles made before swallowing ranges between 9-65 for carrots and 14-44 for Brazil nuts\(^{(12)}\). Moreover, food preparation methods, such as chopping, roasting or salting also influence the number of masticatory cycles required before swallowing\(^{(13)}\).
These differences in masticatory efficiency could influence satiety through several mechanisms. First, studies using rodents report that mastication has a direct effect on satiety through histaminergic activation of the ventromedial hypothalamus and paraventricular nucleus\(^{(14,15)}\). Second, mastication is a key stimulus of cephalic phase responses (CPR)\(^{(16)}\) and increasing masticatory effort before swallowing may increase the CPR of hormones related to appetite such as insulin, cholecystokinin (CCK) and pancreatic polypeptide\(^{(17-19)}\). Third, increasing the number of masticatory cycles would increase oral processing time and recent studies have shown that increasing oral processing time reduces appetite or food intake\(^{(6,20)}\). It will also slow down eating rate, which has been associated with increased satiety by one study\(^{(21)}\) although these results were not confirmed by another study\(^{(22)}\). Taken together, there are good reasons to believe that increasing the number of masticatory cycles before swallowing will increase satiety.

Recent studies report that making a higher number of masticatory cycles before swallowing increases satiety\(^{(23,24)}\). Cassady et al.\(^{(23)}\) found that chewing almonds 40 times before swallowing reduces appetite and modulates plasma concentrations of several hormones compared to chewing 15 times. However, almonds contain a relatively high amount of lipids, which are a key stimulus for several putative satiety hormones\(^{(25)}\) and as mastication increased the release of lipids from the food matrix it may be that increased bioaccessibility of lipids was the primary reason for enhanced satiety rather than mastication. A study conducted by Li et al.\(^{(24)}\) showing that increasing the number of chewing cycles from 15 to 40 when eating pork pie increased satiety in Chinese adults was potentially due, in part, to differences in post-prandial secretion of gut-derived
hormones related to satiety. Further studies are warranted to determine if differences in the number of masticatory cycles made before swallowing influence appetite using different test foods or other population groups.

Based on the previous studies we hypothesized that a higher number of masticatory cycles before swallowing will increase satiety. This effect will be modulated through changes in plasma concentrations of gut derived hormones that are related to appetite.

**Subjects and Methods**

**Subjects**

This study was advertised by a mass e-mail sent to Iowa State University students and staff and by fliers distributed throughout the local community. Individuals interested in the study were invited to attend a screening session to determine their eligibility for the study. During this session, the participant’s height was measured by a stadiometer, and weight was measured using calibrated weighing scales with the participant dressed in a paper gown. The participant was required to void the bladder before this measurement. Inclusion criteria were: male, aged 18-40 years, BMI 20.0-29.9 kg/m², full set of natural teeth, and a willingness to eat the test foods. Potential participants were excluded from the study if they had: presence or history of gastrointestinal disease, presence of other chronic or acute diseases, currently using medication that affects appetite, were a restrained eater (>13 on the restraint section of the three-factor eating questionnaire(26)), allergy or intolerance to the test foods. Participants were instructed to taste and rate the palatability of the test foods using a 9-point scale. Any participants with a score below 5
were excluded. Participants were informed that the purpose of the study was to investigate the effect of chewing on plasma nutrients. When the participants completed the study they were informed about the true purpose of the study and given the option to withdraw their data from the study. This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects were approved by the Iowa State University Institutional Review Board. Written informed consent was obtained from all subjects.

**Test meals**

Freschetta brick oven fire baked 5-cheese pizza (Schwan Food Company, Bloomington, MN, USA) was used as the test food. Nutrient labeling by the manufacturer reported that each pizza provided 51 g carbohydrate, 23 g fat, 22 g protein and 2050 kJ (490 kcal) energy with a total weight of 183 g. Each participant consumed one complete pizza during each test session. The pizza was baked at 204°C (400 F) in a conventional oven for 15 minutes and allowed to cool to a comfortable eating temperature before serving.

An *ad libitum* pasta meal was served three hours after eating the pizza meal. Meals were provided in 3766 kJ (900 kcal) portions, made with 150g Barilla spaghetti (Barilla America Inc., Bannockburn, IL, USA), 375g Barilla Marinara sauce with imported olive oil (Barilla America Inc., Bannockburn, IL, USA), 37.5g shredded parmesan cheese (Wal-Mart Stores Inc., Bentonville, AR, USA) and 5.1g salt (Wal-Mart Stores Inc., Bentonville, AR, USA). Meals were prepared using a standard procedure and mixed well before being served to the participants.
General procedure

A preliminary session was arranged for all eligible participants to determine a suitable pizza portion size (mouthful) for use in the study. Results from this preliminary session indicated a portion size of 3.8×2.5 cm could be safely swallowed by all participants after 15 chewing cycles. For both test sessions, the pizza was cut into 24 portions of 3.8×2.5 cm.

Following the preliminary session, participants attended two test sessions that were separated by at least 7 days. The treatment order was randomized. Participants were required to report to the laboratory at 7:30 AM after an overnight fast. The participants were instructed to refrain from drinking alcoholic beverages in 24 hours before the test session but no other restrictions were placed on their eating or drinking habits. They were also asked to refrain from strenuous physical activity for 24 hours prior to the test session. An indwelling catheter was inserted into their non-dominant arm and following a thirty-minute acclimatization period, a baseline blood draw was made. The participant also completed an appetite questionnaire to determine their baseline subjective appetite. The questionnaire posed four questions: How hungry do you feel right now? How full do you feel right now? How preoccupied with food are you right now? What is your desire to eat right now? Responses were captured using a 100-mm visual analogue scale (VAS). The VAS was anchored with diametrically opposed statements in each end (e.g. not hungry at all, as hungry as I have ever felt). Participants were instructed to draw a vertical marker on the scale at the position they felt reflected their current strength of their appetitive feeling.
Immediately following the baseline measurements, the participants were presented with the pizza test meal. Depending on the test session, participants were instructed to chew each portion 15 or 40 times before swallowing the complete mouthful. A study investigator was present while the participant was eating to confirm the participant followed the eating instructions. Meal duration was measured to the nearest minute and it was 8±1 minutes for the 15 chews session and 20±1 minutes for the 40 chews session.

Immediately after the participant finished the pizza meal, a fresh appetite questionnaire was completed and a blood draw taken (t0). Further blood samples were collected and appetite questionnaires completed at t0+15, 30, 45, 60, 90, 120 and 180 minutes. Throughout the test session, participants were required to remain seated in a quiet room free from food cues and were not allowed to consume other foods or drinks. The participants were allowed to read or use their computer during the test session. While other participants were also in the laboratory at the same time they were isolated from each other by the use of screens. After the final blood draw the catheter was removed and the participants were allowed to rest for five minutes before being presented with the pasta meal. Participants were instructed to eat until comfortably full and they were informed that they could request more of the pasta meal. No instruction regarding mastication was given. Each bowl of food was weighed before and after serving out the sight of subjects, and the amount consumed was recorded.

Hormones and glucose measurement

Blood was drawn into 4 mL EDTA coated vacutainer tubes and mixed with 400 µL 10000 KIU/ml aprotinin and then centrifuged at 3000 g at 4 °C for 15 minutes. The
plasma was then divided into aliquots and stored at -80 °C until analysis. Insulin was assayed by radioimmunoassay (RIA) as previously described\(^\text{(27)}\). Human insulin was used as standards. The assay had a detection range of 0.78 to 200 μU/mL. The intra-assay CV was 13% and the inter-assay CV was 8% at 20 μU/mL. Glucose-dependent insulinotropic peptide (GIP) was assayed by RIA using 1:5000 rabbit anti-human GIP antibody (Phoenix Pharmaceuticals, Burlingame, CA, USA); the assay had a detection range of 0.1 to 6.4 ng/mL, with intra-assay CV of 7% and inter-assay CV of 12% at 0.5 ng/mL. CCK was assayed by RIA using rabbit anti-CCK-8 antibody 92128 diluted by 1:800 (The antibody was a kind gift from Dr. Jens Rehfeld, University of Copenhagen, Denmark). The assay was able to measure CCK from 4 to 128 pg/mL. The intra-assay CV was 8% and inter-assay CV was 15% at 50 pg/mL. Ghrelin was analyzed by RIA using antibody T-4745 purchased from Bachem (Torrance, CA, USA). The assay had a determination range from 0.05 to 12.8 ng/mL. The intra-assay was 10% and inter-assay was 7% at 0.5 ng/mL.\(^{125}\)I-Tracers used for RIAs were purchased from PerkinElmer (Boston, MA, USA). Plasma glucose was assayed using a biochemical analyzer (YSI Life Sciences, Model 2700 select, Yellow Springs, OH, USA).

**Statistical analysis**

Power calculations suggested a sample size of 18 is required to detect a change of 10% in overall mean for subjective appetite, concentrations of biomarkers and food intake, at the power of 0.8 and the significance level of 0.05. An 8-10% reduction in appetite is considered to be practically relevant\(^\text{(28)}\). In this study 15% more participants (n=21) were recruited in case of possible drop-out during the study. All data are presented as mean ±
standard error of the mean (SEM). Statistical Analysis Software (version 9.2, 2008, SAS Institute Inc, Cary, NC, USA) was used to perform the statistical analysis. Plasma hormone data were log-transformed before analysis as they were not normally distributed. A mixed model of repeated measures ANOVA (Proc Mixed, SAS) was used to test overall treatment effect, time effect and treatment × time interaction on subjective appetite ratings and plasma parameters. Baseline values were included as a covariate and subjects was added as a random variable in the model. There was no significant effect of BMI on any of the outcome measures so the data from all participants was pooled. Post-hoc analysis was performed by Bonferroni adjusted pairwise comparison of responses from the same time point. Difference in food intake at the ad libitum meal was tested by a paired t-test.

Results

Participant characteristics

Participants (n=21) had a mean age of 24±1 years (range 18-36 years) with BMI of 24.8±0.6 kg/m² (range 20.3-28.3 kg/m²).

Subjective Appetite

Figure 1 illustrates subjective appetite responses following the 15 or 40 chews treatment. A significant main effect of time was found for all parameters (P<0.05) but there were no statistically significant treatment × time interactions (P>0.05).
There was a significant main effect of chewing on hunger ($F(1,299)=6.92$, $P=0.009$) with hunger being lower following the 40 chews condition. A significant main effect of chewing on preoccupation with food and desire to eat was also found with both being lower following 40 chews ($F(1,299)=8.17$, $P=0.005$ and $F(1,299)=9.59$, $P=0.002$ respectively). There was no main effect of chewing on fullness ($F(1,299)=0.06$, $P=0.813$).

*Glucose and appetite related hormones*

Figure 2 shows plasma concentrations of glucose, insulin, GIP, CCK and ghrelin following the different chewing conditions. A main effect of time was significant for all parameters ($P<0.05$) except CCK ($P=0.073$).

There was a significant main effect of chewing on plasma glucose ($F(1,299)=5.19$, $P=0.024$). Post-hoc analysis revealed that plasma glucose was significantly higher at 0 min when 40 chews was made ($P<0.001$). A significant main effect of chewing on plasma insulin was also found ($F(1,299)=19.55$, $P<0.001$) with post-hoc analysis revealing that insulin was significantly higher at 0 min and 15 min ($P<0.001$ and $P=0.017$ respectively) following the 40 chews condition. There was a significant main effect of chewing on GIP ($F(1,299)=22.81$, $P<0.001$). Post-hoc analysis revealed a higher GIP response at 0 min ($P<0.001$) and 15 min ($P<0.001$) following the 40 chews condition. A significant main effect of chewing on plasma CCK was found ($F(1,299)=4.07$, $P=0.045$). At 180 min, CCK was significantly higher in the 40 chews condition ($P=0.037$). There was a trend towards a significant main effect of chewing on ghrelin ($F(1,299)=3.83$, $P=0.051$) with lower ghrelin following the 40 chews condition.
Food intake

There was no difference in food intake at the subsequent *ad libitum* meal after three hours (40 chews: 417.4±54.5 g vs 15 chews: 410.2±44.5 g, P=0.851).

Discussion

This present study found that increasing the number of chewing cycles before swallowing is associated with reduced postprandial hunger, preoccupation with food and desire to eat. It is also resulted in with a higher post-prandial plasma concentration of CCK and reduced post-prandial ghrelin. However, there was no difference in food intake at a meal served three hours after the test meal. While this study suggests that a higher number of masticatory cycles before swallowing was associated with reduced postprandial appetite, it was also associated with increased plasma concentrations of insulin and glucose.

Data from this present study is supportive of findings from previous studies that increasing the number of masticatory cycles before swallowing increases satiety as measured by subjective appetite questionnaires\(^{23,24}\). These data raise the possibility that efficient eaters (i.e., individuals who use few masticatory cycles to form a bolus) may be at increased risk of weight gain due to reduced satiety. While epidemiological studies report that a fast eating rate is associated with a higher BMI or risk of weight gain\(^{9-11}\) it is not clear to what degree differences in masticatory efficiency contributed to eating rate. Further studies are required to determine if there is a difference in the satiety response between efficient and inefficient masticators.
While this present study found that a higher number of masticatory cycles reduces subjective appetite it is not clear how they are mechanistically linked. In this present study we measured post-prandial plasma concentration of CCK and ghrelin due to their role in the regulation of appetite$^{(25)}$. A higher number of masticatory cycles was associated with increased plasma CCK and reduced ghrelin although it is not clear how mastication influenced secretion of these hormones. One possible explanation is that increasing mastication activity elicits a stronger CPR. A CPR for ghrelin and CCK has been reported by some$^{(18,29)}$ but not all studies$^{(30,31)}$. While the influence of increasing mastication on the CPR warrants further investigation, the CPR is transient and relatively small in magnitude$^{(32)}$ and it is debatable whether it is sufficiently large or long-lasting to influence hormone response over a period of several hours. A more likely explanation is that increasing the number of masticatory cycles before swallowing reduces the size of particles in the swallowed bolus$^{(33,34)}$. Reducing the size of the swallowed particles increases the bioaccessibility of nutrients due to increased breakdown of the food matrix$^{(35)}$. As CCK is stimulated and ghrelin inhibited by the presence of nutrients in the GI tract, greater nutrient bioaccessibility would presumably lead to a pattern of CCK and ghrelin secretion that promotes satiety.

While higher plasma concentrations of CCK and lower plasma ghrelin would be consistent with increased satiety, in this present study there was no correlation between plasma concentrations of these hormones and subjective appetite suggesting they were not causally linked although a lack of correlation is not uncommon in appetite studies$^{(36,37)}$. Other explanations for the reduced subjective appetite may be proposed.
First, several other hormones that were not measured in this study, such as GLP-1 or PYY3-36, contribute to short-term appetite and may have had the dominant effect on appetite. Second, increasing the number of masticatory cycles before swallowing may lead to a reduction in the palatability of the pizza, which may have had a stronger effect on ratings of hunger and desire to eat during the post-prandial period\(^{(38)}\). Third, rodent studies have shown that mastication has a direct effect on satiety center through activation of histamine neuron\(^{(14,15)}\). Further studies are required to determine through which mechanisms mastication contributes to satiety.

Food intake at a meal eaten three hours after completion of the pizza test meal was measured as a further marker of satiety. Despite differences in subjective appetite and plasma hormones there was no effect of masticatory efficiency on food intake. However, in this present study the amount eaten at the *ad libitum* test meal may have been influenced by factors other than appetite masking an actual effect. First, the participants may have stopped eating before reaching satiation so that they could leave the laboratory sooner. Second, participants were not allowed to drink water with the meal. Some studies\(^{(39,40)}\) but not all\(^{(41)}\), have found that restricting fluid intake with a meal lowers food intake. It has been estimated that 75% of fluid ingestion occurs peri-prandially\(^{(42)}\) and restricting fluid intake when eating is atypical of normal behavior for most adults. This may limit the generalizability of the data collected by this present study.

A further finding of this present study is that mastication influences the glycemic response. Chewing the pizza 40 times before swallowing resulted in higher plasma glucose, insulin and GIP compared to chewing 15 times. These results differ from a
previous study in which it was found that chewing pork pie more times before swallowing had no effect on plasma concentrations of glucose or insulin\(^{(24)}\). This discrepancy may be due to differences in the characteristics of the test meals. This explanation is supported by a study, which found that masticatory effort influenced the glycemic response after eating rice but not spaghetti\(^{(43)}\). The glycemic response has been linked to several chronic diseases such as obesity, type 2 diabetes and heart disease\(^{(44)}\). Further research is required to understand the effect of variations in masticatory efficiency on the glycemic response and disease risk.

Nonetheless, there are several limitations to this study. A key limitation of this, and previous studies using a similar experimental design\(^{(23,24)}\), is that the effect of mastication on appetite was not isolated from differences in eating rate, oral processing time or the physical characteristics of the swallowed bolus. Consequently, the observed effects on subjective appetite and plasma hormones cannot be solely attributed to differences in mastication and may have been due to a slower eating rate, a longer oral processing time, differences in the physical characteristics of the swallowed bolus or a combination of these factors. However, these data provide further impetus to examine the role of mastication, eating rate, oral processing time or the physical characteristics of the swallowed bolus on satiety. In addition, pizza was used as the test food, which is not typically eaten as a breakfast food. Pizza was used as it required mastication before swallowing and it provided a mix of macronutrients. Moreover, in this study water was not allowed throughout the test session as gastric distention resulted from water ingestion would confound appetite measurements. While these may have resulted in an atypical
meal, this effect would be consistent across both test sessions and it is unlikely that it would explain the differences in satiety or hormones between the test sessions. The study group also consisted solely of non-obese male participants to maximize statistical power. Further studies are required to determine if different number of masticatory cycles before swallowing influences satiety in females or the obese.

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FIGURE LEGENDS

**Figure 1.** Hunger (A), fullness (B), preoccupation with food (C) and desire to eat (D) following 15 chews and 40 chews conditions. N=21, data were mean±SEM. Main effect of treatment on hunger, preoccupation with food and desire to eat was significant (P=0.009, P=0.005, P=0.002 respectively). No significant main effect of treatment on fullness was found (P=0.813).

**Figure 2.** Plasma level of glucose (A), insulin (B), GIP (C), CCK (D) and ghrelin (E) following 15 chews and 40 chews conditions. N=21, data were mean±SEM. Main effect of treatment on glucose, insulin, GIP and CCK was significant (P=0.024, P<0.001, P<0.001 and P=0.045 respectively). There was a trend toward significant main effect of treatment on ghrelin (P=0.051). * indicates significantly different plasma concentration between treatments at the same time point (P<0.05).
Figure 1.
Figure 2.
CHAPTER 7. THE EFFECTS OF MASTICATORY CYCLES ON POST-PRANDIAL SATIETY AND SUBSEQUENT FOOD INTAKE IN OLDER MEN

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Abstract

Accumulating studies indicate eating slowly and chewing thoroughly suppress appetite in young adults; however, it is not clear how appetite and postprandial metabolism are influenced by changes in eating rate through increasing masticatory cycles in older adults. In a randomized cross-over study, 15 older men (75±2 years) with body mass index of 25.6±0.8 kg/m² consumed a fixed amount of pizza in portions, by chewing 15 or 40 times before swallowing each portion. Appetite questionnaires for assessment of subjective appetite and plasma samples for measurement of appetite-regulating hormones and metabolite were collected during regular interval over three hours following meal consumption. An ad libitum meal was provided three hours later. Compared to 15 chews, eating slowly by making 40 chews per portion during ingestion has reduced hunger (P<0.001), resulted in lower preoccupation with food (P<0.001) and desire to eat (P<0.001). Higher levels of glucose, insulin and glucose-dependent insulinotropic peptide were observed in 40 chews immediately after eating (P<0.05), but they became
significantly lower at 120 and/or 180 min (P<0.05), compared with 15 chews. No difference on cholecystokinin and ghrelin was found (P>0.05). There is a trend toward significance that participants eat more at the subsequent meal in 40 chews (P=0.066). The results suggest eating slowly with 40 chews in older men promotes postprandial satiety but this eating behavior also tended to increase food intake at the subsequent meal. The impaired control of food intake in older adults may partly explain for the results.

**Keywords:** eating rate, mastication, satiety, appetite, aging

**Introduction**

There is a demographic shift towards a more aged population throughout the world [1]. This has implications for society as older adults use a disproportionate amount of health care resources and strategies to maintain good health for as long as possible are required. While the aging process renders a person more susceptible to illness and degenerative diseases, age-related morbidity is not an inevitable part of aging. Rowe and Kahn [2] propose that many diseases of aging could be avoided, or their severity reduced, if the right lifestyle choices, such as diet, are made. It is of some concern that the nutritional habits of older adults are frequently poor and leave them at increased risk of developing various health disorders.

Surveys estimate that while 15% of the free-living elderly are under-nourished up to 85% of older adults who receive institutional care exhibit some degree of under-nutrition [3-5]. The failure to consume adequate energy to meet requirements as been termed the anorexia of aging and is related to an age-related alteration to the physiological appetite
[6]. Older adults are more sensitive to the satiating effects of cholecystokinin [7] and do not respond to internal appetite cues that stimulate eating to compensate for low energy intake or weight loss [8]. Further research is required to fully understand how aging alters appetite.

One area that has gained little attention is if age related changes in eating behavior influence appetite. Accumulating evidence in young adults suggests that increasing the number of masticatory cycles made before swallowing reduces appetite possibly due to differences in satiety hormones [9-10]. Impaired mastication is a feature of aging due to tooth loss or poor fitting dentures [11] and this would likely influence the number of masticatory cycles made before swallowing [12]. It is not known if reducing the efficiency of eating in older adults influences satiety and short-term food intake. Information regarding this issue could help in the development of feeding regimes for older adults.

The objective of the present study is to investigate reducing eating efficiency by increasing the number of masticatory cycles influences satiety in healthy older adults. We hypothesize that increasing the number of masticatory cycles will increase postprandial satiety.

**Methods**

*Participants*
Fifteen older men participated in this study and were recruited by e-mail or fliers that were distributed in the local community. Individuals who were interested in the study were invited to attend a screening session to determine their eligibility. Inclusion criteria for this study were: male; age >65 years; BMI between 20 and 29.9; full set of natural teeth or with well-fitting dentures; and willingness to eat the study foods. Potential participants were excluded from the study if they had: presence or history of gastrointestinal disease, presence of acute disease, using medication that affects appetite, were a restrained eater (>13 on the restraint section of the three-factor eating questionnaire [13]), allergy or intolerance to the test food, or rated palatability of the test food less than 5 on a 9-point scale. This study was approved by the Iowa State University Institutional Review Board and all participants signed an informed consent form before being included in the study.

Test meal

Freschetta brick oven singles fire baked 5-cheese pizza (Schwan Food Company, Bloomington, MN, USA) was used as the test food in this study. Nutrient labeling on the food package reported that each pizza contained 51 g carbohydrate, 23 g fat, 22 g protein and had 2050 kJ (490 kcal) with a total weight of 183 g. The pizza (15×15×1 cm) was baked at 204°C (400 F) in oven for 15 minutes. Each pizza was served in 64 smaller portions (1.9×1.9×1 cm) and each participant consumed the 64 portions in each test session.
An *ad libitum* pasta meal was served three hours later. The meal was prepared in 3766 kJ (900 kcal) portions, made by 150 g of Barilla spaghetti (Barilla America Inc., Bannockburn, IL, USA), 375 g of Barilla Marinara sauce with imported olive oil (Barilla America Inc., Bannockburn, IL, USA), 37.5 g of shredded parmesan cheese (Wal-Mart Stores Inc., Bentonville, AR, USA) and 5.1 g salt. Meals were prepared under a standard procedure and mixed well before presented to subjects.

*General procedure*

To prevent the risk of choke, a preliminary session was conducted for eligible participants to determine a suitable bite size for the study. In this session the pizza was provided in small portions with different sizes and results indicated a bite size of 1.9×1.9×1 cm was suitable for all subjects to swallow it comfortably after 15 chews.

Participants then attended two test sessions that were separated by at least 7 days. The treatment order was randomized.

On each test session, participants were required to attend the laboratory at 7:30am after an overnight fast. An indwelling catheter was inserted into their non-dominant arm and following a thirty-minute acclimatization period a baseline blood draw was made. An appetite questionnaire was provided to assess their baseline subjective appetite ratings. The questionnaire had four questions: How hungry do you feel right now? How full do you feel right now? How preoccupied with food are you right now? What is your desire to eat right now? Responses were captured using a 100 mm visual analogue scale (VAS). The VAS was anchored with diametrically opposed statements in each end (e.g. not at all
hungry, as hungry as I have ever felt). Participants were instructed to draw a vertical marker on the scale at position that they felt reflected their current strength of appetitive feeling.

The participants were then presented with the pizza in 64 equal portions. Depending on test session, participants were instructed to chew each portion 15 or 40 times. Only one portion was allowed each mouthful and subjects must swallow immediately when the number of chews was reached and then repeat the same process for the next portion immediately. No water or drinks were allowed. A researcher was sit with participant to count the number of chews and verify protocol compliance.

Immediately after they finished the pizza meal, an appetite questionnaire was completed and another blood draw was made (t0). Further blood samples and appetite questionnaires were collected at t0+15, 30, 45, 60, 90, 120 and 180 minutes. Throughout the test session, participants were required to remain seated in a quiet room free from food cues and were not allowed to consume other food or drinks. After the final blood draw the catheter was removed and the participants were allowed to rest for five minutes before being presented with the pasta meal. Participants were instructed to eat until comfortably full and were told extra portion was ready if needed. In this meal no instruction on the number of chews was given and no fluid intake was allowed. Each bowl of food was weighed before and after serving out the sight of participants and the amount consumed was recorded.

*Hormone and metabolite measurement*
Blood was drawn into 4 mL EDTA coated vacutainer tubes and mixed with 400 µL 10000 KIU/ml aprotinin and then centrifuged at 3000 g at 4 °C for 15 minutes. The plasma was then divided into aliquots and stored at -80 °C until analysis. Insulin was assayed by radioimmunoassay (RIA) as described before [14]. Human insulin was used as standards. The assay was able to detect insulin ranging from 0.78 to 200 µU/mL. The intra-assay CV was 6% while inter-assay CV was 5% at 20 µU/mL. Glucose-dependent insulinotropic peptide (GIP) was assayed by RIA using 1:8000 human GIP antibody (Phoenix Pharmaceuticals, Burlingame, CA, USA); the assay had a determination range from 0.05 to 6.4 ng/mL, with intra-assay CV of 7% and inter-assay CV of 6% at 0.5 ng/mL. Cholecystokinin (CCK) was assayed by RIA using antibody 92128 diluted by 1:2000. The antibody was a gift from Dr. Jens Rehfeld. The assay was able to measure CCK from 4.0 to 128 pg/mL. The intra-assay CV was 7% and inter-assay CV was 9% at 50 pg/mL. Ghrelin was analyzed by RIA using 1:10000 diluted antibody T-4747 purchased from Bachem (Torrance, CA, USA). The assay had a determination range from 0.05 to 12.8 ng/mL. The intra-assay was 11% and inter-assay was 14% at 0.5 ng/mL. Tracers used for RIAs were purchased from PerkinElmer (Boston, MA, USA). Plasma glucose was assayed using a biochemical analyzer (YSI Life Sciences, Model 2700 select, Yellow Springs, OH, USA).

Statistical analysis

All data are presented as mean ± standard error of the mean. SAS v9.2 (SAS Institute, Cary, NC, USA) was used to perform statistical analysis. To adjust for variation in baseline value, data change from baseline were used. A mixed model of repeated
measures ANOVA (Proc Mixed, SAS) was used to test overall treatment effect, time effect and treatment×time interaction. Subjects were added as a random variable in the model. Post-hoc analysis was performed by Bonferroni adjusted pairwise comparison of responses from the same time point. Difference in food intake at the subsequent meal was tested using a paired t-test. Statistical significance was set at P<0.05.

Results

Participant characteristics

Participants had age of 75±2 years and their BMI was 25.6±0.8 kg/m². One participant did not consume the ad libitum meal after providing the last blood draw and completing the final appetite questionnaire at 180 min, because he wanted to leave laboratory earlier for personal issues. Therefore, the number of subjects used for appetite data analysis was 15, and it was 14 for analysis of food intake at the subsequent meal.

Subjective Appetite

Figure 1 shows the subjective appetite responses under different chewing conditions. A significant main effect of time was found on all parameters (P<0.0001) but there was no treatment by time interaction in any of these parameters (P>0.05).

Hunger was lower in the 15 chews condition compared with 40 chews (F(1,210)=14.94, P<0.001). No main effect of treatment was found on fullness (F(1,210)=2.85, P=0.093). Compared with 15 chews, preoccupation with food was lower in the 40 chews condition (F(1,210)=18.55, P<0.001). A significant main effect of treatment on desire to eat was
found \((F(1,210)=11.26, \ P<0.001)\), subjects had less desire to eat in the 40 chews condition.

*Satietiy related hormones and metabolites*

Figure 2 shows postprandial plasma concentration of glucose, insulin, GIP, CCK and ghrelin in both test sessions. Main effect of time was significant on glucose, insulin and GIP \((P<0.05)\) but not on CCK and ghrelin \((P>0.05)\). Treatment by time interactions were significant on glucose, insulin and GIP \((P<0.05)\).

There was a significant main effect of treatment on postprandial plasma concentration of glucose \((F(1,210)=4.22, \ P=0.041)\). Immediately after meal, glucose concentration was significantly higher in 40 chews \((P=0.002)\). However after 15 minutes it became lower and it was further reduced below baseline at 120 and 180 min, whereas in 15 chews, glucose concentration was constantly above baseline during three hours after meal. At 180 min, glucose was significantly higher in 15 chews \((P=0.008)\). The postprandial glycemic response showed a peak at 30 min in 15 chews whereas it occurred at 0 min in 40 chews.

A significant main effect of treatment on insulin was found \((F(1,210)=5.60, \ P=0.019)\). Insulin was maintained above baseline for three hours after meal in both conditions. Higher insulin was found in 40 chews at 0 min \((P<0.001)\) compared with 15 chews. However, the difference in insulin between test sessions diminished gradually afterwards. At 120 min and 180 min, lower insulin level was observed in 40 chews \((P<0.05)\). The
postprandial peak insulin occurred at 15 min in 40 chews where it was at 45 min in 15 chews.

Postprandial GIP concentration was higher than baseline level for 3 hours after meal in both conditions. No significant main effect of treatment on GIP concentration was found (F(1,210)=1.05, P=0.306). However, in the 40 chews condition, GIP was significantly higher at 0 min (P=0.002) and it became significantly lower at 120 min and 180 min (P<0.05). The peak was observed at 45 min in 40 chews where it was at 60 min in 15 chews.

No main effect of treatment was found on CCK (F(1,210)=0.04, P=0.851). The peak of CCK was found at 30 min in the 40 chews condition, it occurred at 90 min in 15 chews. No main effect of treatment on ghrelin was found (F(1,210)=1.26, P=0.263). Ghrelin was suppressed below baseline before 120 min in 40 chews; in 15 chews it reached a peak above baseline at 60 min.

Food intake

A trend toward significance was observed on the food intake at the subsequent meal. In the 40 chews condition, subject had an increase in the amount of food intake at subsequent meal. (40 chews: 380.6±38.6 g vs 15 chews: 318.5±32.4 g, P=0.066).

Discussion

The present study found that increasing the number of masticatory cycles in older men during a fixed-portion meal further reduced postprandial subjective appetite and
promoted satiety. Differences in the plasma concentration of glucose, insulin and GIP were observed between conditions with a significant interaction effect, i.e., in 40 chews they were higher immediately after meal but lower after 2-3 hours. Interestingly, although participants have lower appetite scores in the 40 chews condition, they tended to eat more at the subsequent meal.

Anorexia of aging is characterized by early satiation and reduced amount of food intake, which are results from dysregulation of food intake and a complex interaction of endocrine system and central nervous system [6, 15]. Although etiology of anorexia of aging has not been fully elucidated, it is believed older adults are associated with a significant impairment in the ability to control food intake [16-17]. In the present study, we found increasing masticatory cycles during a fixed portion meal promotes food intake at the subsequent meal in older men. This may appear counter-intuitive (i.e., in the 40 chews condition, participants had less appetite, however, they consumed more at the subsequent meal). The conflicting results on hedonic rating of appetite and food intake may be partly due to aging-related dysregulation of appetite.

The increase in glucose further stimulates secretion of insulin from pancreas, and this is consistent with the result in insulin and GIP which had shown a higher response at 0 min in the 40 chews condition. GIP is an incretin and it is secreted to facilitate disposal of ingested nutrients and stimulate insulin secretion [20]. In this study, it was found at 120 and/or 180 minutes, glucose, insulin and GIP became significantly lower in the 40 chews condition. This is because the test meal was provided in a fixed preload under both conditions. The more elevated level of glucose immediately following meal in 40 chews
would result in a lower glucose level later. The decline in glucose afterwards induced a similar response in GIP and insulin. The lower concentration of plasma glucose in the 40 chews condition at 180 min could partly explain why participants consumed more food at the subsequent meal, since decline in blood glucose is associated with stimulation of appetite [21]. However, it is not known why a similar change on subjective appetite was not observed.

In human it has been found that masticatory force has a positive relationship with saliva flow rate [22], and mastication increases saliva secretion rate [19]. Although in this study we did not measure saliva production, participants mentioned they could feel more saliva was ingested when they swallowed food bolus in the 40 chews condition, suggesting that increasing masticatory cycles resulted in increased production of saliva. Older adults usually have a feeling of dry mouth [23], therefore, by increasing masticatory cycles, it might be helpful to stimulate salivary production to aid ingestion.

In the present study it was found subjects reported consistently higher ratings on hunger, preoccupation with food, and desire to eat when 15 chews were made for each portion of food during ingestion. In a study of almond by Cassady et al., no dose response between mastication cycles and postprandial hedonic ratings on appetite was found [9]. Several factors could account for the differences between the two studies. The Cassady et al. study used normal body weight adults with age < 50, and a mixed population of males and females, whereas in the present study only males but both normal weight and overweight subjects were included. Moreover, difference in test food may account for discrepancy in these results. Almond is a high fat food whereas the test meal in the
present study consisted of large amount of carbohydrates. Fat and carbohydrate have different satiating effect [24-25] and this may partly account for the difference in the results.

Several studies have found eating rate affects appetite in young adults [26-27]. To our knowledge this is the first study to investigate the effect of eating rate/masticatory cycles on satiety in older adults. Karl et al. [28] conducted an eating rate study using a fixed amount of meal in adults with age <55, they found no effect of eating rate on postprandial appetite, appetite-regulating hormones and subsequent food intake. In our study, we found although eating slowly in the 40 chews condition promoted subjective satiety, no effect on CCK or ghrelin was found. Difference in study population may account for the difference in the results. In addition, methods used to extend meal duration were different. By now it is not clear whether the methods to slow down eating rate could have an impact on experimental results, and if they have the same mechanisms for the results. In their study subjects consumed the meal at a pace according to a programmed eating curve with no instruction on chewing behavior was given [28]. Further study on comparison of different methods to slow down eating rate on appetite is required.

In conclusion, our study suggests for older men, eating slowly by increasing masticatory cycles could suppress postprandial appetite but also induce increase of food intake at the subsequent meal. Although further work is required to fully elucidate mechanisms for impaired regulation of appetite and food intake in older adults, increasing masticatory cycles could be a potential strategy to promote food intake in this population.
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References


FIGURE LEGENDS

**Figure 1.** Change of hunger (A), fullness (B), preoccupation with food (C) and desire to eat (D) following 15 chews and 40 chews conditions. N=15, data were mean±SEM. Main effect of treatment on hunger, preoccupation with food and desire to eat was significant (P<0.001, P<0.001, P<0.001 respectively). No significant main effect of treatment on fullness was found (P>0.05).

**Figure 2.** Change of glucose (A), insulin (B), GIP (C), CCK (D) and ghrelin (E) following 15 chews and 40 chews conditions. N=15, data were mean±SEM. Main effect of treatment on glucose and insulin was significant (P=0.041, P=0.019, respectively). No significant main effect of treatment on GIP, CCK and ghrelin was found (P>0.05). Significant treatment by time interaction was found on glucose, insulin and GIP (P=0.002, P<0.001, P<0.001, respectively). The asterisk (*) indicates significantly different plasma concentration between treatments at the same time point (P<0.05).
Figure 1.
Figure 2.
CHAPTER 8. GENERAL CONCLUSIONS

General Conclusions

Identifying factors that affect appetite is essential for development of better dietary and behavioral strategies for body weight management. The studies we conducted have clearly shown that ingestive behavior has a significant impact on appetite.

The results from the studies involved in the dissertation have demonstrated that:

(1) Body weight is a variable explaining for the inter-individual variation in habitual mastication performance. Specifically, there is a negative association between BMI and the habitual number of chews made before swallowing.

(2) Mastication performance is influenced by aging and food hardness. The older adults have reduced mastication efficiency compared with young adults. Harder food requires more chews before they can be swallowed.

(3) There is little variation in the particle size of the bolus at the swallowing threshold. Aging, body weight and food hardness do not influence the particle size distribution of the food bolus at the swallowing threshold.

(4) Increasing the number of chews per mouthful significantly reduces meal size in healthy lean, overweight and obese young adults. However, it does not influence the meal size in the older adults.

(5) The ingestive behavior, characterized by eating slowly and chewing thoroughly through increasing the number of chews per mouthful, promotes post-prandial satiety and influences glycemic response in both young and older adults.
The satiating effect resulted from such an ingestive behavior is short-lived in young adults, and it does not influence food intake three hours later. By contrast, the older adults tends to eat more at the subsequent meal, even though they feel less hungry after they have increased the number of chews during the previous fixed-amount meal.

In consistent with the result from the study by Tureli et al. [1], we have shown body weight is a variable explaining for the habitual mastication performance. In view of this finding, body weight should be controlled in participant recruitment or data analysis in future mastication or dental studies. Right now most of the studies investigating mastication performance in human have not provided body weight information of their participants or used body weight as a covariate in their data analysis.

The results from the study investigating the effect of mastication on postprandial satiety in young adults are generally consistent with the previous studies that have shown increasing the number of chews promotes postprandial satiety [2-3]. While the previous studies did not provide a subsequent meal for the participants, we have shown there was no effect on the subsequent food intake provided three hours later. This suggests the satiating effect is short-lived, nonetheless, it is possible that when people increase their mastication activity during the first meal, the satiating effect may result in a reduction in inter-meal snacking.

The results from the study investigating the effect of mastication on satiation in young adults also support the results reported by previous studies showing meal size was reduced when participants made more chews per mouthful [3-4]. A significant difference
in the experimental design compared with the previous studies is that we used the habitual number of chews rather than a pre-specified masticatory cycle, in consideration of the considerable variation in the habitual number of chews among the population. This allows the development of individualized strategies. Moreover, a major concern in the previous studies is that the excessive chewing may be uncomfortable thus the participants stopped eating and ate less when more chews per mouthful were required. By contrast, we have measured the appetite ratings at meal termination, which has clearly shown that participants had a similar degree of satiation in all test sessions when they finished the ad libitum meal.

To our knowledge, it is the first time that the effect of mastication on appetite in older adults was investigated. The studies provide further evidences for the impaired appetite control in this population, i.e., they could eat more food when they feel less hungry; the number of chews have an impact on satiation in young adults but not older adults. Moreover, the results suggest it is possible that the impaired mastication partly accounts for the development of anorexia of aging in this population. Compared with young adults, older adults need more masticatory cycles until a suitable bolus is formed; given the same pre-specified number of masticatory cycles made before swallowing, they must use a smaller bite size so that they can swallow without the risk of choking. Those ingestive behaviors (increasing the number of chews and smaller bite size) have been demonstrated to promote satiation in young adults [3-6], and they could partly explain for the reduced food intake in older adults.
The overall results indicate the potential role of higher mastication efficiency (less number of chews during ingestion) in contributing to the positive energy balance, and the effect on appetite may act as one of the mediators. It is possible that people eat faster and chew less before they swallow the bolus, resulting in insufficient cues for development of satiation signals, which lead to larger meal size and/or lower degree of postprandial satiety, and in long-term, to weight gain.

In overall conclusion, the research involved in the dissertation has demonstrated the appetite-suppressing effect of increased mastication. Increasing the mastication activity during ingestion can be a strategy to curb appetite. For the purpose of body weight management, choosing harder foods that require more chews, eating slowly and chewing thoroughly, could be the advices formulated based on the findings from the present research.

**Recommendations for Future Research**

*Epidemiology*

It has been reported that the hardness of the habitual diet was negatively associated with the waist circumference in free-living Japanese women [7]. However, no similar studies have been conducted in western countries. Considering the difference in habitual food items between Asia and western countries, it is worth investigating the association of hardness of western diet and body weight.

Epidemiological studies have shown that eating rate was positively associated with body mass index in Japan [8-12] and New Zealand [13]. Nonetheless, no studies in Europe or
North America have been published. In view of the difference in obesity epidemic in different ethnic groups, as well as different countries and regions, the study in western countries to elucidate the association between eating rate and body weight is warranted.

Clinical Nutrition

In the current research we have shown mastication influences appetite and food intake. Another possible route that mastication may affect energy balance is through energy expenditure. However, the effect of mastication on diet induced thermogenesis has received little attention. No human study in this area has been conducted. An animal study has shown rats fed with soft chow which developed greater adiposity was because of the deficiency of thermogenesis [14]. This is interesting and it may provide some hints for research in human subjects.

Animal studies suggest mastication can activate the satiety center through histamine neurons [15-16]. With the development of functional neuro-imaging techniques, it is now possible to conduct studies to elucidate the neurological mechanisms how mastication contributes to the development of satiation in human. A search in literature has suggested currently there are no relevant studies published; it is crucial to design such studies to provide convincing evidence in explaining the direct effect of mastication on appetite.

As the long-term goal is to develop dietary and behavioral strategies for body weight management, clinical trials investigating the effect of ingestive behavior characterized by eating slowly and chewing thoroughly on body weight in overweight and obese participants are warranted. Currently no studies have been conducted to elucidate the
effect of long-term modification in mastication on body weight, although a study has shown by using a mandometer to provide real time feedback to remind children to eat slowly, it resulted in a significantly lower mean BMI standard deviation score after 12 months [17].

References


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