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The Effects of Manual Tooth Brushing on Parotid and Submandibular/Sublingual Gland Salivary Flow Rates in Healthy Young and Older Adults

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Graduate Program in Health and Rehabilitation Sciences
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Abstract

Saliva is crucial for the maintenance of oral health. Individuals with reduced salivary flow may experience a distortion in taste, difficulty swallowing, and impaired articulation of speech. Research has shown that tooth brushing increases whole salivary flow rates in older adults. It is important to determine whether this increase results from the modulation of parotid gland salivary flow, submandibular and sublingual gland salivary flow, or both. Saliva produced from the parotid gland aids in digestive processes, while saliva secreted from the submandibular and sublingual glands promotes protection of the oral cavity. A within-subjects methodology was used to examine the effects of tooth brushing on gland-specific salivary flow rates in healthy young and older adults. Tooth brushing was associated with increased salivary flow from both the parotid and submandibular and sublingual glands in young and older adults. Tooth brushing may hold potential as a therapeutic approach to increasing salivary flow rates.

Keywords

“tooth brushing, saliva, aging, oral sensory stimulation, parotid glands, submandibular/sublingual glands, salivary flow, oral health, rehabilitation”

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Table of Contents

Abstract.....	ii
Acknowledgments.....	iii
Table of Contents.....	iv
List of Tables.....	vii
List of Figures.....	viii
List of Appendices.....	ix
Chapter 1.....	1
1 Introduction.....	1
1.1 Saliva Composition and Functions.....	1
1.2 Salivary Gland Anatomy and Physiology.....	2
1.3 Neural Control of Salivary Flow.....	2
1.4 Aging and Salivary Flow.....	4
1.5 Gender and Salivary Flow.....	4
1.6 Effects of Oral Sensation on Salivary Physiology.....	5
1.6.1 Gustatory Stimulation of the Oral Cavity.....	5
1.6.2 Olfactory Stimulation of the Oral Cavity.....	5
1.6.3 Mechanical Stimulation of the Oral Cavity.....	5
Chapter 2.....	9
2 Methodology.....	9
2.1 Apparatus and Materials.....	9
2.2 Saliva Collection.....	9
2.3 Manual Tooth Brushing Technique.....	10
2.4 Experimental Paradigm.....	10
2.5 Additional Study Procedure.....	12

2.6	Calculation of Salivary Flow Rates	12
2.7	Statistical Analyses	12
Chapter 3	14
3	Results	14
3.1	Participants.....	14
3.1.1	Inclusion and Exclusion Criteria.....	14
3.1.2	Participant Demographics.....	15
3.2	Observations During Experimental Sessions.....	18
3.2.1	Experimental Procedure.....	18
3.2.2	Salivettes®	18
3.2.3	Swallowing Occurrence	19
3.3	Statistical Analyses	19
3.3.1	Left Parotid Gland Salivary Flow Rate.....	19
3.3.2	Right Parotid Gland Salivary Flow Rate	25
3.3.3	Submandibular/Sublingual Gland Salivary Flow Rate.....	31
3.3.4	Submandibular/Sublingual Gland Adjusted Salivary Flow Rate.....	38
Chapter 4	46
4	Discussion	46
4.1	Major Findings.....	46
4.1.1	Baseline Salivary Flow Rates	47
4.1.2	Left and Right Parotid Glands	47
4.1.3	SMSL Glands.....	51
4.1.4	SMSL Glands Adjusted	52
4.1.5	Comparison of Parotid and SMSL Gland Findings	53
4.2	Limitations of Study	53
4.3	Clinical Implications.....	55

4.4 Suggestions for Future Studies	55
4.5 Conclusion	56
References.....	57
Appendices.....	65
Curriculum Vitae	75

List of Tables

Table 1. Characteristics of Young Adult Participants	15
Table 2. Characteristics of Older Adult Participants	16
Table 3. Left Parotid Gland Salivary Flow Rates (Mean \pm Standard Error of the Mean)	22
Table 4. Right Parotid Gland Salivary Flow Rates (Mean \pm Standard Error of the Mean)....	28
Table 5. Submandibular/Sublingual Gland Salivary Flow Rates (Mean \pm Standard Error of the Mean)	34
Table 6. Submandibular/Sublingual Gland Adjusted Salivary Flow Rates (Mean \pm Standard Error of the Mean)	42

List of Figures

Figure 1. Experimental Protocol	11
Figure 2. Left Parotid Gland Salivary Flow.....	24
Figure 3. Right Parotid Gland Salivary Flow	29
Figure 4. SMSL Gland Salivary Flow	36
Figure 5. SMSL Gland Adjusted Salivary Flow	43

List of Appendices

Appendix A: Ethics Approval.....	65
Appendix B: Letter of Information and Consent Form	66
Appendix C: Participant Health Questionnaire.....	71
Appendix D: Oral Examination Form.....	72
Appendix E: Data Collection Form	73

Chapter 1

1 Introduction

1.1 Saliva Composition and Functions

Saliva is an exocrine secretion, which has several important functions. Its properties help with the processes of mastication, digestion, and swallowing (Nagler, 2004). The maintenance of a neutral pH in the oral cavity is achieved by numerous electrolytes (sodium, potassium, calcium, magnesium, bicarbonate, and phosphate). This is necessary to ensure the oral cavity environment promotes beneficial bacterial growth, while clearing the oral cavity of organisms known to cause dental caries (Marsh, Do, Beighton, & Devine, 2016). Proteins and enzymes protect the oral cavity from bacteria, viruses, and fungi (Nagler, 2004). They cleanse the oral cavity by interfering with attachment of the microorganisms to oral cavity structures (Humphrey & Williamson, 2001).

Adequate salivary flow is necessary for the maintenance and protection of the oral cavity. Salivary flow rates vary greatly across individuals under both unstimulated and stimulated conditions. However, salivary flow rate greater than 0.1 mL/min is considered normal during unstimulated, or resting conditions. Under stimulated conditions, salivary flow rate greater than 0.2 mL/min is regarded as normal (Humphrey & Williamson, 2001). The submandibular and sublingual glands contribute greatly to the unstimulated salivary flow rate, with the submandibular glands contributing 65% and the sublingual glands contributing 7% to 8% of total salivary secretions. Additionally, the parotid glands contribute 20%, while the minor salivary glands are responsible for producing less than 10% of salivary secretions during resting conditions. In response to stimulation, flow rate from the parotid glands increases to produce greater than 50% of salivary secretions (Humphrey & Williamson, 2001). Hyposalivation is classified as salivary flow rate that is less than 0.1 mL/min at rest or less than 0.7 mL/min under stimulated conditions (Saleh, Figueiredo, Cherubini, & Salum, 2014). The oral cavity tissues become dry and the salivary glands begin to atrophy, which can lead to a distortion of the sense of taste, difficulty swallowing and impaired articulation of speech (Scully & Felix, 2005).

Secretions from the parotid gland are serous in nature, contain no mucins, but are rich in amylase and proline-rich proteins. Saliva from the parotid glands facilitates the digestion of food. In contrast, secretions from the submandibular and sublingual glands are mixed serous and mucous in nature (Humphrey & Williamson, 2001), and are rich in mucin and cystatin (Carpenter, 2013). Submandibular and sublingual gland saliva promotes protection of the oral cavity.

1.2 Salivary Gland Anatomy and Physiology

There are three main paired salivary glands that are responsible for producing approximately 90% of total salivary secretions—the parotid, submandibular, and sublingual glands. The parotid glands are the largest salivary glands, which are located at the back of the mouth, inside each cheek. The submandibular and sublingual glands are found underneath the tongue (Tucker, 2007). Minor salivary glands located in the buccal, labial, palatal, and lingual regions of the oral cavity also contribute to salivary secretion (Eliasson & Carlén, 2010). The combination of saliva secreted from the major and minor salivary glands, gingival crevicular fluid, mucosal cells, oral bacteria, and food debris, constitutes whole saliva (Sreebny & Vissink, 2010).

The salivary glands consist of acinar cells that are responsible for the production of saliva, and the ductal cells, which transport saliva to the mouth. A signal is sent from the brain to the myoepithelial cells, which initiates constriction of the acinar cells. The acinar cells secrete salt into the ductal lumen of the salivary gland, comprising the first secretory event (Carpenter, 2013; Humphrey & Williamson, 2001). Simultaneously, water enters the cells via aquaporin channels, which creates a fluid that is isotonic with respect to serum. The ductal cells resorb the salt, modifying the isotonic saliva into a hypotonic saliva (Carpenter, 2013).

1.3 Neural Control of Salivary Flow

Taste and mechanical stimulation relay sensory information through the afferent fibers of the facial, glossopharyngeal, and trigeminal nerves. The facial and glossopharyngeal nerves synapse in the nucleus tractus solitarius (NTS), while the trigeminal nerve

synapses in the trigeminal nucleus. Next, signals are sent to the superior and inferior salivatory nuclei in the medulla oblongata (Proctor, 2016). Salivary gland secretion is predominantly regulated by efferent parasympathetic and sympathetic nerves of the autonomic nervous system (ANS) (Proctor & Carpenter, 2007). The ANS also controls the secretion of tears and sweat, the contraction of gastrointestinal sphincters, blood pressure, and heart rate; all processes vastly under involuntary control (Sreebny & Vissink, 2010). The parasympathetic nerves originate in the salivatory nucleus of the medulla. Those originating in the superior salivatory nucleus travel via the facial nerve, synapse in the submandibular ganglion, and innervate the submandibular and sublingual salivary glands. Fibers originating in the inferior salivatory nucleus travel via the glossopharyngeal nerve, synapse in the otic ganglion and supply the parotid gland. In contrast, sympathetic nerves originate outside the cortex, in the thoracolumbar region of the spinal cord. Sympathetic nerves synapse in the superior cervical ganglion before supplying the submandibular, sublingual, and parotid salivary glands (Sreebny & Vissink, 2010).

Parasympathetic nerves are responsible for the secretion of acetylcholine (ACh), a neurotransmitter which interacts with muscarinic cholinergic receptors (mAChRs) to cause salivary secretion (Proctor & Carpenter, 2007). It has been shown that two subtypes of mAChRs, M1 and M3 receptors, mediate the secretion of whole saliva (Gautam, Heard, Cui, Miller, & Bloodworth, 2004; Nakamura et al., 2004). Sympathetic nerves play less of a role in causing fluid secretion, but their importance in producing salivary protein secretion has been demonstrated by studies showing that the protein concentration of saliva is decreased following acute sympathetic denervation compared to glands without denervation (Matsuo, Garrett, Proctor, & Carpenter, 2000). Sympathetic nerves release noradrenaline, which acts through alpha1- and beta1-adrenoceptors. It is evident that parasympathetic stimulation has a great role in evoking the secretion of water and electrolytes (Garrett, 1987), while sympathetic stimulation tends to have greater effect in modulating the protein composition of saliva (Proctor & Carpenter, 2007). However, it has been shown that parasympathetic impulses have the ability to produce significant protein secretion (Asking & Gjørstrup, 1987).

1.4 Aging and Salivary Flow

It is well established that with increasing age, changes in the cellular structures of the salivary glands occur (Vissink, Spijkervet, & Amerongen, 1996). There is an increased volume of fat and fibrovascular tissue in the sublingual, submandibular and parotid glands, and a reduction in the volume of acini (Azevedo, Damante, Lara, & Lauris, 2005; Moreira, Azevedo, Lauris, Taga, & Damante, 2006; Scott, Flower, & Burns, 1987; Scott, 1977).

A decline in salivary flow rate with increasing age has been reported in some studies (Gutman & Ben-Aryeh, 1974; Moritsuka et al., 2006). However, others have not reported the same outcome (Fischer & Ship, 1999; Heft & Baum, 1984; Tylenda, Ship, Fox, & Baum, 1988). A recent meta-analysis found that unstimulated and stimulated whole and submandibular and sublingual gland salivary flow rates are significantly lower in older adults compared to younger adults. There were no significant differences in parotid gland salivary flow rates between the young and older adults (Affoo, Foley, Garrick, Siqueira, & Martin, 2015).

It has been postulated that systemic diseases (i.e, Sjögren's syndrome) and their treatments (medication usage, chemotherapy, head and neck radiation) contribute more to reduced salivary flow than does the process of aging (Sreebny & Schwartz, 1997). For example, in patients who have received radiation treatment for head and neck cancer, unstimulated salivary flow rate can decrease by up to 45% of its normal value (Gonnelli et al., 2016).

1.5 Gender and Salivary Flow

Studies have shown that females have lower mean salivary flow rates than males. The smaller gland sizes in females compared to males, may be responsible for this finding (Bergdahl, 2000; Percival, Challacombe, & Marsh, 1994). The difference in flow rate has been reported to be between 0.1 mL/min and 0.2 mL/min (Bergdahl, 2000; Narhi et al., 1992).

1.6 Effects of Oral Sensation on Salivary Physiology

The secretion of saliva is modulated by specific stimuli. Gustatory, olfactory, and mechanical stimuli may meet the threshold necessary for the neural control system to lead to salivary flow (Humphrey & Williamson, 2001).

1.6.1 Gustatory Stimulation of the Oral Cavity

Taste buds are found in the papillae of the tongue, soft palate, epiglottis, esophagus, nasopharynx, and the buccal wall (Ekström, Hylén, Massimo, & Irene, 2012). They are responsive to various stimuli including sour, sweet, salty, bitter, and umami taste. It has been shown that the stimuli have different effects on the flow rate, ionic, and organic composition of saliva—sour stimuli have been shown to produce the greatest increase in salivary flow rate, while bitter stimuli are the least likely to affect salivary flow (Hodson & Linden, 2006).

1.6.2 Olfactory Stimulation of the Oral Cavity

Molecules of nasal airflow are responsible for stimulating olfactory receptors, which are located in the cribriform plate (Ekström et al., 2012). The literature examining the effects of olfactory stimuli on salivary flow is limited. Some studies have shown that odours have no effect on resting and stimulated parotid salivary flow (Lee & Linden, 1992), while other studies have reported an effect of odours on whole salivary flow rates (Kerr, 1961).

1.6.3 Mechanical Stimulation of the Oral Cavity

It has been shown that the act of chewing can stimulate salivary flow, which can aid lubrication of the oral mucosa and in the management of dental caries (Dawes & Kubieniec, 2004). Wang and colleagues (2012) investigated the relationship between gum chewing, salivary flow, and dental caries severity in adults. They found that frequent gum chewing over the previous year was associated with a higher unstimulated salivary flow rate and lower caries severity.

Hiraba and colleagues (2008) examined the effect of facial vibrotactile stimulation on salivary flow. The mechanical stimulus was delivered to facial skin overlying the masseter muscles. It was found that vibration at 89 Hz increased salivation in the left and right parotid, submandibular, and sublingual glands by more than 50% compared to baseline salivary flow rates.

In a subsequent study, Hiraba and colleagues (2014) investigated the effects of the vibrotactile stimulation on the parasympathetic nervous system. They reported that vibration at 89 Hz resulted in lower pulse frequency, contracted pupils, and increased salivary secretion in comparison to vibration at 114 Hz, classic music, and noise. This finding suggests that mechanical stimulation at 89 Hz activates the parasympathetic nervous system.

Mechanoreceptors are located throughout the oral tissues, including the mucosa, periodontal ligament, tongue, palate, and lips (Jacobs et al., 2002; Nordin & Hagbarth, 1989). They are responsive to various mechanical stimuli including touch, pressure, vibration and proprioception (Dong, Shiwaku, Kawakami, & Chudler, 1993; Nordin & Hagbarth, 1989; Trulsson & Johansson, 2002). Mechanical stimulation of the oral cavity via tooth brushing has been shown to stimulate salivary flow in healthy young adults (Hoek, Brand, Veerman, & Nieuw Amerongen, 2002; Ligtenberg, Brand, Bots, & Nieuw Amerongen, 2006). Hoek and colleagues (2002) examined the effect of tooth brushing on the flow rate and protein composition of whole saliva. The Bass method was employed as a standardized protocol for tooth brushing. This tooth-brushing technique involved directing the toothbrush towards the gum line at a 45° angle, and making small circular motions to brush the teeth. Salivary flow rate was shown to significantly increase during the initial five minutes after tooth brushing, and decrease after fifteen minutes. Thus, tooth brushing elicited a brief increase in whole salivary flow rate. No significant changes were observed in the total protein and amylase concentrations.

Ligtenberg and colleagues (2006) examined the effects of tooth brushing on whole salivary flow rate, pH, and buffering capacity. Participants used the Bass method for brushing and were divided into groups for brushing with either water, menthol-free

toothpaste, anti-caries toothpaste, or Parodontax®. It was found that brushing with water increased salivary secretion significantly for 60 minutes. After brushing with toothpaste, salivary secretion rates increased significantly when compared to brushing with water. This finding was most likely a result of gustatory stimulation from the toothpaste. Salivary pH and buffering capacity was shown to increase, and was likely a result of the increased salivary flow rate.

The effects of electric tooth brushing and manual tooth brushing on salivary flow rate in individuals who experienced medication-induced xerostomia were examined by Papas et al. (2006). Electric tooth brushing was associated with greater salivary flow rates for up to 45 minutes post-stimulation. A study by Affoo and colleagues (2015a) found that whole salivary flow rate significantly increased during a two-minute tooth-brushing period and during the five-minute period immediately following tooth brushing in healthy older adults. These effects were observed after brushing with either a manual toothbrush or an electric toothbrush. No significant difference was found between the maximum salivary flow rate increase associated with the manual tooth brushing compared to the maximum salivary flow rate increase associated with electric tooth brushing.

A previous study examining the effects of tooth brushing on whole salivary flow rates in healthy older adults demonstrated a significant increase in salivary flow rate from baseline to tooth brushing, which continued for up to five minutes (Affoo, 2015a). It would be beneficial to determine if the increase in whole salivary flow associated with manual tooth brushing, reported by Affoo et al. (2015a) is gland-specific, since saliva produced from the different glands plays different roles in maintaining oral health. Therefore, this study sought to examine whether the increase in salivary flow as a result of manual tooth brushing is attributable to an increase in (i) parotid gland salivary flow or, (ii) submandibular/sublingual gland salivary flow or, (iii) both. Additionally, given the limited understanding of the effects of aging on salivary flow, the study also investigated whether the effects of manual tooth brushing on salivary flow are similar in healthy young adults compared to older adults.

Evidence from previous literature and from the results of a previous study examining the effects of tooth brushing on whole salivary flow rates in healthy older adults (Affoo, 2015a), allowed for predictions to be made about the gland-specific salivary flow rates resulting from manual tooth brushing. It was hypothesized that salivary flow rate from the left and right parotid glands would be modulated by tooth brushing, while the submandibular/sublingual gland flow rate would not be altered by tooth brushing. This prediction was based on previous research showing that (i) the relative proportion of parotid saliva in whole saliva generally increases with increases in whole salivary flow (Humphrey & Williamson, 2001), and (ii) this phenomenon has been documented when the modulatory technique was chewing, a mechanical stimulus (Dodds, Hsieh, & Johnson, 1991) that is, in that regard, similar to tooth brushing. Additionally, it was expected that the increases in parotid gland salivary flow rates would persist for up to five minutes following tooth brushing. A previous study examining the effects of tooth brushing on whole salivary flow rates in healthy older adults demonstrated a significant increase in salivary flow rate from baseline to tooth brushing, which continued for up to five minutes (Affoo, 2015a).

Chapter 2

2 Methodology

2.1 Apparatus and Materials

The experiment was conducted in the Swallowing Laboratory at Elborn College, Western University. The participant was seated in a lowered office chair in front of a low table. Three sensors were positioned on the participant: a belt-mounted respiratory movement sensor was positioned around the participant's neck (Model 1585, CT2 Pediatric Piezo Respiratory Effort Sensor (Pro-Tech Services, Inc.) (Licence No. 69444)) to register swallow-related movements of the larynx. A second belt-mounted respiratory movement sensor was positioned around the participant's upper abdomen (Model 1582, CT2 Adult Piezo Respiratory Effort Sensor (Pro-Tech Services, Inc.) (Licence No. 69444)) to register swallow-related respiratory movements during the study. An omnidirectional electret microphone (F-SM Snore Electret Microphone, Pro-Tech Services, Inc.) (Licence No. 69446)) was affixed to the participant's neck with medical tape to record the swallow-related acoustic signal. These three physiologic signals were recorded continuously throughout the experimental session using an AS40 Comet Series PSG/EEG Portable System (Astro-Med Inc. Licence No. 65827). Swallows were identified on the basis of a distinct pattern of laryngeal (i.e., neck) movement, respiratory apnea, and a neck-recorded acoustic signal. The participant was also video recorded in the lateral plane, which assisted researchers in determining whether participants swallowed during the saliva-collection periods.

Colgate Sensitive Pro-Relief manual toothbrushes were employed in all studies.

2.2 Saliva Collection

Saliva was collected during eight collection periods using clean, pre-weighed Salivette® cotton rolls, each roll tethered with dental floss, which was taped to the facial skin over the participant's right or left cheek with a small piece of medical tape. At the beginning of each saliva collection period, three Salivette® rolls were placed in the oral cavity for the duration of the collection period: one roll in each of the left and right maxillary buccal

cavities near Stensen's duct which drains the parotid salivary gland, and one roll (divided in two halves), was placed in the sublingual areas at midline near Wharton's duct, which drains the submandibular and sublingual salivary glands. The Salivette® rolls were removed from the oral cavity at the end of each saliva collection period. They were placed in pre-weighed, autoclaved beakers and weighed immediately after each saliva collection period.

2.3 Manual Tooth Brushing Technique

The modified Bass technique for manual tooth brushing was utilized in this study. It was previously used in the study conducted by Affoo and colleagues (2015a) that examined the effects of tooth brushing on whole saliva in healthy older adults. It has been shown that the removal of supragingival plaque from all, lingual, and buccal sites, is significantly greater when the modified Bass technique is applied, compared to other tooth-brushing practices (Poyato-Ferrera, Segura-Egea, & Bullón-Fernández, 2003).

The modified Bass tooth-brushing technique was performed by the researcher (KMT) on all study participants as follows. The oral cavity was divided into four distinct quadrants, upper right, upper left, lower right, and lower left, and brushing followed this order. The total time for the tooth-brushing condition was two minutes, and thus, thirty seconds was spent brushing in each quadrant. Tooth brushing involved brushing of the buccal, lingual, and occlusal surfaces of the teeth, as well as the tongue and hard palate. The toothbrush was directed towards the base of the tooth at the gum line at a 45° angle. The brush was moved using short strokes, in small circular motions, with the brush head remaining in contact with the gingivae and the teeth. When thirty seconds approached, the toothbrush was rolled down over the teeth (Poyato-Ferrera et al., 2003). Following brushing of the lower left quadrant, the tongue and hard palate were brushed with two to three brush strokes.

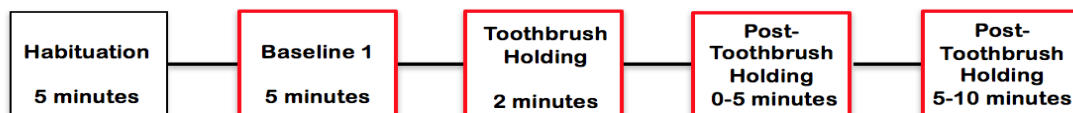
2.4 Experimental Paradigm

There were ten, five-minute experimental periods for each study participant, eight of which involved saliva collection (see Figure 1). Each session was divided into a control

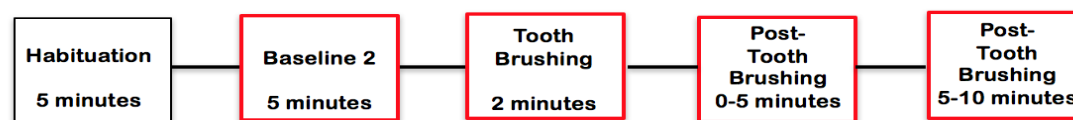
and an experimental phase. The session began with the participant rinsing her/his mouth with distilled water. Subsequently, the participant was seated and the sensors placed on the participant's body. This was followed by a five-minute habituation period during which the participant became acquainted with the laboratory environment. A five-minute baseline period followed, which involved saliva collection as the participant sat at rest. During the baseline period, KMT soaked the toothbrush in water. Thirty seconds prior to the end of the baseline period, the toothbrush was removed from the water and blotted with gauze. Next, a control condition was performed. The control condition involved saliva collection while the participant held the toothbrush stationary in the oral cavity with bristles facing down on the superior surface of their tongue for two minutes. Two, five-minute saliva collections were performed at 0-5 minutes and 5-10 minutes following the toothbrush holding period. A rest period followed, which allowed the participant to sit quietly for a five-minute "washout" period, to increase the likelihood that any effect from the toothbrush would not influence subsequent experimental periods. Another five-minute baseline saliva collection was performed prior to toothbrush stimulation. A toothbrushing experimental period followed, during which the researcher brushed the participant's teeth, tongue, and hard palate using the Bass Method, as described above. Tooth brushing was performed without dentifrice. Two, five-minute saliva collections were performed at 0-5 minutes and 5-10 minutes following the tooth-brushing period. Participants were instructed not to swallow their saliva and to make minimal orofacial movements during and immediately following the saliva collection periods.

Figure 1. Experimental Protocol

Control Phase



Experimental Phase



2.5 Additional Study Procedure

To investigate the chance that residual water on the toothbrush following soaking contributed to the weight of the Salivette® located near the SMSL glands, an additional procedure was performed following saliva collection from all study participants. First, the toothbrush was placed in a pre-weighed beaker and the weight was recorded. Next, the toothbrush was soaked in water for five minutes. The toothbrush was blotted using gauze to absorb excess water, as was completed during the experimental paradigm described above. The toothbrush was returned to the beaker and the weight was recorded. This was performed five times. The five trials were averaged to determine the weight of water that may have contributed to the recorded weight of the Salivette® near the SMSL glands. The calculated flow rate was subtracted from SMSL gland salivary flow during the two collection periods when the toothbrush was present in the oral cavity—toothbrush holding and tooth brushing.

2.6 Calculation of Salivary Flow Rates

Prior to saliva collection, each Salivette® and its accompanying plastic container, were placed into an autoclaved beaker, and weighed. The weight recorded (in grams) was noted as the pre-weight measurement. Upon removal of Salivettes® from the oral cavity, each was placed back into its plastic container, and returned to the same beaker used for obtaining the pre-weight measurement. The weight recorded was noted as the post-weight measurement. The pre-weight measurements were subtracted from the post-weight measurements for each Salivette® (left parotid, right parotid, and SMSL) for each collection period (see Appendix E). The measurements were divided by collection time, to obtain flow rates in g/minute.

2.7 Statistical Analyses

A three-way mixed ANOVA ($\alpha = 0.05$) was performed for each of the left parotid, right parotid, and SMSL salivary glands. Salivary flow rate was the dependent variable, and treatment condition, collection period, and age were the independent variables. Treatment condition was a repeated-measures independent variable with two levels: control (i.e., toothbrush holding) and experimental (i.e., tooth brushing). Collection period was a

repeated-measures independent variable with four levels: baseline, toothbrush-holding/brushing, 0-5 minutes post-toothbrush holding/brushing, and 5-10 minutes post-toothbrush holding/brushing. Age was a between-groups independent variable with two levels: young and old. Interaction effects of each ANOVA guided the post hoc tests performed. Comparisons were performed using paired samples t-tests. Bonferroni adjustments were applied by dividing alpha by the number of comparisons.

All analyses were completed using IBM SPSS Statistics 23.

Chapter 3

3 Results

3.1 Participants

Twenty-five healthy young adults (19 females, 6 males, age range= 20-26 years, mean age= 22.3 years) and twenty-five healthy older adults (18 females, 7 males, age range= 63-86 years, mean age= 71.8 years) volunteered to participate in the study. A sample size power calculation indicated that 50 participants was sufficient to detect a moderate effect size of a four-level within-subject independent variable using a 0.05 alpha level with power of 0.80.

Participants were instructed to eat a typical breakfast and complete their morning tooth brushing one hour prior to their scheduled appointment time and to refrain from eating or drinking anything thereafter prior to the experiment. Study sessions were held between 8:30 am and 11:30 am, with each lasting approximately 60 minutes. All participants provided written informed consent in accordance with Research Ethics at Western University (see Appendix B). Information pertaining to participants' health was collected prior to the start of the experiment (see Appendix C). Additionally, all participants underwent a clinical examination of their mouth by an experienced speech-language pathologist (see Appendix D). Participants were recruited from Western University, the Retirement Research Association, the Ladies Retirement Research Association, and the Senior Alumni Program at Western University. All participants were compensated \$20 for their participation in the study.

3.1.1 Inclusion and Exclusion Criteria

Participants in the healthy young adult group were between the ages of 18 and 30 years. Participants in the healthy older adult group were between the ages of 60 and 90 years. Individuals were ineligible to participate in the study if they had less than 20 natural teeth, had a history of illness potentially affecting salivary flow (e.g., neurological, respiratory, gastrointestinal, systemic, autoimmune), or had history of surgery or medical

treatment potentially affecting salivary flow (e.g., radiation therapy to head/neck, surgery to the head/neck). Participants were also non-smoking and free of major systemic disease.

3.1.2 Participant Demographics

Table 1. Characteristics of Young Adult Participants

Participant	Age (Years)	Gender (M/F)	Health Conditions/Illnesses	Medications
1	24	F	--	Alesse 21, Accutane 40 mg
2	23	F	--	--
3	21	M	--	--
4	20	F	--	Prozac 50mg, Wellbutrin 100mg, Clonazepam 10 mg, Birth Control (Seasonale)
5	24	F	--	--
6	22	F	--	--
7	22	F	--	--
8	21	F	--	--
9	21	F	--	Birth Control, Eletriptan prn
10	22	F	--	--
11	20	F	--	Birth Control
12	20	F	--	--
13	21	F	--	--
14	22	F	--	--
15	22	M	--	--
16	26	F	--	Birth Control
17	23	M	--	--
18	21	F	Hashimoto	--

19	21	F	--	--
20	21	M	--	--
21	25	F	--	Birth Control
22	24	F	--	Birth Control, Alesse
23	25	F	--	Birth Control
24	22	M	--	--
25	25	M	--	Motrin 2x400mg

Table 2. Characteristics of Older Adult Participants

Participant	Age (Years)	Gender (M/F)	Health Conditions/Illnesses	Medications
1	75	M	--	Atacand 18 mg
2	73	F	Heart condition, high blood pressure, celiac disease	Coversyl 8 mg, Synthroid 0.137 mg, Amlodipine 5 mg, Bystolic 2.5 mg, Rabeprazole 20 mg, Pradaya 150 mg
3	69	F	--	--
4	75	M	--	Lipitor 20 mg
5	74	F	--	SDZ-Telmisartan, HCT
6	68	F	--	Alendronate 70 mg (1x/week)
7	69	F	--	Pariet 20 mg, Aspirin 80 mg
8	74	M	Ulcerative colitis	Entyvio infusion 1/8weeks
9	74	F	--	Rosuvastatin 10 mg, Levothyroxine SOD 88 mg (Synthroid)

10	67	F	High blood pressure, mild stroke (Jan. 2015)	Mylan-Pantoprazole 40 mg (acid), Sandoz-Telmisartan 80 mg (BP), Teva-Rosuvastatin 10 mg (cholesterol), Apo-Clopidogrel 75 mg (blood thinner)
11	74	F	High blood pressure	Celebrex, Blood pressure, Cholesterol
12	68	F	--	Prolia
13	74	F	--	Lovastatin, Macrobid
14	75	M	High blood pressure	PMS-Finasteride 5 mg, APO-Atorvastatin 10 mg, SDZ-Ramipril 5 mg, APO-Hydro 25 mg, APO-Metoprolol 50 mg, APO-Omeprazole 20 mg, SDZ-Tamsulosin CR 0.4 mg, APO-Amlodipine 10 mg, Mylan-Beclor AQ 50 mcg, APO-Salvent 100 mcg, APO-Ramipril 10 mg, Teva-Chloroquine 250 mg
15	87	M	Diabetes	Ratio-Metformin 500 mg, Teva-Rosuvastatin 20 mg, Co-Ramipril 2.5 mg, Ditropan XL 5 mg (anticholinergic)
16	66	F	--	--
17	73	F	High blood pressure	APO-Hydro 25 mg, APO-Cephalex 500 mg, APO-Naproxen 250 mg, APO-Citalopram 20 mg, Climara 25 0.025 mg/24h, Synthroid 0.088 mg, SDZ-Ramipril 2.5 mg, PMS-Ramipril HCTZ 2.5/12.5 mg
18	67	F	--	Lansoprazole 30 mg, Pulmicort inhaler 400 mcg
19	68	F	--	Symbicort 200 mcg, Actonel DR 35 mg, Synthroid 125 mcg, Apo-Mometasone Aqueous 50 mcg/spray

20	76	F	--	Act-Ramipril 5 mg, Sandoz-Ezetimibe 10 mg, Teva-Rosuvastatin 20 mg, Tecta 40 mg, Teva-Bisoprolol 5 mg
21	63	F	--	Levothyroxin 50 mcg, Co-Rosuvastatin 5 mg
22	77	F	--	--
23	76	M	--	Ramipril 10 mg, Lipitor 10 mg, Flomax
24	69	F	High blood pressure	Coversyl Plus 8.2 mg, Rosuvastatin 5 mg, Lorazepam 0.5 mg
25	64	M	--	--

3.2 Observations During Experimental Sessions

3.2.1 Experimental Procedure

The younger adults tended to tolerate the length of the study better than the older adults. Participants were asked to sit comfortably in an office chair, to minimize their movement, particularly movements of the mouth, and keep their eyes open during the experiment. In general, the younger adults were able to adhere to these instructions well, while the older adults displayed more difficulty in remaining stationary and keeping their eyes open.

Although instructed to refrain from talking during saliva collection periods, several of the young and older adult participants spoke, often to ask questions.

3.2.2 Salivettes®

The younger adults appeared to have less complaints about the Salivettes® in the oral cavity throughout the experiment. In general, it was more difficult to place Salivettes® in the mouths of the older adult participants. Consequently, additional time was spent placing the Salivettes® in proper position prior to the start of some saliva collection periods. One participant from the older adult group withdrew from the study as they felt it was an uncomfortable method of collection. Some sheering of the cotton from the

Salivettes® resulted following the removal of Salivettes® from the oral cavity. Sheering of the cotton most often resulted from the left parotid and right parotid glands. It was observed that salivary flow decreased from these areas over time, causing the areas to become dry and the cotton to adhere to the oral mucosa. This was evident in both the young and older adult participants. Occasionally the floss used to tether the Salivettes® would detach from the cotton roll. These two events occurred with similar frequency amongst the young adult and older adult participants.

3.2.3 Swallowing Occurrence

Participants were instructed not to swallow during/immediately following saliva collection periods. Older adults reported the urge to swallow more often than the young adults. At times, swallows were observed in both the young adult and older adult groups.

3.3 Statistical Analyses

3.3.1 Left Parotid Gland Salivary Flow Rate

A three-way mixed ANOVA was performed using left parotid gland salivary flow rate as the dependent variable. The independent variables were treatment condition, collection period, and age. Treatment condition was a repeated-measures independent variable with two levels: control (i.e., toothbrush holding) and experimental (i.e., tooth brushing). Collection period was a repeated-measures independent variable with four levels: baseline, toothbrush holding/brushing, 0-5 minutes post-toothbrush holding/brushing, and 5-10 minutes post-toothbrush holding/brushing. Age was a between-groups independent variable with two levels: young and old. Mauchly's test of sphericity indicated that collection period did not satisfy the assumption of sphericity. Therefore, the Greenhouse-Geisser correction was applied to the degrees of freedom associated with the main effect of collection period and the collection period by treatment condition interaction effect. Data are reported as mean \pm standard error throughout.

The three-way mixed ANOVA yielded a significant main effect of treatment condition [$F(1, 48) = 5.21, p < 0.05$], a significant main effect of collection period [$F(1.71, 81.86) = 41.10, p < 0.05$], and a significant main effect of age [$F(1, 48) = 6.26, p < 0.05$]. There

were two significant two-way interaction effects. Specifically, the treatment condition by collection period interaction was significant [$F(1.66, 79.67) = 5.30, p < 0.05$], and the age by collection period interaction was significant [$F(1.71, 81.86) = 4.16, p < 0.05$]. The other two-way interaction and the three-way interaction were not statistically significant.

The significant two-way interactions prevented direct interpretation of the main effects. Therefore, tests of simple main effects were performed. These simple main effects (presented below) were made using paired t-tests.

i) Simple Main Effects Relating to Treatment Condition by Collection Period Two-Way Interaction

The significant interaction between treatment condition and collection period was examined by making paired comparisons among the four collection periods within and across each treatment condition. Visual observation of histograms depicting the collection periods (see Figure 2i) revealed that salivary flow rates during the 0-5 minute post-toothbrush holding/brushing and 5-10 minute post-toothbrush holding/brushing periods were similar, and thus, comparisons of toothbrush holding and brushing were only performed against the 0-5 minute post-toothbrush periods in the control and experimental conditions to allow for the alpha value to be less conservative. Bonferroni adjustments were applied by manually dividing alpha by the number of comparisons ($\alpha = 0.05/8$). In the control condition, salivary flow rate during the two-minute toothbrush-holding period ($M = 0.0794 \pm 0.012$ g/min) was significantly greater ($p_{\text{adj}} < 0.006$) than that during the baseline ($M = 0.0468 \pm 0.008$ g/min), and the 0-5 minute post-toothbrush-holding ($M = 0.0472 \pm 0.008$ g/min) periods. Salivary flow rate during the 0-5 minute post-toothbrush holding ($M = 0.0472 \pm 0.008$ g/min), and the 5-10 minute post-toothbrush holding ($M = 0.0452 \pm 0.007$ g/min) periods were not significantly greater ($p_{\text{adj}} > 0.006$) than that during the baseline period ($M = 0.0468 \pm 0.008$ g/min). Similarly, in the experimental condition, the salivary flow rate during the two-minute toothbrushing period ($M = 0.1180 \pm 0.017$ g/min) was significantly greater ($p_{\text{adj}} < 0.006$) than that during the baseline ($M = 0.0452 \pm 0.007$ g/min) and 0-5 minute post-tooth brushing ($M = 0.0516 \pm 0.010$ g/min) periods. Salivary flow rate during the 0-5 minute post-tooth

brushing ($M = 0.0516 \pm 0.010$ g/min), and the 5-10 minute post-tooth brushing ($M = 0.0432 \pm 0.007$ g/min) periods were not significantly greater ($p_{\text{adj}} > 0.006$) than that during the baseline period ($M = 0.0452 \pm 0.007$ g/min).

In addition, the simple main effect of condition was tested by comparing the salivary flow rate in the control and experimental conditions at each collection period. Bonferroni adjustments were applied by manually dividing alpha by the number of comparisons ($\alpha = 0.05/4$). Salivary flow rate during the two-minute tooth-brushing period in the experimental condition ($M = 0.1180 \pm 0.017$ g/min) was significantly greater ($p_{\text{adj}} < 0.013$) than that during the two-minute toothbrush holding period in the control condition ($M = 0.0794 \pm 0.012$ g/min). There were no significant differences in salivary flow rates between control and experimental conditions during the baseline, 0-5 minute post-toothbrush holding/brushing, nor 5-10 minute post-toothbrush holding/brushing periods.

ii) Simple Main Effects Relating to Age by Collection Period Two-Way Interaction

The significant interaction between age and collection period was examined by making paired comparisons among the four collection periods within and between each age group. Visual observation of histograms depicting the collection periods (see Figure 2ii) revealed that salivary flow rates during the 0-5 minute post-toothbrush holding/brushing and 5-10 minute post-toothbrush holding/brushing periods were similar, and thus, comparisons of toothbrush holding and brushing were only performed against the 0-5 minute post-toothbrush periods in the control and experimental conditions to allow for the alpha value to be less conservative. Bonferroni adjustments were applied by manually dividing alpha by the number of comparisons ($\alpha = 0.05/8$). In the young adults, salivary flow rate during the two-minute toothbrush period ($M = 0.0670 \pm 0.009$ g/min) was significantly greater ($p_{\text{adj}} < 0.006$) than that during the baseline ($M = 0.0304 \pm 0.005$ g/min), and the 0-5 minute post-toothbrush ($M = 0.0312 \pm 0.004$ g/min) periods. Salivary flow rate during the 0-5 minute post-toothbrush ($M = 0.0312 \pm 0.004$ g/min), and the 5-10 minute post-toothbrush ($M = 0.0312 \pm 0.005$ g/min) periods were not significantly greater ($p_{\text{adj}} > 0.006$) than that during the baseline period ($M = 0.0304 \pm 0.005$ g/min). Similarly,

in the older adults, the salivary flow rate during the two-minute toothbrush period ($M = 0.1304 \pm 0.018$ g/min) was significantly greater ($p_{\text{adj}} < 0.006$) than that during the baseline ($M = 0.0616 \pm 0.009$ g/min), and the 0-5 minute post-toothbrush ($M = 0.0676 \pm 0.011$ g/min) periods. Salivary flow rate during the 0-5 minute post-toothbrush ($M = 0.0676 \pm 0.011$ g/min), and the 5-10 minute post-toothbrush ($M = 0.0572 \pm 0.009$ g/min) periods were not significantly greater ($p_{\text{adj}} > 0.006$) than that during the baseline period ($M = 0.0616 \pm 0.009$ g/min).

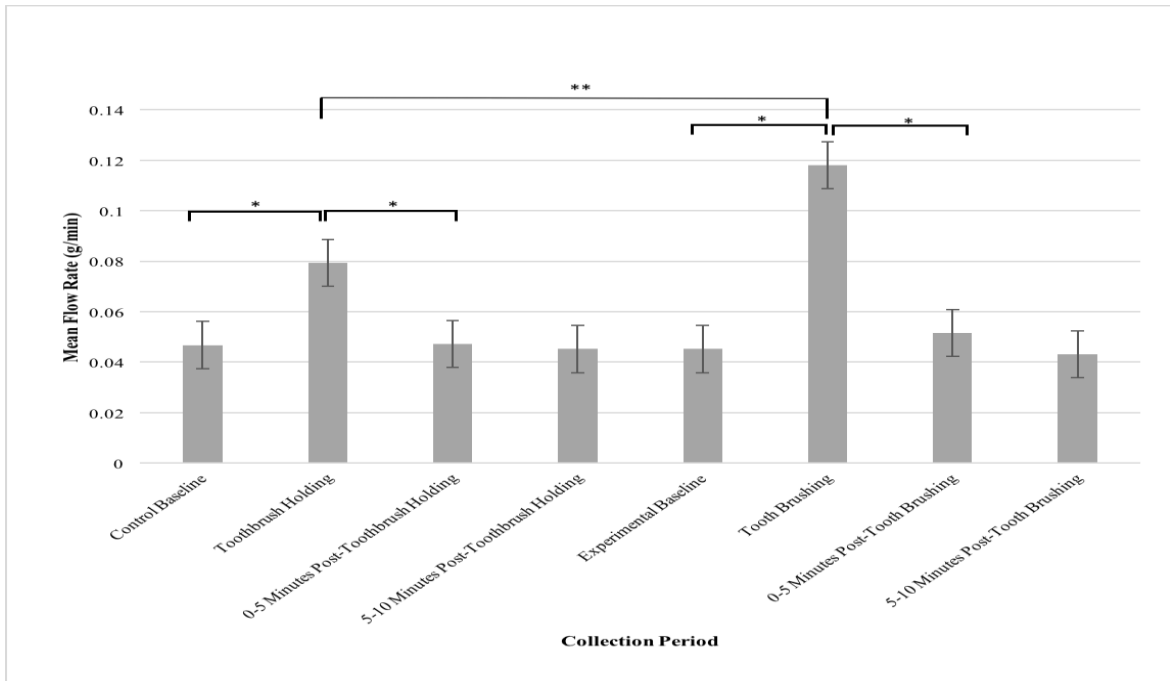
In addition, the simple main effect of age was tested by comparing the salivary flow rates of the young adults with the older adults at each collection period. Bonferroni adjustments were applied by manually dividing alpha by the number of comparisons ($\alpha = 0.05/4$). Salivary flow rate in the older adults was significantly greater ($p_{\text{adj}} < 0.013$) than salivary flow rate in the young adults during the baseline ($M = 0.0616 \pm 0.009$ g/min; $M = 0.0304 \pm 0.005$ g/min), two-minute toothbrush ($M = 0.1304 \pm 0.018$ g/min; $M = 0.0670 \pm 0.009$ g/min), 0-5 minute post-toothbrush ($M = 0.0676 \pm 0.011$ g/min; $M = 0.0312 \pm 0.004$ g/min), and the 5-10 minute post-toothbrush ($M = 0.0572 \pm 0.008$ g/min; $M = 0.0312 \pm 0.005$ g/min) periods.

Table 3. Left Parotid Gland Salivary Flow Rates (Mean \pm Standard Error of the Mean)

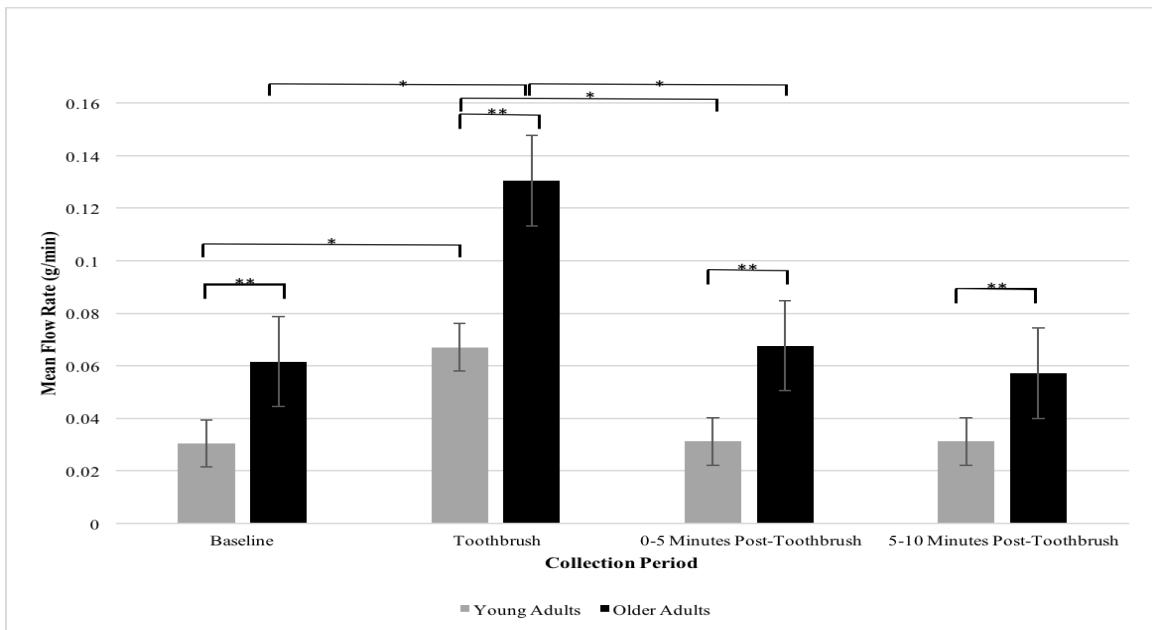
		Collection Period			
Condition	Age	1	2	3	4
Control	Young	0.0312 (± 0.008)	0.0620 (± 0.007)	0.0320 (± 0.006)	0.0304 (± 0.006)
	Old	0.0624 (± 0.013)	0.0968 (± 0.022)	0.0624 (± 0.013)	0.0600 (± 0.013)

	Control Mean	0.0468 (±0.008)	0.0794 (±0.012)	0.0472 (±0.008)	0.0452 (±0.007)
Experimental	Young	0.0296 (±0.005)	0.0720 (±0.085)	0.0304 (±0.025)	0.0320 (±0.038)
	Old	0.0608 (±0.013)	0.1640 (±0.027)	0.0728 (±0.018)	0.0544 (±0.011)
	Experimental Mean	0.0452 (±0.007)	0.1180 (±0.017)	0.0516 (±0.010)	0.0432 (±0.007)
	Young Mean	0.0304 (±0.005)	0.0670 (±0.009)	0.0312 (±0.004)	0.0312 (±0.005)
	Old Mean	0.0616 (±0.009)	0.1304 (±0.018)	0.0676 (±0.011)	0.0572 (±0.008)
	Period Mean	0.0460 (±0.005)	0.0987 (±0.010)	0.0494 (±0.006)	0.0442 (±0.005)

Figure 2. Left Parotid Gland Salivary Flow



i. Comparison of treatment conditions and collection periods, * denotes significance at $\alpha=0.006$; ** denotes significance at $\alpha=0.013$.



ii. Comparison of age and collection periods, * denotes significance at $\alpha=0.006$, ** denotes significance at $\alpha=0.013$.

3.3.2 Right Parotid Gland Salivary Flow Rate

A three-way mixed ANOVA was performed using right parotid gland salivary flow rate as the dependent variable. The independent variables were treatment condition, collection period, and age. Treatment condition was a repeated-measures independent variable with two levels: control (i.e., toothbrush holding) and experimental (i.e., tooth brushing). Collection period was a repeated-measures independent variable with four levels: baseline, toothbrush holding/brushing, 0-5 minutes post-toothbrush holding/brushing, and 5-10 minutes post-toothbrush holding/brushing. Age was a between groups independent variable with two levels: young and old. Mauchly's test of sphericity indicated that collection period did not satisfy the assumption of sphericity. Therefore, the Greenhouse-Geisser correction was applied to the degrees of freedom associated with the main effect of collection period and the treatment condition by collection period interaction effect. Data are reported as mean \pm standard error throughout.

The three-way mixed ANOVA yielded a significant main effect of treatment condition [$F(1, 48) = 4.23, p < 0.05$], a significant main effect of collection period [$F(1.61, 77.44) = 39.38, p < 0.05$], and a significant main effect of age [$F(1, 48) = 11.32, p < 0.05$]. All two-way interactions were statistically significant. Specifically, the treatment condition by collection period was significant [$F(1.76, 84.25) = 5.05, p < 0.05$], the age by collection period interaction was significant [$F(1.61, 77.44) = 8.16, p < 0.05$], and the age by treatment condition was significant [$F(1, 48) = 7.26, p < 0.05$]. Similarly, the three-way interaction between treatment condition, collection period, and age on right parotid gland salivary flow was statistically significant, [$F(1.76, 84.25) = 4.06, p < 0.05$].

3.3.2.1 Post Hoc Comparisons

The significant two-way and three-way interactions prevented direct interpretation of the main effects. Therefore, tests of simple main effects and simple simple main effects were performed.

There was a statistically significant simple two-way interaction between treatment condition and collection period for the older adults [$F(1.54, 36.89) = 5.33, p < 0.05$] but not for the young adults [$F(2.44, 58.51) = 0.11, p > 0.05$].

Young Adults

As the simple two-way interaction between treatment condition and collection period was not statistically significant for the young adults, main effects of the two-factor ANOVA were interpreted. There was a significant simple main effect of collection period [$F(1.28, 30.77) = 11.79, p < 0.05$] on right parotid gland salivary flow, however, the simple main effect of treatment condition was not statistically significant.

i) Comparison of Collection Periods

Pairwise comparisons were performed for collection periods in the control and experimental conditions. Visual observation of histograms depicting the collection periods (see Figure 3i) revealed that salivary flow rates during the 0-5 minute post-toothbrush holding/brushing and 5-10 minute post-toothbrush holding/brushing periods were similar, and thus, comparisons of toothbrush holding and brushing were only performed against the 0-5 minute post-toothbrush period in the control and experimental conditions to allow for the alpha value to be less conservative. Bonferroni adjustments were applied by manually dividing alpha by the number of comparisons ($\alpha = 0.05/8$). The salivary flow rate during the two-minute toothbrush holding period ($M = 0.0660 \pm 0.012$ g/min) was significantly greater ($p_{\text{adj}} < 0.006$) than the 0-5 minute post-toothbrush-holding period ($M = 0.0320 \pm 0.005$ g/min). The salivary flow rate during the two-minute tooth-brushing period ($M = 0.0660 \pm 0.015$ g/min) was significantly greater ($p_{\text{adj}} < 0.006$) than the salivary flow rate during the baseline period in the experimental condition ($M = 0.0280 \pm 0.005$ g/min) and the 0-5 minute post-tooth brushing period ($M = 0.0304 \pm 0.005$ g/min).

Older Adults

As the simple two-way interaction between treatment condition and collection period was statistically significant for the older adults, tests of simple simple main effects were performed using paired t-tests.

The simple main effect of period for older adults was statistically significant in the control condition [$F(1.98, 47.46) = 10.93, p < 0.05$] and also in the experimental condition [$F(1.49, 35.69) = 23.24, p < 0.05$]. Thus, collection period had an effect on right parotid gland salivary flow rate in the older adults for both the control and experimental conditions. Paired comparisons were made among the four collection periods within and across each treatment condition for the older adults.

i) Comparison of Baselines

Paired samples t-tests indicated that salivary flow rate during the baseline in the control condition ($M = 0.0736 \pm 0.014$ g/min) was not significantly different ($p > 0.05$) from the salivary flow rate during the baseline in the experimental condition ($M = 0.0584 \pm 0.011$ g/min) in the older adults. Based on this finding, the baselines from the control and experimental conditions were averaged for each participant, and a single baseline was created and used for subsequent analyses. The averaged baseline for the older adults was $M = 0.0660 \pm 0.012$ g/min. The averaged baseline is referred to as “baseline” in the following sections of the thesis.

ii) Comparison of Collection Periods

Simple pairwise comparisons were performed between the various collection periods for the older adults in the control and experimental conditions (see Figure 3ii). Bonferroni adjustments were applied. The salivary flow rate during the two-minute toothbrush holding period ($M = 0.1280 \pm 0.023$ g/min) was significantly greater ($p < 0.05$) than that during the baseline ($M = 0.0660 \pm 0.012$ g/min), 0-5 minute post-toothbrush holding ($M = 0.0584 \pm 0.012$ g/min), and 5-10 minute post-toothbrush holding ($M = 0.0608 \pm 0.011$ g/min) periods. Similarly, the salivary flow rate during the two-minute tooth-brushing period ($M = 0.1920 \pm 0.029$ g/min) was significantly greater ($p < 0.05$) than that during the baseline ($M = 0.0660 \pm 0.012$ g/min), 0-5 minute post-tooth brushing ($M = 0.0720 \pm 0.016$ g/min), and 5-10 minute post-tooth brushing ($M = 0.0640 \pm 0.012$ g/min) periods for the older adults.

Comparison of Young and Older Adults

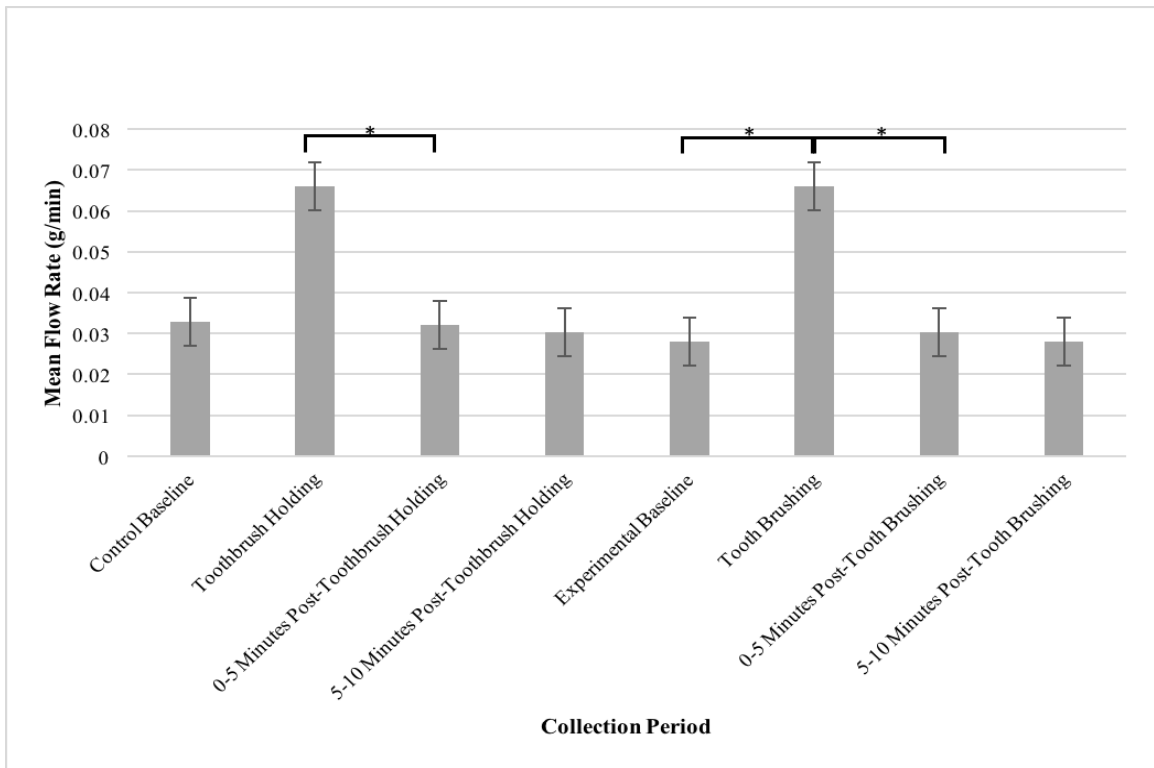
Independent samples t-tests were performed (see Figure 3iii). Bonferroni adjustments were applied by manually dividing alpha by the number of comparisons ($\alpha = 0.05/8$). The t-tests indicated that salivary flow rate among the older adults was significantly greater ($p_{adj} < 0.006$) than in the young adults during the tooth-brushing period in the experimental condition ($M = 0.1920 \pm 0.029$ g/min; $M = 0.0660 \pm 0.015$ g/min).

Table 4. Right Parotid Gland Salivary Flow Rates (Mean \pm Standard Error of the Mean)

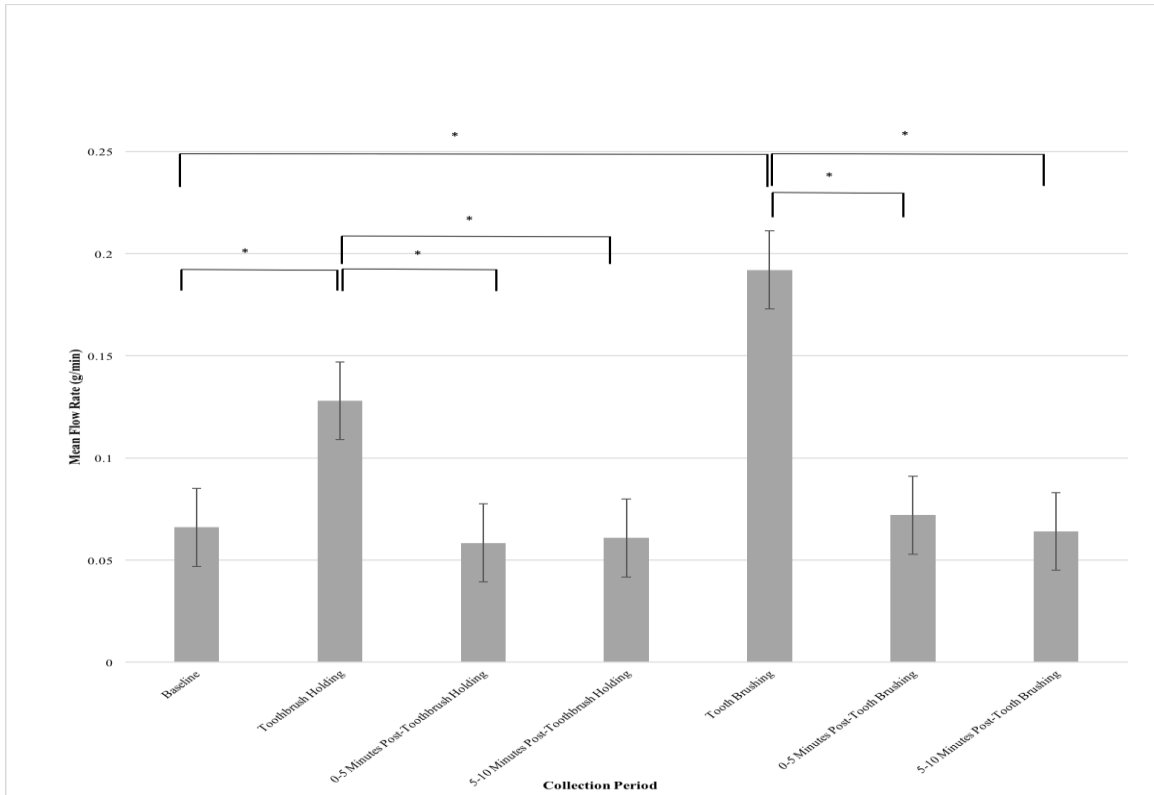
		Collection Period			
Condition	Age	1	2	3	4
Control	Young	0.0328 (± 0.005)	0.0660 (± 0.012)	0.0320 (± 0.005)	0.0304 (± 0.005)
	Old	0.0736 (± 0.014)	0.1280 (± 0.023)	0.0584 (± 0.012)	0.0608 (± 0.011)
	Control Mean	0.0532 (± 0.008)	0.0970 (± 0.013)	0.0452 (± 0.007)	0.0456 (± 0.006)
Experimental	Young	0.0280 (± 0.005)	0.0660 (± 0.015)	0.0304 (± 0.005)	0.0280 (± 0.006)
	Old	0.0584 (± 0.011)	0.1920 (± 0.029)	0.0720 (± 0.016)	0.0640 (± 0.012)
	Experimental Mean	0.0432 (± 0.006)	0.1290 (± 0.018)	0.0512 (± 0.009)	0.0460 (± 0.007)

	Young Mean	0.0304 (±0.004)	0.0660 (±0.009)	0.0312 (±0.003)	0.0292 (±0.004)
	Old Mean	0.0660 (±0.012)	0.1600 (±0.018)	0.0652 (±0.010)	0.0624 (±0.008)
	Period Mean	0.0482 (±0.005)	0.1130 (±0.009)	0.0482 (±0.005)	0.0458 (±0.004)

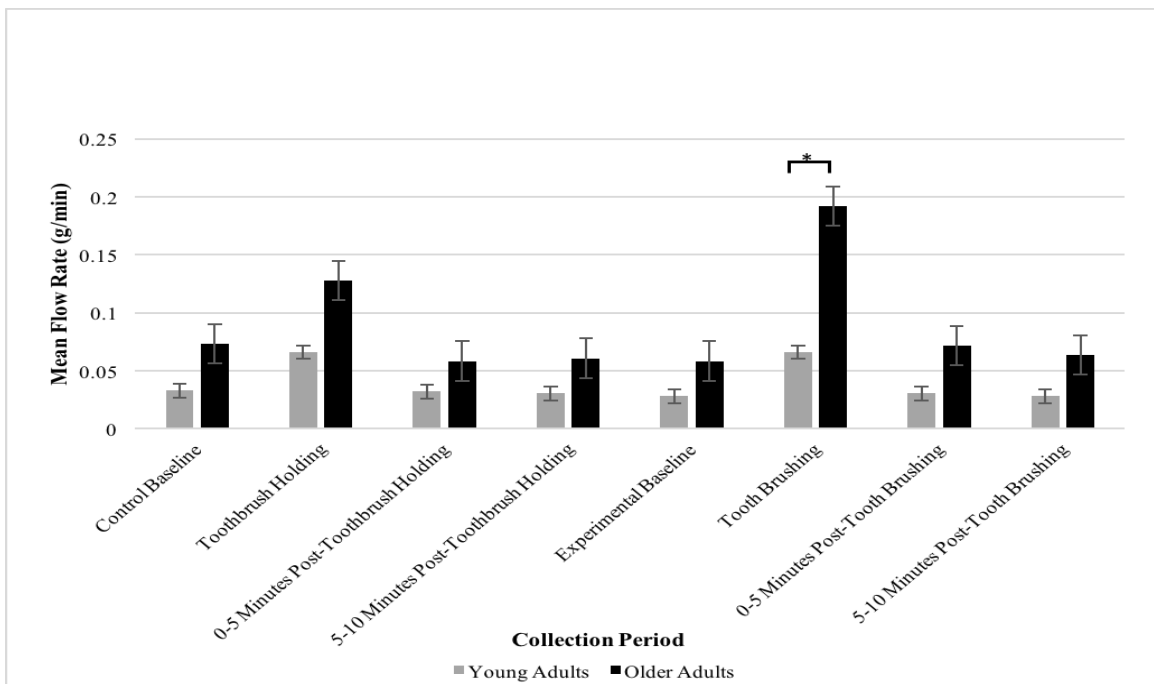
Figure 3. Right Parotid Gland Salivary Flow



i. Comparison of collection periods in young adults, * denotes significance at $\alpha=0.006$.



ii. Comparison of collection periods in older adults, * denotes significance at $\alpha=0.05$.



iii) Comparison of age and collection periods, * denotes significance at $\alpha=0.006$.

3.3.3 Submandibular/Sublingual Gland Salivary Flow Rate

A three-way mixed ANOVA was performed using SMSL gland salivary flow rate as the dependent variable. The independent variables were treatment condition, collection period, and age. Treatment condition was a repeated-measures independent variable with two levels: control (i.e., toothbrush holding) and experimental (i.e., tooth brushing). Collection period was a repeated-measures independent variable with four levels: baseline, toothbrush holding/brushing, 0-5 minutes post-toothbrush holding/brushing, and 5-10 minutes post-toothbrush holding/brushing. Age was a between-groups independent variable with two levels: young and old. Mauchly's test of sphericity indicated that collection period did not satisfy the assumption of sphericity. Therefore, the Greenhouse-Geisser correction was applied to the degrees of freedom associated with the main effect of collection period and the treatment condition by collection period interaction effect. Data are reported as mean \pm standard error throughout.

The three-way mixed ANOVA yielded a significant main effect of treatment condition [$F(1, 48) = 8.02, p < 0.05$], and a significant main effect of collection period [$F(1.26, 60.69) = 158.72, p < 0.05$]. The main effect of age was not statistically significant. There were two significant two-way interaction effects. Specifically, the treatment condition by collection period was significant [$F(1.64, 78.83) = 12.56, p < 0.05$], and the age by collection period interaction was significant [$F(1.26, 60.69) = 4.35, p < 0.05$]. The two-way interaction between age and treatment condition was not statistically significant. The three-way interaction between treatment condition, collection period, and age on SMSL gland salivary flow was statistically significant, [$F(1.64, 78.83) = 4.82, p < 0.05$].

3.3.3.1 Post Hoc Comparisons

The significant two-way and three-way interactions prevented direct interpretation of the main effects. Therefore, tests of simple main effects and simple simple main effects were performed. These simple main effects and simple simple main effects were made using paired t-tests.

There was a statistically significant simple two-way interaction between treatment condition and collection period for the older adults [$F(1.74, 41.80) = 13.83, p < 0.05$], but not for the young adults, [$F(1.51, 36.23) = 2.19, p > 0.05$].

Young Adults

As the simple two-way interaction between treatment condition and collection period was not statistically significant for the young adults, main effects of the two-factor ANOVA were interpreted. There was a significant simple main effect of collection period [$F(1.18, 28.39) = 52.61, p < 0.05$] on SMSL gland salivary flow, however, the simple main effect of treatment condition was not statistically significant.

i) Comparison of Collection Periods

Pairwise comparisons were performed for collection periods in the control and experimental conditions. Visual observation of histograms depicting the collection periods (see Figure 4i) revealed that salivary flow rates during the 0-5 minute post-toothbrush holding/brushing and 5-10 minute post-toothbrush holding/brushing periods were similar, and thus, comparisons of toothbrush holding and brushing were only performed against the 0-5 minute post-toothbrush period in the control and experimental conditions to allow for the alpha value to be less conservative. Bonferroni adjustments were applied by manually dividing alpha by the number of comparisons ($\alpha = 0.05/8$). The salivary flow rate during the two-minute toothbrush holding period ($M = 0.5400 \pm 0.054$ g/min) was significantly greater ($p_{\text{adj}} < 0.006$) than the salivary flow rate during the baseline period in the control condition ($M = 0.2432 \pm 0.028$ g/min) and the 0-5 minute post-toothbrush holding period ($M = 0.2720 \pm 0.026$ g/min). The salivary flow rate during the two-minute tooth-brushing period ($M = 0.5820 \pm 0.066$ g/min) was significantly greater ($p_{\text{adj}} < 0.006$) than the salivary flow rate during the baseline period in the experimental condition ($M = 0.2104 \pm 0.024$ g/min) and the 0-5 minute post-tooth brushing period ($M = 0.3032 \pm 0.027$ g/min). The salivary flow rate during the 0-5 minute post-tooth brushing ($M = 0.3032 \pm 0.027$ g/min), and the 5-10 minute post-tooth brushing periods ($M = 0.2936 \pm 0.029$ g/min) were significantly greater ($p_{\text{adj}} < 0.006$) than the

salivary flow rate during the baseline period in the experimental condition ($M = 0.2104 \pm 0.024$ g/min).

Older Adults

As the simple two-way interaction between treatment condition and collection period was statistically significant for the older adults, tests of simple simple main effects were performed using paired t-tests.

The simple simple main effect of period for the older adults was statistically significant in the control condition, [$F(1.38, 33.02) = 50.97, p < 0.05$], and also in the experimental condition, [$F(1.51, 36.23) = 114.04, p < 0.05$]. Thus, collection period had an effect on SMSL gland salivary flow rate in the older adults for both the control and experimental conditions. Paired comparisons were made among the four collection periods within and across each treatment condition for the older adults.

i) Comparison of Baselines

Paired samples t-tests indicated that there was a statistically significant difference ($p < 0.05$) between the salivary flow rate at baseline in the control ($M = 0.2576 \pm 0.032$ g/min) and experimental ($M = 0.2240 \pm 0.030$ g/min) conditions for the older adults. Based on this finding, the baselines from the control and experimental conditions could not be averaged for the older adults.

ii) Comparison of Collection Periods

Simple simple pairwise comparisons were performed between the various collection periods for older adults in the control and experimental conditions (see Figure 4ii). Bonferroni adjustments were applied. The salivary flow rate during the two-minute toothbrush holding period ($M = 0.5680 \pm 0.063$ g/min) was significantly greater ($p < 0.05$) than that during the baseline ($M = 0.2576 \pm 0.031$ g/min), 0-5 minute post-toothbrush holding ($M = 0.2632 \pm 0.031$ g/min), and 5-10 minute post-toothbrush holding ($M = 0.2384 \pm 0.028$ g/min) periods. The salivary flow rate during the two-minute toothbrushing period ($M = 0.7520 \pm 0.063$ g/min) was significantly greater ($p < 0.05$) than that

during the baseline ($M = 0.2240 \pm 0.030$ g/min), 0-5 minute post-tooth brushing ($M = 0.2776 \pm 0.030$ g/min), and 5-10 minute post-tooth brushing ($M = 0.2600 \pm 0.031$ g/min), periods for the older adults. Additionally, the salivary flow rate during the 0-5 minute post-tooth brushing period ($M = 0.2776 \pm 0.030$ g/min) was significantly greater ($p < 0.05$) than that during the baseline period ($M = 0.2240 \pm 0.030$ g/min).

As the baselines could not be averaged in the control and experimental conditions, collection periods could not be compared across the control and experimental conditions. An alternate approach was employed in attempts to compare the relative effects of the toothbrush holding, and tooth brushing, conditions, as follows. Difference scores were calculated from the salivary flow rates during the (i) baseline period in the control condition and (ii) two-minute toothbrush holding period (ii-i), and for the salivary flow rate during the (iii) baseline period in the experimental condition and (iv) two-minute tooth-brushing period (iv-iii) (see Figure 4iii). A paired samples t-test indicated that the difference between salivary flow rate during the two-minute tooth-brushing period and baseline in the experimental condition ($M = 0.5280 \pm 0.041$ g/min) was significantly greater ($p < 0.05$) than the difference between salivary flow rate during the two-minute toothbrush holding period and baseline in the control condition for the older adults ($M = 0.3104 \pm 0.042$ g/min).

Comparison of Young and Older Adults

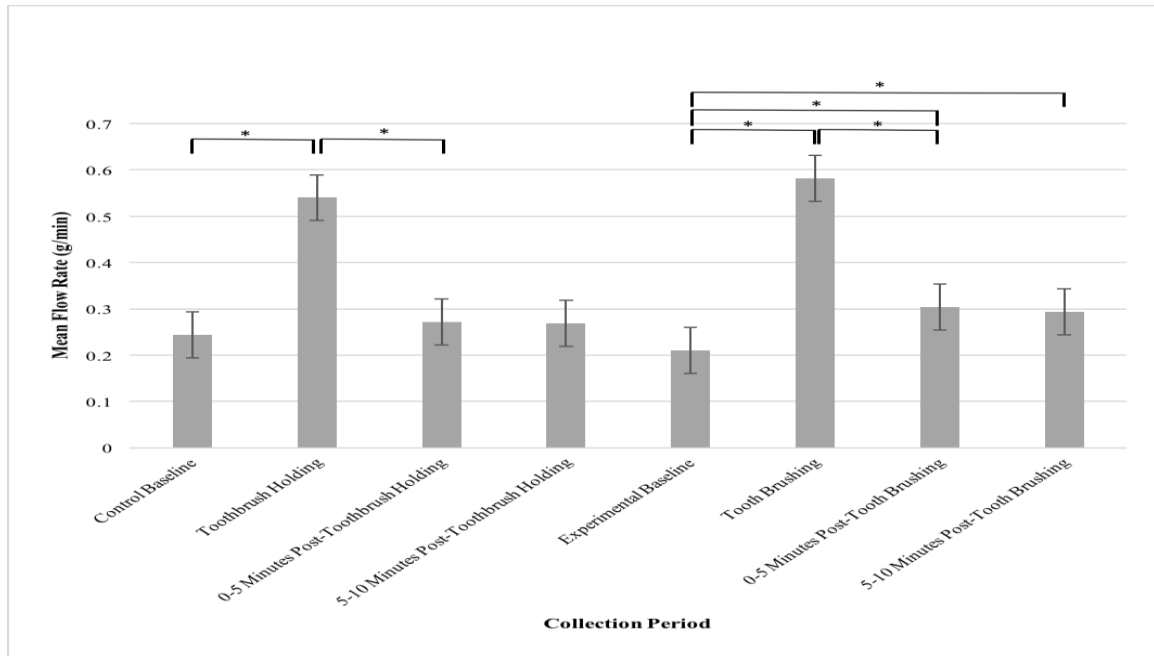
Independent samples t-tests indicated that SMSL gland salivary flow rate among the young adults did not differ significantly ($p > 0.006$) from SMSL gland salivary flow rate among the older adults during any of the collection periods (see Figure 4iv).

Table 5. Submandibular/Sublingual Gland Salivary Flow Rates (Mean \pm Standard Error of the Mean)

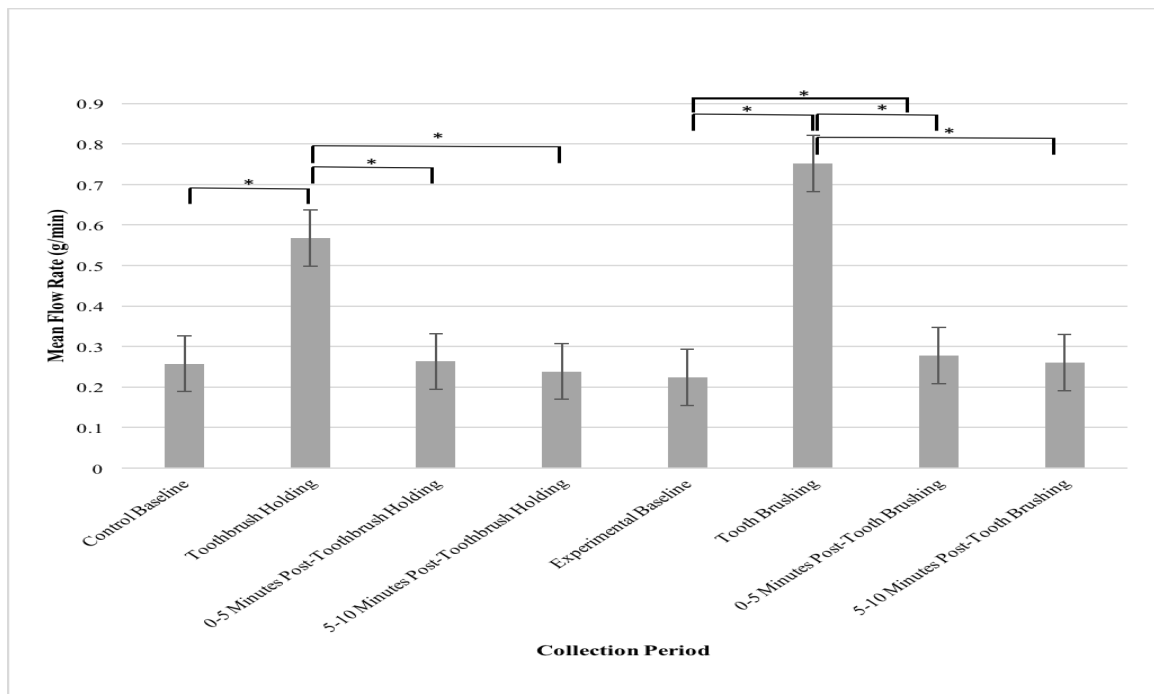
		Collection Period			
Condition	Age	1	2	3	4

Control	Young	0.2432 (±0.028)	0.5400 (±0.054)	0.2720 (±0.026)	0.2680 (±0.025)
	Old	0.2576 (±0.031)	0.5680 (±0.063)	0.2632 (±0.031)	0.2384 (±0.028)
	Control Mean	0.2504 (±0.021)	0.5540 (±0.041)	0.2676 (±0.020)	0.2532 (±0.019)
Experimental	Young	0.2104 (±0.024)	0.5820 (±0.066)	0.3032 (±0.027)	0.2936 (±0.029)
	Old	0.2240 (±0.030)	0.7520 (±0.063)	0.2776 (±0.030)	0.2600 (±0.031)
	Experimental Mean	0.2172 (±0.019)	0.6670 (±0.047)	0.2904 (±0.020)	0.2768 (±0.021)
	Young Mean	0.2268 (±0.018)	0.5610 (±0.042)	0.2876 (±0.018)	0.2808 (±0.019)
	Old Mean	0.2408 (±0.022)	0.6600 (±0.050)	0.2704 (±0.021)	0.2492 (±0.020)
	Period Mean	0.2338 (±0.014)	0.6105 (±0.034)	0.2790 (±0.014)	0.2650 (±0.015)

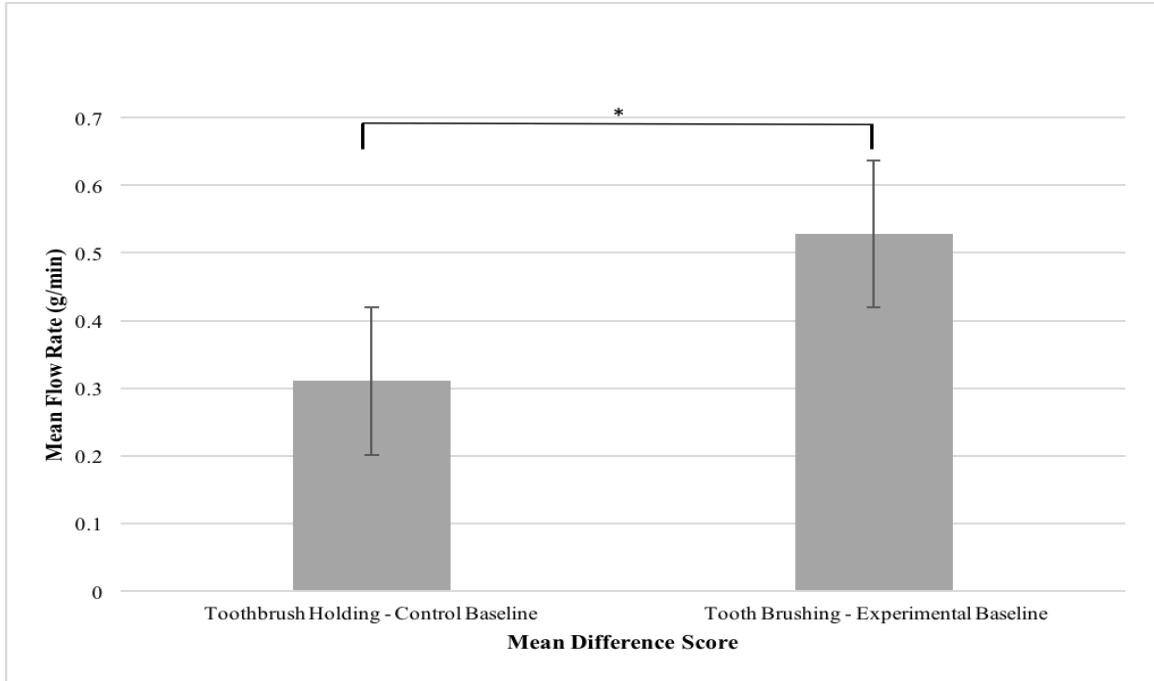
Figure 4. SMSL Gland Salivary Flow



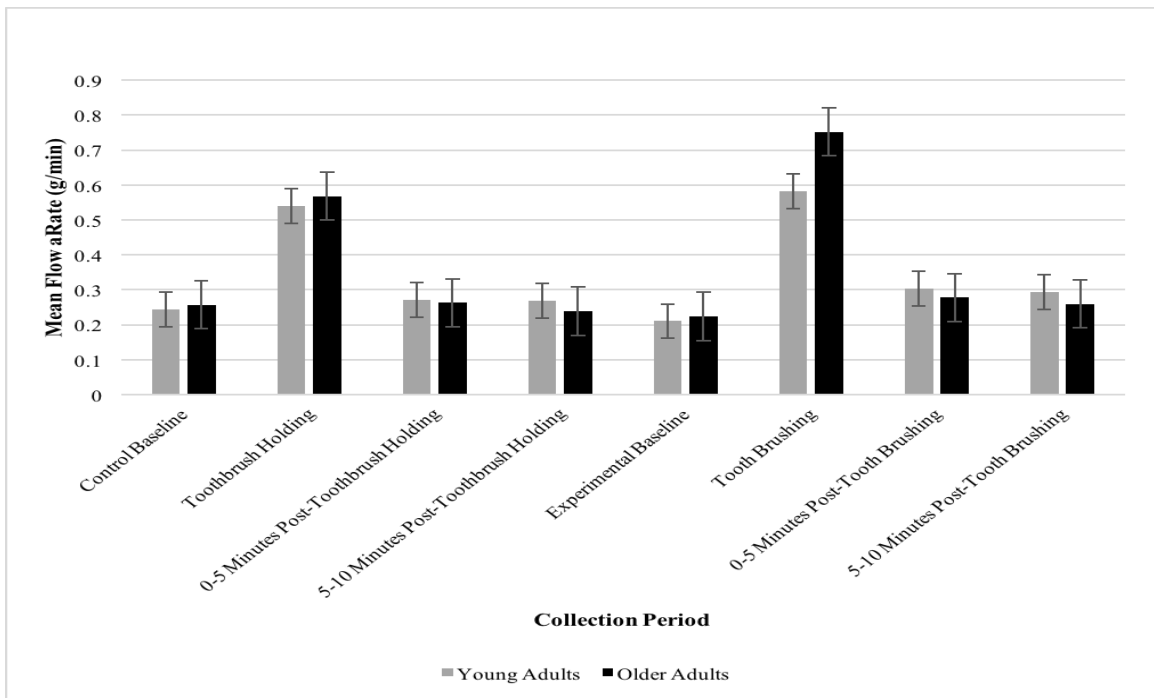
i) Comparison of collection periods in young adults, * denotes significance at $\alpha=0.006$.



ii) Comparison of collection periods in older adults, * denotes significance at $\alpha=0.05$.



iii) Comparison of treatment conditions in older adults, * denotes significance at $\alpha=0.05$.



iv) Comparison of age and collection periods.

3.3.4 Submandibular/Sublingual Gland Adjusted Salivary Flow Rate

An additional analysis was performed to account for the amount of water that may have contributed to the SMSL gland salivary flow rates in the toothbrush holding and tooth brushing collection periods (as described in Methodology, see pg. 12). The values are referred to as SMSL gland “adjusted” salivary flow rates for the rest of the thesis.

A three-way mixed ANOVA was performed using SMSL gland adjusted salivary flow rate as the dependent variable. The independent variables were treatment condition, collection period, and age. Treatment condition was a repeated-measures independent variable with two levels: control (i.e., toothbrush holding) and experimental (i.e., tooth brushing). Collection period was a repeated-measures independent variable with four levels: baseline, toothbrush holding/brushing, 0-5 minutes post-toothbrush-holding/brushing, and 5-10 minutes post-toothbrush holding/brushing. Age was a between groups independent variable with two levels: young and old. Mauchly’s test of sphericity indicated that collection period did not satisfy the assumption of sphericity. Therefore, the Greenhouse-Geisser correction was applied to the degrees of freedom associated with the main effect of collection period and the treatment condition by collection period interaction effect. Data are reported as mean \pm standard error throughout.

The three-way mixed ANOVA yielded a significant main effect of treatment condition [$F(1, 48) = 8.02, p < 0.05$], and a significant main effect of collection period [$F(1.26, 60.69) = 109.69, p < 0.05$]. The main effect of age was not statistically significant. There were two significant two-way interaction effects. Specifically, the treatment condition by collection period was significant [$F(1.64, 78.83) = 12.56, p < 0.05$], and the age by collection period interaction was significant [$F(1.26, 60.69) = 4.35, p < 0.05$]. The two-way interaction between age and treatment condition was not statistically significant. The three-way interaction between treatment condition, collection period, and age on SMSL gland adjusted salivary flow was statistically significant, [$F(1.64, 78.83) = 4.82, p < 0.05$].

3.3.4.1 Post Hoc Comparisons

The significant two-way and three-way interactions prevented direct interpretation of the main effects. Therefore, tests of simple main effects and simple simple main effects were performed. These simple main effects and simple simple main effects were made using paired t-tests.

There was a statistically significant simple two-way interaction between treatment condition and collection period for the older adults [$F(1.74, 41.80) = 13.83, p < 0.05$], but not for the young adults, [$F(1.51, 36.23) = 2.19, p > 0.05$].

Young Adults

As the simple two-way interaction between treatment condition and collection period was not statistically significant for the young adults, main effects of the two-factor ANOVA were interpreted. There was a significant simple main effect of collection period [$F(1.18, 28.39) = 34.07, p < 0.05$] on SMSL gland adjusted salivary flow, however, the simple main effect of treatment condition was not statistically significant.

i) Comparison of Collection Periods

Pairwise comparisons were performed for collection periods in the control and experimental conditions. Visual observation of histograms depicting the collection periods (see Figure 5i) revealed that salivary flow rates during the 0-5 minute post-toothbrush holding/brushing and 5-10 minute post-toothbrush holding/brushing periods were similar, and thus, comparisons of toothbrush holding and brushing were only performed against the 0-5 minute post-toothbrush period in the control and experimental conditions to allow for the alpha value to be less conservative. Bonferroni adjustments were applied by manually dividing alpha by the number of comparisons ($\alpha = 0.05/8$). The salivary flow rate during the two-minute tooth-brush holding period ($M = 0.4800 \pm 0.054$ g/min) was significantly greater ($p_{adj} < 0.006$) than the salivary flow rate during the baseline period in the control condition ($M = 0.2432 \pm 0.028$ g/min) and the 0-5 minute post-toothbrush holding period ($M = 0.2720 \pm 0.026$ g/min). The salivary flow rate during the two-minute tooth-brushing period ($M = 0.5220 \pm 0.066$ g/min) was significantly

greater ($p_{\text{adj}} < 0.006$) than the salivary flow rate during the baseline period in the experimental condition ($M = 0.2104 \pm 0.024$ g/min) and the 0-5 minute post-tooth brushing period ($M = 0.3032 \pm 0.027$ g/min). The salivary flow rate during the 0-5 minute post-tooth brushing ($M = 0.3032 \pm 0.027$ g/min) and the 5-10 minute post-tooth brushing periods ($M = 0.2936 \pm 0.029$ g/min) were significantly greater ($p_{\text{adj}} < 0.006$) than the salivary flow rate during the baseline period in the experimental condition ($M = 0.2104 \pm 0.024$ g/min).

Older Adults

As the simple two-way interaction between treatment condition and collection period was statistically significant for the older adults, tests of simple simple main effects were performed using paired t-tests.

The simple simple main effect of period for the older adults was statistically significant in the control condition, [$F(1.38, 33.02) = 33.48, p < 0.05$], and also in the experimental condition, [$F(1.51, 36.23) = 88.43, p < 0.05$]. Thus, collection period had an effect on SMSL gland adjusted salivary flow rate in the older adults for both the control and experimental conditions. Paired comparisons were made among the four collection periods within and across each treatment condition for the older adults.

i) Comparisons of Baselines

Paired samples t-tests indicated that there was a statistically significant difference ($p < 0.05$) between the salivary flow rate at baseline in the control ($M = 0.2576 \pm 0.032$ g/min) and experimental ($M = 0.2240 \pm 0.030$ g/min) conditions for the older adults. Based on this finding, the baselines from the control and experimental conditions could not be averaged for the older adults.

ii) Comparisons of Collection Periods

Simple simple pairwise comparisons were performed between the various collection periods for older adults in the control and experimental conditions (see Figure 5ii). Bonferroni adjustments were applied. The salivary flow rate during the two-minute

toothbrush holding period ($M = 0.5080 \pm 0.063$ g/min) was significantly greater ($p < 0.05$) than that during the baseline ($M = 0.2576 \pm 0.031$ g/min), 0-5 minute post-toothbrush holding ($M = 0.2632 \pm 0.031$ g/min), and 5-10 minute post-toothbrush holding ($M = 0.2384 \pm 0.028$ g/min) periods. The salivary flow rate during the two-minute toothbrushing period ($M = 0.6920 \pm 0.063$ g/min) was significantly greater ($p < 0.05$) than that during the baseline ($M = 0.2240 \pm 0.030$ g/min), 0-5 minute post-tooth brushing ($M = 0.2776 \pm 0.030$ g/min), and 5-10 post-tooth brushing ($M = 0.2600 \pm 0.031$ g/min) periods for the older adults. Additionally, the salivary flow rate during the 0-5 minute post-tooth brushing period ($M = 0.2776 \pm 0.030$ g/min) was significantly greater ($p < 0.05$) than that during the baseline period ($M = 0.2240 \pm 0.030$ g/min).

As the baselines could not be averaged in the control and experimental conditions, collection periods could not be compared across the control and experimental conditions. An alternate approach was employed in attempts to compare the relative effects of the toothbrush holding and tooth brushing conditions, as follows. Difference scores were calculated from the salivary flow rates during the (i) baseline period in the control condition and (ii) two-minute toothbrush holding period (ii-i), and for the salivary flow rate during the (iii) baseline period in the experimental condition and (iv) two-minute tooth-brushing period (iv-iii) (see Figure 5iii). A paired samples t-test indicated that the difference between salivary flow rate during the two-minute tooth-brushing period and baseline in the experimental condition ($M = 0.4680 \pm 0.041$ g/min) was significantly greater ($p < 0.05$) than the difference between salivary flow rate during the two-minute toothbrush holding period and baseline in the control condition for the older adults ($M = 0.2504 \pm 0.042$ g/min).

Comparison of Young and Older Adults

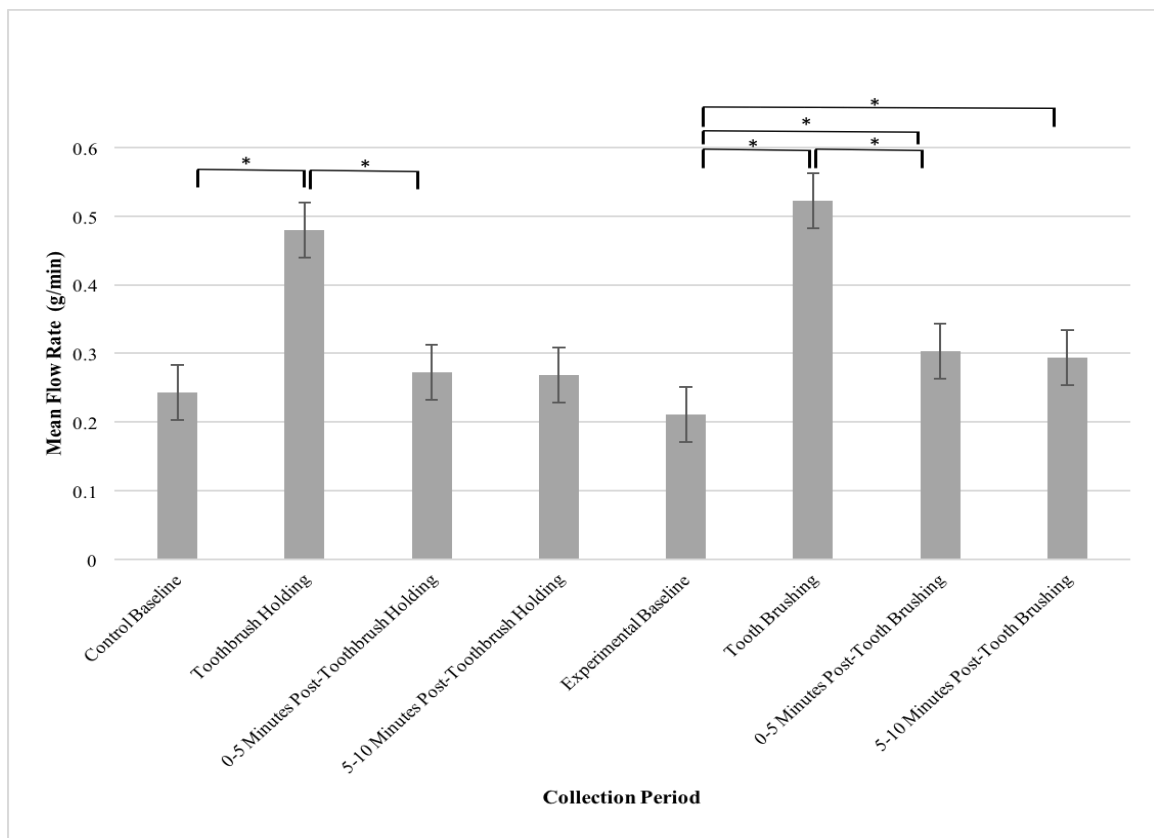
Independent samples t-tests indicated that SMSL gland adjusted salivary flow rate among the young adults did not differ significantly ($p > 0.006$) from SMSL gland adjusted salivary flow rate among the older adults during any of the collection periods (see Figure 5iv).

Table 6. Submandibular/Sublingual Gland Adjusted Salivary Flow Rates (Mean \pm Standard Error of the Mean)

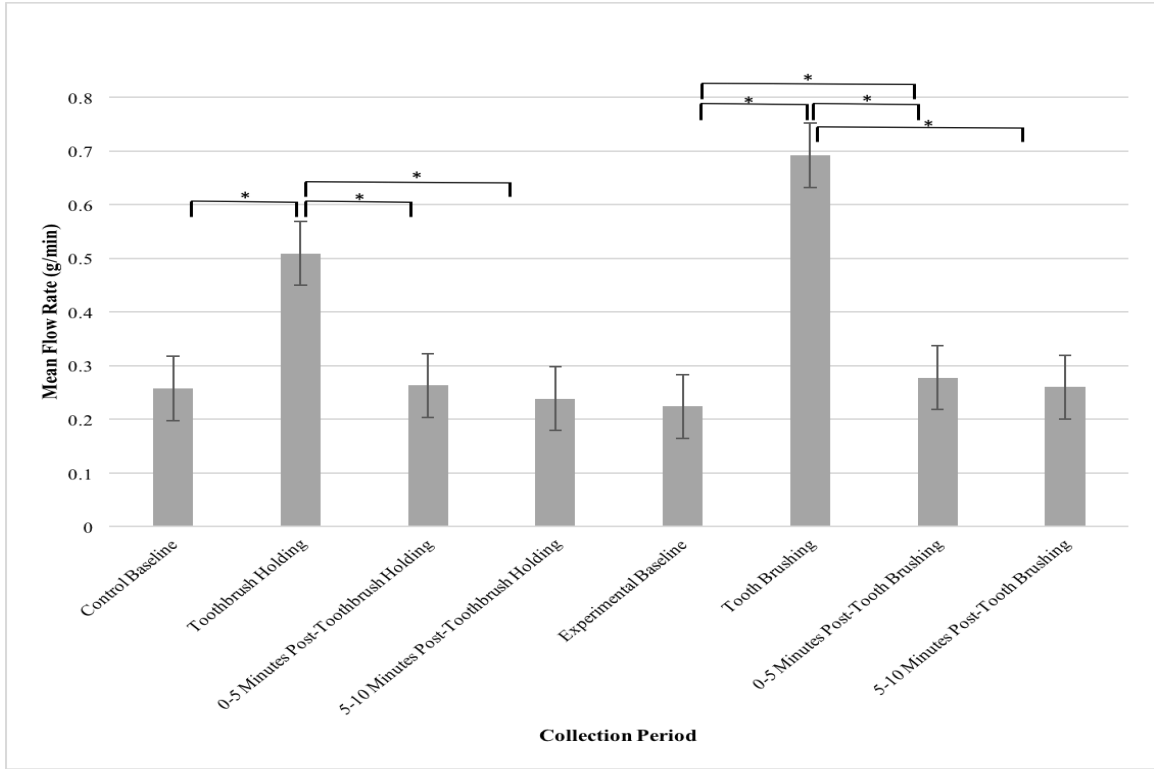
		Collection Period			
Condition	Age	1	2	3	4
Control	Young	0.2432 (± 0.028)	0.4800 (± 0.054)	0.2720 (± 0.026)	0.2680 (± 0.025)
	Old	0.2576 (± 0.031)	0.5080 (± 0.063)	0.2632 (± 0.031)	0.2384 (± 0.028)
	Control Mean	0.2504 (± 0.020)	0.4940 (± 0.041)	0.2676 (± 0.020)	0.2532 (± 0.019)
Experimental	Young	0.2104 (± 0.024)	0.5220 (± 0.066)	0.3032 (± 0.027)	0.2936 (± 0.029)
	Old	0.2240 (± 0.030)	0.6920 (± 0.063)	0.2776 (± 0.030)	0.2600 (± 0.031)
	Experimental Mean	0.2172 (± 0.019)	0.6070 (± 0.047)	0.2904 (± 0.020)	0.2768 (± 0.021)
	Young Mean	0.2268 (± 0.018)	0.5010 (± 0.042)	0.2876 (± 0.018)	0.2808 (± 0.019)

	Old Mean	0.2408 (±0.022)	0.6000 (±0.046)	0.2704 (±0.021)	0.2492 (±0.021)
	Period Mean	0.2338 (±0.014)	0.5505 (±0.034)	0.2790 (±0.014)	0.2650 (±0.015)

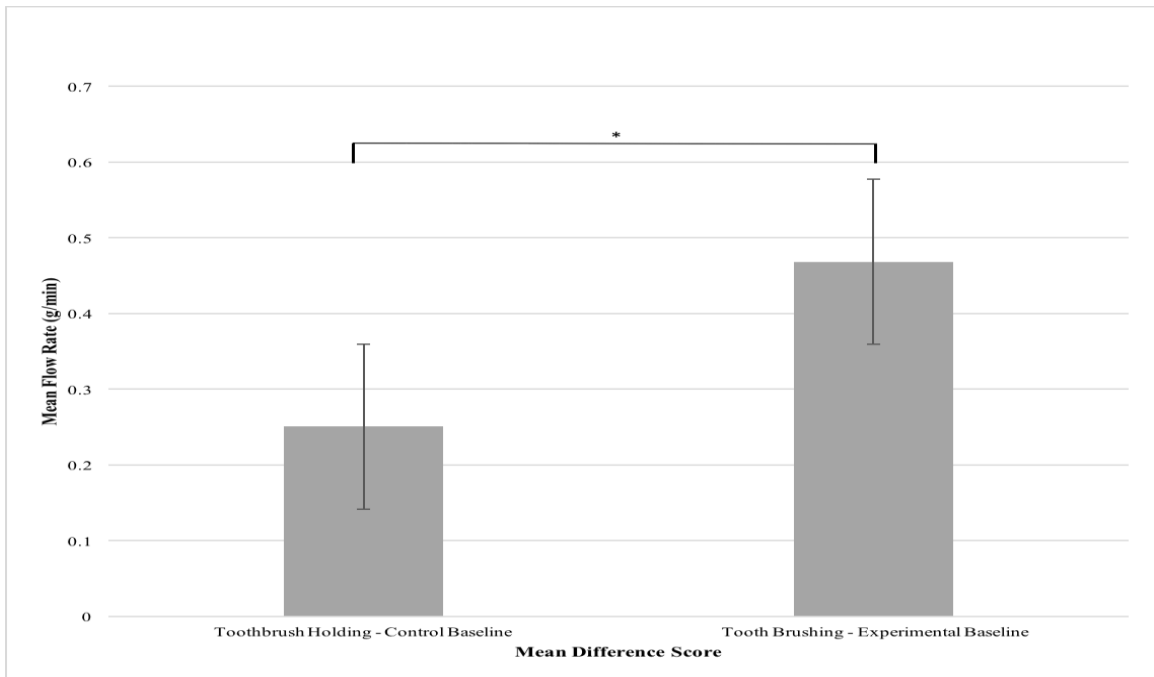
Figure 5. SMSL Gland Adjusted Salivary Flow



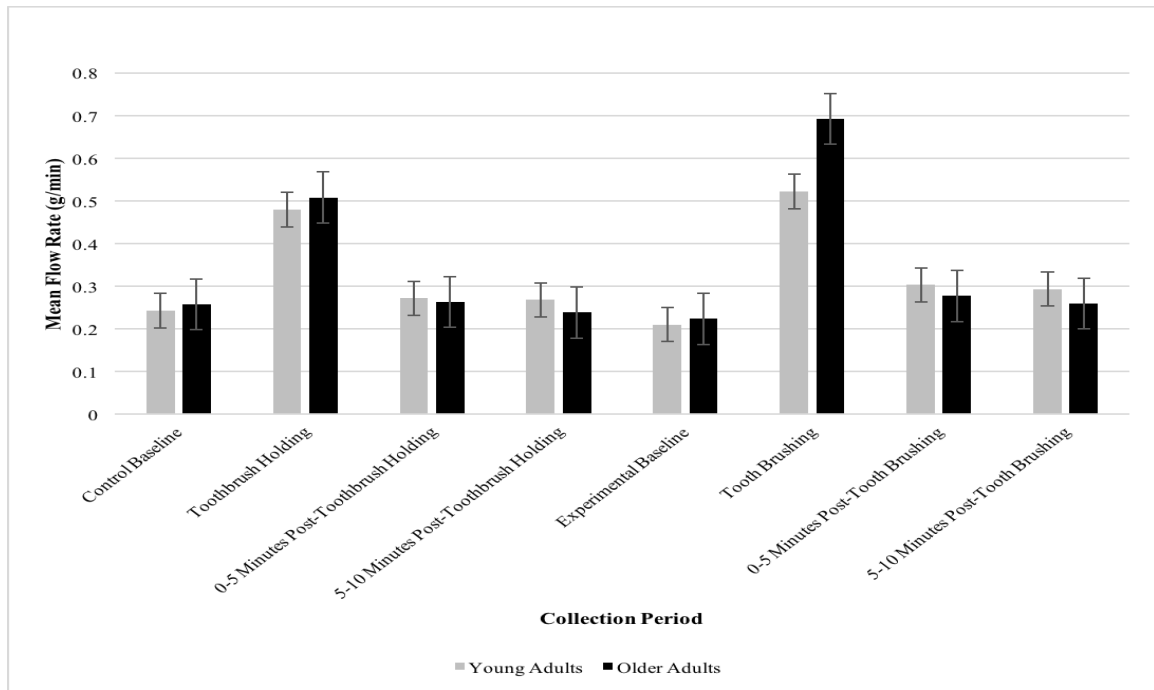
i) Comparison of collection periods in young adults, * denotes significance at $\alpha=0.006$.



ii) Comparison of collection periods in older adults, * denotes significance at $\alpha=0.05$.



iii) Comparison of treatment conditions in older adults, * denotes significance at $\alpha=0.05$.



iv) Comparison of age and collection periods.

Chapter 4

4 Discussion

The aim of the present study was to determine whether manual tooth brushing modulates the rate of flow of saliva from the (i) parotid glands (left and/or right) or, (ii) submandibular/sublingual glands, or (iii) both. This was investigated by measuring salivary flow rates from the parotid and submandibular/sublingual salivary glands during a number of periods before, during and following tooth brushing. A second goal of the study was to examine whether modulation of whole salivary flow rates, that has been documented in healthy older adults (Affoo, 2015a), is seen also in healthy young adults.

In order to determine whether modulation of salivary flow rate associated with tooth brushing is gland-specific, saliva was collected separately from each salivary gland. It was predicted that salivary flow rate from the left and right parotid glands would be modulated by tooth brushing, while the submandibular/sublingual gland flow rate would not be altered by tooth brushing. This prediction was based on previous research showing that (i) the relative proportion of parotid saliva in whole saliva generally increases with increases in whole salivary flow (Humphrey & Williamson, 2001), and (ii) this phenomenon has been documented when the modulatory technique was chewing, a mechanical stimulus (Dodds et al., 1991) that is, in that regard, similar to tooth brushing. It was also expected that the increases in parotid gland salivary flow rates would persist for up to five minutes following tooth brushing. A previous study examining the effects of tooth brushing on whole salivary flow rates in healthy older adults demonstrated a significant increase in salivary flow rate from baseline to tooth brushing, which continued for up to five minutes (Affoo, 2015a). This was the basis for the current prediction regarding the duration of salivary flow rate increase in the present study.

4.1 Major Findings

The present study found that salivary flow rates were increased in association with manual tooth brushing. This is consistent with the findings from Affoo et al. (2015a) who examined the effects of manual tooth brushing on whole salivary flow rate in healthy

older adults. With respect to the hypothesis being tested, the current study showed that tooth brushing was associated with statistically significant increases in salivary flow rates for the left parotid, right parotid, and submandibular/sublingual glands. Finally, manual tooth brushing produced greater parotid gland salivary flow rates in the healthy older adults compared with the healthy young adults, while no difference was observed between young and older adults for SMSL gland salivary flow rate.

4.1.1 Baseline Salivary Flow Rates

The unstimulated left and right parotid gland salivary flow rates observed in the present study, are generally in line with those reported in previous literature for healthy young adults. Left and right parotid gland salivary flow rate was 0.030 mL/min, which is similar to the 0.027 mL/min previously reported by Fischer and colleagues (1999). In contrast, unstimulated left and right parotid gland salivary flow rates in healthy older adults, appear to be greater than those previously reported. In the present study, it was reported that unstimulated left and right parotid gland salivary flow rates were approximately 0.060 mL/min—almost double the 0.033 mL/min that was reported by Fischer and colleagues (1999).

Unstimulated SMSL gland salivary flow rates observed in the present study are generally consistent with those previously reported for both healthy young and older adults. The present study observed flow rates to be approximately 0.22 mL/min, which is similar to the 0.20 mL/min previously reported by Tylanda et al. (1988).

4.1.2 Left and Right Parotid Glands

4.1.2.1 Summary of Findings in the Left Parotid Gland

The present study found that salivary flow rates were significantly increased from baseline during toothbrush holding, tooth brushing, and also when flow rates from the toothbrush holding and tooth-brushing periods were averaged. Furthermore, the mean salivary flow rate during the tooth-brushing period was significantly greater than that during the toothbrush holding period. Thus, both stationary holding of a toothbrush on the tongue, and manual tooth brushing, are associated with increases in left parotid gland

salivary flow rate; tooth brushing is associated with a greater modulatory effect than stationary toothbrush holding.

However, these increases in salivary flow rates were short-lived, as salivary flow decreased immediately following the toothbrush holding and tooth-brushing periods. This suggests that manual tooth brushing is associated with a brief increase in left parotid gland salivary flow rate.

Comparisons of the younger and older adults' salivary flow rates during the various collection periods, averaged across conditions, indicated that the older adults had significantly greater salivary flow rates during the baseline (control and experimental), toothbrush (holding and brushing), 0-5 minute post-toothbrush (holding and brushing), and 5-10 minute post-tooth brush (holding and brushing) periods. Thus, the older adults showed greater unstimulated (i.e., resting) salivary flow rates, greater stimulated salivary flow rates, and greater post-stimulation salivary flow rates compared with the younger adults for the left parotid gland.

4.1.2.2 Summary of Findings in the Right Parotid Gland

Due to interaction effects, salivary flow rates for the young and older adults were analyzed separately for the right parotid gland.

The present study found that, in the young adults, the salivary flow rate during the toothbrush holding period was not significantly increased from baseline, although it approached statistical significance. In contrast, tooth brushing was associated with a significant increase in salivary flow rate from baseline. Interaction effects did not allow for statistical comparison across conditions, however, observation of the descriptive data suggested that the salivary flow rates during the toothbrush holding and tooth-brushing periods were generally similar.

In the older adults, the salivary flow rates during both (i) the toothbrush holding, and (ii) the tooth-brushing, periods were significantly increased from baseline. A comparison of the salivary flow rates during the toothbrush holding, and tooth-brushing, periods approached significance. Thus, stationary holding of a toothbrush on the tongue, and

manual tooth brushing, were associated with increased salivary flow rates in right parotid gland salivary flow rate in the older adults.

These responses were short-lived, as salivary flow decreased immediately following the toothbrush holding and tooth-brushing periods. Thus, manual tooth brushing appears to be associated with brief increases in right parotid gland salivary flow rates in both young and older adults.

Comparison of the younger and older adults across the various collection periods revealed that the salivary flow rate for the older adults was significantly greater than salivary flow rate for the young adults during the tooth-brushing period. It is noteworthy that the older adults displayed greater salivary flow rates than the younger adults for all saliva collection periods. This suggests that the older adults had greater right parotid gland salivary flow rate than the younger adults at rest, during stimulation by toothbrush holding and tooth brushing, and up to ten minutes post-stimulation.

4.1.2.3 Left and Right Parotid Glands

Findings were similar for the left and right parotid glands. Tooth brushing was associated with increased salivary flow rates for both the left and right parotid glands. The effects did not continue beyond the tooth-brushing period. Therefore, manual tooth brushing appears to be associated with a brief increase in parotid gland salivary flow rate.

The present findings also suggest that older adults may have greater resting parotid gland salivary flow rates than young adults, that is, in the absence of stimulation. To our knowledge, this is a novel finding.

The young adults demonstrated similar salivary flow rates when holding the toothbrush stationary on their tongue, and during tooth brushing. In contrast, for the older adults, the difference between salivary flow rates for the toothbrush holding, and tooth-brushing periods, approached significance. This apparent difference in salivary flow rate responses between the younger and older adults may be related to physiological changes with aging that occur in the oral cavity. Mechanoreceptors are located throughout the oral tissues, including the mucosa, periodontal ligament, tongue, palate, and lips (Jacobs et al., 2002;

Nordin & Hagbarth, 1989). They are responsive to various mechanical stimuli including touch, pressure, vibration and proprioception (Dong et al., 1993; Nordin & Hagbarth, 1989; Trulsson & Johansson, 2002). It has been shown that the ability to detect light touch decreases with age, and increased thresholds have been identified on the hard palate (Calhoun, Gibson, Hartley, Minton, & Hokanson, 1992; Newman, 1979; Weiffenbach, Tyllenda, & Baum, 1990). This may explain why the younger adults elicited similar salivary flow rates in response to holding the toothbrush stationary on the tongue and tooth brushing. This finding suggests that the young adults may have lower touch and pressure stimulation thresholds than the older adults in terms of mechanical stimulation eliciting parotid gland salivary flow.

Another important finding was that the older adults displayed greater salivary flow rates than the young adults across all collection periods for both left and right parotid glands. This is generally consistent with reviews suggesting that parotid gland salivary flow does not decrease with increasing age (Baum, Ship, & Wu, 1992). The present finding is also generally in line with a recent meta-analysis by Affoo et al. (2015b) which reported that parotid gland salivary flow rate was not lower for older adults, compared with younger adults. Furthermore, a study conducted by Navazesh and colleagues (1992) found that chewing produced significantly higher salivary flow rates in older adults compared to younger adults. Given that the parotid glands are responsible for producing more than 50% of total salivary secretions under stimulated conditions (Humphrey & Williamson, 2001) (i.e., in response to taste, smell, and visual and mechanical stimuli), this finding aligns with the higher parotid gland salivary flow rates that were identified in the older adults compared to younger adults in the present study. However, Navazesh et al. (1992) also found that unstimulated whole salivary flow was significantly lower in the older adults. This finding does not align with the present study, which also found that unstimulated parotid gland salivary flow rate was higher in older adults compared to young (see above). Future studies are needed to confirm age-related changes in whole, and gland-specific, unstimulated and stimulated salivary flow rates.

It has been shown that the salivary glands undergo structural changes as part of the aging process. Increased amounts of fat and fibrovascular tissue are evident, and the numbers of

acini are reduced (Vissink et al., 1996). Taken together, these changes may result in decreased salivary output. However, it has been demonstrated that the parotid glands contain a “reserve” functional capacity, which may help to offset the reduced salivary flow that may be present as a result of structural changes seen with normal aging (Vissink et al., 1996). Several studies support this view, and have reported that parotid salivary gland function does not decline with age (Fischer & Ship, 1999; Heft & Baum, 1984).

4.1.3 SMSL Glands

4.1.3.1 Summary of Findings in the SMSL Glands

The present study found that, in the young adults, salivary flow rates during the toothbrush holding, and tooth-brushing, periods were significantly increased from baseline. Interaction effects did not allow for statistical comparison across conditions. However, observation of the descriptive data suggested that the salivary flow rates during the toothbrush holding and tooth-brushing periods were generally similar.

In the older adults, the salivary flow rates during both (i) the toothbrush holding, and (ii) tooth-brushing, periods were significantly increased from baseline. The difference between tooth brushing and baseline was significantly greater than the difference between stationary toothbrush holding and baseline. This suggests that, while stationary holding of a toothbrush on the tongue, and manual tooth brushing are both associated with increased salivary flow rates in the SMSL gland in the older adults, tooth brushing has a greater modulatory effect.

Although salivary flow decreased immediately following the toothbrush holding and tooth-brushing periods in both the young and older adults, salivary flow rate was increased from baseline for up to ten minutes post-tooth brushing in the young adults, and up to five minutes post-tooth brushing in the older adults. Thus, manual tooth brushing appears to be associated with brief increases in SMSL gland salivary flow rate, which may display a more prolonged effect in young adults compared to older adults.

Comparison of the younger and older adults for the various collection periods revealed that the young and older adults had similar SMSL gland salivary flow rates for all saliva

collection periods, that is, at rest, during stimulation by toothbrush holding or tooth brushing, and up to ten minutes post-stimulation.

4.1.3.2 Submandibular/Sublingual Glands

Manual tooth brushing was associated with increased salivary flow rates for the SMSL gland. The effects continued for up to ten minutes in the young adults, and five minutes in the older adults. The longer duration of modulation of salivary flow rate in the young adults, compared to the older adults, suggests that manual tooth brushing may produce a longer duration increase in SMSL gland salivary flow rate in young adults.

The present findings also suggest that young and older adults may have similar resting SMSL gland salivary flow rates.

The young adults demonstrated similar salivary flow rates when holding the toothbrush stationary on their tongue, and during tooth brushing. In contrast, for the older adults, tooth brushing was associated with a greater increase in salivary flow rate than was toothbrush holding. This apparent difference in salivary flow rate responses between the younger and older adults may be related to physiological changes with aging that occur in the oral cavity. The young adults may have lower touch and pressure stimulation thresholds than the older adults in terms of mechanical stimulation eliciting SMSL gland salivary flow, as previously discussed for the left and right parotid glands.

In contrast to the parotid glands, the young and older adults showed similar SMSL gland salivary flow rates for each of the collection periods examined. The similarities in flow rates between healthy young and older adults may suggest that the SMSL glands are resilient to the effects of aging. However, this finding is inconsistent with findings previously reported in a meta-analysis by Affoo et al. (2015b), which found that SMSL gland salivary flow rate was lower in older adults.

4.1.4 SMSL Glands Adjusted

The results for the SMSL gland salivary flow rate, are identical to results obtained when the data were corrected for a possible contribution of residual water on the toothbrush. This indicates that the weight of the water on the toothbrush did not affect the

calculations of SMSL gland salivary flow rates during the toothbrush holding and brushing periods.

4.1.5 Comparison of Parotid and SMSL Gland Findings

The current study found that manual tooth brushing was associated with increased salivary flow rates from both the parotid and SMSL glands in young and older adults. The increase in parotid gland salivary flow rate did not continue beyond the tooth-brushing period for both age groups. In contrast, the increase in SMSL gland salivary flow rate remained for up to ten minutes post-tooth brushing in the young adults and up to five minutes post-tooth brushing in the older adults. Older adults had higher unstimulated and stimulated parotid gland salivary flow rates than young adults, whereas SMSL gland salivary flow rates were similar across the two age groups.

4.2 Limitations of Study

This study identified the gland-specific salivary flow rates in response to manual tooth brushing, and explored whether the effects were age-specific. Although the research provided insights to these questions, some limitations exist. One particular limitation regarding study materials was the sheering of cotton from the Salivettes® that resulted following removal of the rolls from the oral cavity. Salivary flow diminished over time, causing areas of the oral cavity to become dry and the cotton to adhere to the oral mucosa. This may have caused inaccurate (i.e., low) weights of Salivettes® to be recorded, as some cotton remained in the oral cavity and thus, the weight was not accounted for. Although this may have slightly influenced the results, this occurred with similar frequency amongst the young and older adults.

Another limitation was that the Salivettes® may not have successfully collected all of the saliva in the oral cavity. The SMSL gland salivary secretions pooled in the floor of the mouth, and it was frequently observed that some saliva remained following the removal of the Salivettes®.

This was not observed during saliva collection from the parotid glands, given the location of the parotid glands in the oral cavity, and thus the inability for saliva to pool in an area.

Despite this limitation, increases in SMSL gland salivary flow rate were observed in both young and older adults. The volume of saliva that was not accounted for may have contributed to greater SMSL gland salivary flow rates. Therefore, values reported for SMSL gland salivary flow rate are potentially more conservative.

An additional limitation is the study analyses that were performed. We wanted to examine the interactions of the three factors. Thus, our statistical analyses were more complex than if we had chosen to analyze just two of these factors. Following the three-way ANOVAs, it was necessary to perform post-hoc tests, which included several paired t-tests. While this approach allowed us to analyze three factors, the adjusted significance levels (α) were very conservative. This may have contributed to certain contrasts not reaching statistical significance. Although this limitation is present, the three-factor analysis provided a wealth of information that would not have been revealed with a simpler analysis.

An additional limitation is the number of males and females within the two age groups included in our study. Although the number of males and females were not even in each group, numbers of each sex were similar across groups. In the young adult group, there were nineteen females, and six males, while there were eighteen females and seven males in the older adult group. It is not believed that this factor influenced our study results, as the number of females and males were similar between the two age groups.

Another consideration is that the older adults in our study may have been healthier than typical older adults. A large number of the older adults in our study were members of an exercise group, who exercised approximately three times a week. Exercise has been shown to alter salivary secretion (Chicharro, Lucia, Perez, Vaquero, & Urena, 1998). This could potentially explain why the older adults in our study displayed higher unstimulated salivary flow rates (i.e., at baseline), and stimulated salivary flow rates than the young adults from the parotid glands.

One final limitation is that we did not complete separate statistical analyses for medicated and non-medicated individuals. Many medications, such as antidepressants, diuretics, analgesics, antihistamines, antihypertensives, antianxiety drugs, and appetite suppressants

are capable of reducing salivary flow (Sreebny & Schwartz, 1997). Some older adults in our study reported that they were taking one or more of these medications. However, medication use cannot explain the greater parotid gland salivary flow rate found in the older adults, and the lack of differences in SMSL gland salivary flow rate between young and older adults that were observed. As medication usage is more prevalent in older adults, the inclusion of medicated older adults in our study may make our findings more representative of the older adult population in general.

4.3 Clinical Implications

The present study found that there is an increase in salivary flow rate associated with manual tooth brushing from both the parotid and SMSL glands. The increase in parotid gland salivary flow rate was short-lived, with salivary flow rate immediately decreasing following tooth brushing. However, SMSL gland salivary flow rate was increased from baseline for ten minutes in the young adults and for five minutes in the older adults. This finding suggests that tooth brushing may provide an approach to increasing salivary flow in both young and older adults. Further studies are needed to determine if similar results are obtained in individuals who have hyposalivation and/or xerostomia.

4.4 Suggestions for Future Studies

Future studies should quantify the constituents of saliva collected from each salivary gland to distinguish between saliva collected from the parotid and SMSL glands. The parotid and SMSL glands produce saliva with different components, therefore, analyzing the saliva for the respective constituents will ensure that the method of saliva collection used in the present study (i.e., Salivettes®) accurately collected saliva from each gland.

Additionally, future research should include a larger sample size of healthy young adults aged 18-30 years and healthy older adults aged 60-90 years. It is necessary to replicate these results with regards to our findings of higher parotid gland salivary flow rates in older adults compared to young adults, and similar SMSL gland salivary flow rates between young and older adults.

4.5 Conclusion

The current study found that manual tooth brushing was associated with increased salivary flow rates from both the parotid and SMSL glands in young and older adults. The increase in parotid gland salivary flow rate was brief, as the effect did not continue beyond the tooth-brushing period for both age groups. In contrast, the increase in SMSL gland salivary flow rate remained for up to ten minutes post-tooth brushing in the young adults and up to five minutes post-tooth brushing in the older adults. Furthermore, in the younger adults, holding the toothbrush stationary on the tongue produced a similar effect to tooth brushing, which was observable across all salivary glands. This finding suggests the possibility that older adults may require greater mechanical stimulation (i.e., tooth brushing) than young adults to elicit an increase in salivary flow rate. The present study also found that older adults had higher unstimulated and stimulated parotid gland salivary flow rates than young adults, whereas SMSL gland salivary flow rates were similar across the two age groups. Manual tooth brushing may hold potential as a therapeutic approach to increasing salivary flow rates.

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Gerontol, 45(4), M121–5.

Appendices

Appendix A: Ethics Approval



**Western
Research**

Research Ethics

Western University Health Science Research Ethics Board
HSREB Delegated Initial Approval Notice

Principal Investigator: Dr. Ruth Martin

Department & Institution: Health Sciences/Communication Sciences & Disorders, Western University

Review Type: Delegated

HSREB File Number: 107216

Study Title: Examination of Potential Salivary Effects of Oral Sensory Stimulation: The Effects of Oral Stimulation Associated with Manual Tooth Brushing on Parotid- and Submandibular/Sublingual Gland Salivary Flow Rates in Healthy Young and Older Adults

HSREB Initial Approval Date: December 09, 2015

HSREB Expiry Date: December 09, 2016

Documents Approved and/or Received for Information:

Document Name	Comments	Version Date
Western University Protocol		2015/11/03
Letter of Information & Consent		2015/11/03
Advertisement	Saliva Study Advertisement 1	2015/11/03
Advertisement	Saliva Study Advertisement 2	2015/11/03
Recruitment Items	Telephone Script	2015/11/03
Recruitment Items	Email Script	2015/11/03

The Western University Health Science Research Ethics Board (HSREB) has reviewed and approved the above named study, as of the HSREB Initial Approval Date noted above.

HSREB approval for this study remains valid until the HSREB Expiry Date noted above, conditional to timely submission and acceptance of HSREB Continuing Ethics Review.

The Western University HSREB operates in compliance with the Tri-Council Policy Statement Ethical Conduct for Research Involving Humans (TCPS2), the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use Guideline for Good Clinical Practice Practices (ICH E6 R1), the Ontario Personal Health Information Protection Act (PHIPA, 2004), Part 4 of the Natural Health Product Regulations, Health Canada Medical Device Regulations and Part C, Division 5, of the Food and Drug Regulations of Health Canada.

Members of the HSREB who are named as Investigators in research studies do not participate in discussions related to, nor vote on such studies when they are presented to the REB.

The HSREB is registered with the U.S. Department of Health & Human Services under the IRB registration number IRB 00000940.

Appendix B: Letter of Information and Consent Form



The Effects of Manual Tooth Brushing on Parotid and Submandibular/Sublingual
Gland Salivary Flow Rates in Healthy Young and Older Adults

October 2015

Ruth E. Martin, MHS, PhD
Professor, School of Communication Sciences and Disorders
Department of Physiology & Pharmacology
The University of Western Ontario
London, Ontario Canada

Version 2 Date: November 3, 2015 Initials of Participant: _____

Page 1 of 5

LETTER OF INFORMATION

Study Background

You are being invited to participate in a research study looking at the effects of tooth brushing on salivary flow from various glands within the mouth.

We are testing the hypothesis that whole saliva will be altered post-mechanical stimulation of the oral cavity by manual tooth brushing, displaying a greater contribution from the parotid gland in healthy young and healthy older adults by measuring the amount of saliva produced by the parotid- and submandibular/sublingual glands after a 2-minute tooth brushing period.

We are looking for healthy young adults aged 18 to 30 years and healthy older adults aged 60 to 90 years. Participants must be non smokers, have at least 20 or more natural teeth, have no history of illness potentially affecting salivary flow (e.g. autoimmune diseases, HIV/AIDS, diabetes, Alzheimer's disease), have no history of surgery or medical treatment potentially affecting salivary flow (e.g. surgical or radiotherapy for head and neck cancer), and currently taking no medication that affects salivary flow (e.g. antidepressants, diuretics, analgesics, antihistamines, anti-hypertensives, anti-anxiety, appetite suppressants).

This research study will be conducted in Professor Ruth Martin's lab, Room 2528, Elborn College, The University of Western Ontario. You will be asked to participate in one study session that will be 45 minutes in duration. You will be asked to arrive at the Laboratory between 9:00 am and 12:00 pm, depending on your scheduled experiment time. You will be asked to complete your breakfast meal and morning tooth brushing one hour prior to your scheduled experiment time and refrain from eating and drinking anything thereafter prior to the experiment.

Oral Examination

If you agree to participate in the study, you will be asked to complete a verbal questionnaire that asks questions about your mouth, such as whether you have a dental appliance. The questionnaire will be administered by a member of the research team. You will also have a clinical examination of your mouth. This examination will be performed by an experienced speech-language pathologist who is a member of our research team and will be approximately 5 minutes in duration. The results of the examination will be conveyed to you at your request.

Oral Stimulation Protocol

Providing that your oral examination indicates that you meet the criteria for study participation, you will be asked to sit in an upright chair or a dental chair. A dental bib will be placed on your chest to prevent soiling of your clothes with saliva.

If you agree to participate in the study, a research assistant will perform a standardized tooth brushing technique using a new Colgate Sensitive Pro-Relief manual toothbrush provided by the researcher. The tooth brushing will be completed for two minutes. Saliva will be collected during the tooth brushing period and following the tooth brushing session at periodic intervals for up to 10 minutes after the tooth brushing session for a total of 8 saliva collections. Saliva

will be collected using Salivette® cotton rolls, with each roll tethered with dental floss and secured by tape on your right or left cheek. The experimenter will place the Salivette® rolls in your mouth at the beginning of each saliva collection period, and the rolls will be removed at the end of each saliva collection period. You will be asked not to swallow during these experimental periods.

Risks and Discomforts

There are no reports of pain or discomfort during the saliva collection procedure.

Benefits to Study Participation

The results from this study will help us to understand better the physiology of salivation. This data may lead to new tools to prevent or combat oral diseases caused by reduced volume of saliva.

Right to Refuse or Withdraw

Participation in the study is voluntary. You may refuse to participate, refuse to answer any questions, or withdraw from the study at any time. The procedures to be used in this study are designed for research purposes and are not intended to provide you any direct benefit.

If, during the course of this study, new information becomes available that may relate to your willingness to continue to participate, this information will be provided to you by the investigator.

Confidentiality

All scientific data and information obtained for the purpose of this study may be used for publications, and/or teaching. Your name will not appear in any publications or document generated from this study. We will attempt to keep all of your private information confidential and your information will not be shared by other institutions or resources. In addition, private information in hard copies will be kept in a locked filing cabinet and information in electronic versions will be kept on a computer under controlled access with a password.

All information obtained in this study will be held in strict confidence and participant confidentiality will be maintained. If you desire, you may request that your data and records related to this study be destroyed at a later date. Your name will not appear in any publications or presentations of the findings of this study. If you would like to receive copies of these publications, please contact Professor Martin at the telephone number on the last page of this letter. Representatives of the University of Western Ontario Health Sciences Research Ethics Board may contact you or may require access to your study related records to monitor the conduct of the research.

Compensation

Participants in this study are reimbursed for all costs incurred in relation to the study (e.g., parking, mileage, transportation). You will be given \$20 cash for your participation in this study. If the cost exceeds \$20 (due to travel distance, for example), please keep all relevant receipts and present them to one of the researchers. A cheque will then be issued for reimbursement.

Measures taken to Limit Conflict of Interest

There are no conflicts of interest.

Alternatives to Study Participation

An alternative to the procedures described above is not to participate in the study and continue on just as you do now.

If you have any questions or would like additional information about this study, please contact Dr. Ruth Martin, School of Communication Sciences and Disorders, University of Western Ontario, London, Ontario, N6G 1H1 (telephone: 519-661-2111 ext. 88186).

If you have questions regarding the conduct of this study or your rights as a research participant, you may contact The Office of Research Ethics, University of Western Ontario, 519-661-3036.

Signing of Consent Form

If you agree to participate in this study, please sign the consent form. You do not waive any legal rights by signing the consent form. You will be given a copy of the Letter of Information and the Consent Form for your records once it has been signed.

Sincerely,

Ruth E. Martin, M.H.Sc., Ph.D.

Professor
School of Communication Sciences and Disorders
Department of Physiology & Pharmacology
University of Western Ontario

The Effects of Manual Tooth Brushing on Parotid and Submandibular/Sublingual Gland Salivary Flow Rates in Healthy Young and Older Adults

Ruth E. Martin, M.H.Sc., Ph.D.
Professor
School of Communication Sciences and Disorders
Department of Physiology & Pharmacology
Graduate Program in Neuroscience
University of Western Ontario

CONSENT FORM

I have read the Letter of Information and have had the nature of the study explained to me. I agree to participate in the study entitled, "The Effects of Manual Tooth Brushing on Parotid and Submandibular/Sublingual Gland Salivary Flow Rates in Healthy Young and Older Adults" being conducted by Dr. Ruth Martin. All questions have been answered to my satisfaction.

Research Participant (please print): _____

Signature: _____

Date: _____

Printed Name of Person Responsible for Obtaining Signed Consent: _____

Signature of Person Responsible for Obtaining Signed Consent: _____

Appendix C: Participant Health Questionnaire

PI: Dr. Ruth Martin

The Effects of Manual Tooth Brushing on Parotid and Submandibular/Sublingual Gland Salivary Flow Rates in Healthy Young and Older Adults

Participant Questionnaire

Alphanumeric Identifier:

Date of Examination:

1. Do you have any health conditions or illnesses? (e.g, diabetes, a heart condition, Sjogren's syndrome, high blood pressure)
2. Have you had any surgeries? (If so, what surgeries?)
3. Do you currently take any medicine? (If so, what medications and dosage?)
4. Do you have any allergies? (If so, what?)
5. Do you drink any alcohol? (If yes, how much do you drink a day?)
6. Do you smoke cigarettes? (If yes, how many per day?)
7. Did you take any food or drink, suck any candy, or brush your teeth in the hour before your appointment today?
8. Do you have dentures (complete or partial denture)?
9. Have you had teeth extracted? (If so, how many and when?)
10. Do you have dry mouth?
11. Have you experienced any change in your sense of taste?
12. Do you have any condition or illness that affects your mouth?

Appendix D: Oral Examination Form

PI: Dr. Ruth Martin

The Effects of Manual Tooth Brushing on Parotid and
Submandibular/Sublingual Gland Salivary Flow Rates in Healthy Young
and Older Adults

Oral Examination Form

Alphanumeric Identifier:

Date of Examination:

Upper Teeth

Number:

Missing Teeth:

Denture: complete partial

Prostheses:

Condition of teeth:

Condition of gingiva:

Oral Secretions:

Lower Teeth

Number:

Missing Teeth:

Denture: complete partial

Prostheses:

Condition of teeth:

Condition of gingiva:

Oral Secretions:

Other Observations

Condition of tongue:

Condition of oral mucosa:

Appendix E: Data Collection Form

The Effects of Manual Tooth Brushing on Parotid and Submandibular/Sublingual Gland
Salivary Flow Rates in Healthy Young and Older Adults

Data Collection Form

Alphanumeric Identifier:

Date of Examination:

Collection Period	Collection Time (minutes)	Saliva	Stimulation	Beaker No.	Postweight (grams)	Prewriteight (grams)	Flow Rate/Minute
Baseline One	5	Left Parotid	None	1			
	5	Right Parotid	None	1			
	5	SMSL	None	1			
Toothbrush Control	2	Left Parotid	Control	2			
	2	Right Parotid	Control	2			
	2	SMSL	Control	2			
First Post-Toothbrush Control	5	Left Parotid		3			
	5	Right Parotid		3			
	5	SMSL		3			
Second Post-Toothbrush Control	5	Left Parotid	None	4			
	5	Right Parotid	None	4			
	5	SMSL	None	4			
Baseline Two	5	Left Parotid	None	5			

	5	Right Parotid	None	5			
	5	SMSL	None	5			
Tooth brushing Experimental	2	Left Parotid	Experiment	6			
	2	Right Parotid	Experiment	6			
	2	SMSL	Experiment	6			
First Post-Tooth brushing Experimental	5	Left Parotid		7			
	5	Right Parotid		7			
	5	SMSL		7			
Second Post-Tooth brushing Experimental	5	Left Parotid	None	8			
	5	Right Parotid	None	8			
	5	SMSL	None	8			

Salivary Flow Rate= $\frac{\text{Postweight Measure} - \text{Preweight Measure}}{\text{Collection Time}}$ = _____ g/min

Curriculum Vitae

Personal Information

Name: Kristen Marie Trottier, B.Sc.H, M.Sc. candidate
Main Affiliation: The Graduate Program of Health and Rehabilitation Sciences
Speech and Language Sciences
Western University

Education

2014- Expected 2016 Master of Science
The Graduate Program of Health and Rehabilitation Sciences
Speech and Language Sciences
Western University, London, Ontario, Canada
Supervisor: Dr. Ruth Martin
M.Sc. Thesis: “The Effects of Manual Tooth Brushing on
Parotid and Submandibular/Sublingual Gland Salivary Flow
Rates in Healthy Young and Older Adults”

2010-2014 Bachelor of Science
Departments of Biomedical Sciences/Human Health and
Nutritional Sciences
Honours Major Bio-Medical Science, Minor Neuroscience
University of Guelph, Guelph, Ontario, Canada

Research Experience

- 2014-Present Researcher in Dr. Ruth Martin's Swallowing Laboratory
Western University
Assisted Dr. Ruth Martin and laboratory members with research projects.
Completed grant proposals, ethics applications, participant recruitment and data collection.
Completed Tri-Council Policy Statement: Ethical Conduct for Research Involving Humans.
- 2013-2014 Researcher in Dr. Craig Bailey's Neuroscience Laboratory
University of Guelph
Conducted fourth year research project: "The Effect of Allopregnanolone on Nicotinic Acetylcholine Receptors in Functioning Neurons".
Delivered formal seminar presentations and wrote research project papers.
-

Teaching Experience

- 2015 Teaching Assistant

Western University

Faculty of Health Sciences

Communication Sciences and Disorders 9610A/9620A

Attended labs and helped students identify head and neck structures.

Marked assessments and provided constructive feedback to students in a timely manner.
- 2014 Teaching Assistant

Western University

Faculty of Health Sciences

Communication Sciences and Disorders 9640: Neurologically Based Speech Disorders (Motor Speech Disorders)

Marked assessments and provided constructive feedback to students in a timely manner.

2014

Teaching Assistant

Western University

Faculty of Health Sciences

Health Sciences 3052: A Brief History of Drug Use in the Western World

Marked assessments and provided constructive feedback to students in a timely manner.

Leadership Experience

2015-Present

Councillor for the Society of Graduate Students

One of four students appointed to represent the department of Health and Rehabilitation Sciences in the graduate student union.

Responsible for attending monthly meetings and conveying important information including events, campaigns, and services, to students in the Health and Rehabilitation Sciences Graduate Program.

2016

Marketing Sub-Committee Co-Leader

Co-lead an advertising team for the Health and Rehabilitation Sciences Graduate Student Research Conference.

Helped with the design of posters and compilation of the program booklet.

Collaborated with members of other sub-committees to review abstracts and successfully plan the event.

2015-2016

Vice President of Student Development for the Health and Rehabilitation Graduate Student Society

Organized academic workshops throughout the year for students. This included scheduling faculty presenters and promoting the events through poster advertisements, emails, and social media.

Presentations

2016

Oral presentation at the Health and Rehabilitation Sciences Speech and Language Science seminar. Thesis Research: “The Effects of Manual Tooth Brushing on Parotid and Submandibular/Sublingual Gland Salivary Flow Rates in Healthy Young and Older Adults”.

2016

Oral presentation at the Health and Rehabilitation Sciences Graduate Student Research Conference. Thesis Research: “The Effects of Manual Tooth Brushing on Parotid and Submandibular/Sublingual Gland Salivary Flow Rates in Healthy Young and Older Adults”.

Faculty of Health Sciences, Western University

London, Canada

2015
Poster presentation at the Health and Rehabilitation Sciences Graduate Student Research Conference. "Examination of Potential Salivary Effects of Oral Sensory Stimulation on Swallowing Rates of Individuals with Post-Stroke Dysphagia".

Faculty of Health Sciences, Western University

London, Canada

Awards

2014-2016
Western Graduate Research Scholarship
Valued at \$10,000 for each academic year.

2015
Canadian Institutes of Health Research
Alternate for one of the fifteen CIHR awards granted to master's students at Western University. Selection for awards is based on academic excellence, research potential, personal characteristics and interpersonal skills.