Characterization of Metallic Flavor in Drinking Water: An Interdisciplinary Exploration through Sensory Science, Medicine, Health, and the Environment

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By

Susan Mirlohi

Abstract

Scientific explorations can lead to life changing discoveries or light the path for new discoveries as scientists continue to carry or pass on the torch of knowledge to current and future generations. This torch of knowledge radiates in many directions, as the path of discovery often demands a multidimensional perspective. This research explored the many aspects of metallic flavor in drinking water through applications of sensory science, medicine, health, and the environment.

Humans interact with their environment through the five senses and are often exposed to contaminants through multiple routes; oral intake of trace metal contaminants through drinking water is a likely source. The biochemical mechanism by which humans are able to detect the flavor of strongly metallic agents such as iron has been previously elucidated, but little is known about population variability in the ability to sense metallic flavors. This research evaluated sensory thresholds and biochemical indicators of metallic flavor perception in healthy adults for ferrous iron in drinking water; 61 subjects aged 19 – 84 years, participated. The findings demonstrated an age-dependent sensitivity to iron indicating as people age they are less sensitive to metallic perception; impairment of olfactory functions is a contributing factor.

Unlike in healthy adults, where human senses are often protective of overexposure to contaminants, and supportive of sensations of everyday life's pleasures, cancer patients often suffer from chemosensory dysfunctions. Metallic phantom taste is a commonly experienced sensation, yet very little studied aspect of this debilitating disorder. Impact of cancer therapy on chemosensory functions of patients with malignant brain tumors undergoing combined modality treatment (CMT) was explored. The results indicated that chemosensory dysfunctions of the patients can range from minimal to moderate

impairment with maximum impairment developing during the 6-week CMT. Study of salivary constituents may provide clues on to the causes of chemosensory dysfunctions.

On health aspects, implication of individual sensitivity to metallic flavor on beverage choices and overall water consumption was assessed in 33 healthy adults through self-reported beverage questionnaire. The results indicated that among the elderly reduced intake of drinking water coincided with reduced sensitivity to metallic flavor. The findings have important health implications in terms of hydration status and beverage choices.

Finally, with environmental exposure relevance, preliminary findings on sensory properties of zerovalent iron nanoparticles (nZVI) indicated that oral exposure to nZVI may induce sensory properties different from that of ferrous iron, likely predictive of a diminished detection of metallic flavor by humans. Further research is warranted in this area.

Extended Abstract

Scientific explorations can lead to life changing discoveries or light the path for new discoveries as scientists continue to carry or pass on the torch of knowledge to current and future generations. This torch of knowledge radiates in many directions, as the path of discovery often demands a multidimensional perspective. This research explored the many aspects of metallic flavor in drinking water through applications of sensory science, medicine, health, and the environment.

Humans interact with their environment through the five senses and are often exposed to contaminants through multiple routes. Oral intake of trace metal contaminants through drinking water is a probable source of exposure. The biochemical mechanism by which humans are able to detect the flavor of strongly metallic agents such as iron has been previously elucidated, but little is known about population variability in the ability to sense metallic flavors. This research evaluated sensory thresholds and biochemical indicators of metallic flavor perception in healthy adults for ferrous (Fe^{2+}) iron in drinking water; 61 subjects aged 19 – 84 years, participated. Metallic flavor thresholds for individuals and sub-populations based on age were determined. Average thresholds for individuals younger and older than 50 years of age (grouped by the daily recommended nutritional guidelines for iron intake) were significantly different ($p = 0.013$); the population thresholds for each group were 0.045 mg/L Fe^{2+} and 0.499 mg/L Fe^{2+}, respectively. Few subjects <50 years were also insensitive to metallic flavor. Standardize olfactory assessment found poor sensitivity for Fe^{2+} corresponded with conditions of mild, moderate, and total anosmia. The findings demonstrate an age-dependent sensitivity to iron indicating as people age they are less sensitive to metallic perception.

Unlike in healthy adults, where human senses are often protective of overexposure to contaminants, and supportive of sensations of everyday life's pleasures, cancer patients often suffer from chemosensory (taste and smell) dysfunctions due to the disease itself and/or side effects of cancer treatment, especially those impacting the sensory organs. Metallic phantom taste is a commonly experienced sensation, yet very little studied

aspect of this debilitating disorder. Through application of sensory science and diagnostic medicine, this research explored the impacts of cancer therapy on chemosensory functions of patients with malignant brain tumors undergoing combined modality (radiation & chemotherapy) treatment (CMT). The study quantified taste and smell abnormalities (TSA) in a group of 22 glioma patients during the various phase of CMT, from 0 to 30-weeks post therapy, and explored salivary markers of oxidative stress as measured by iron-induced salivary lipid oxidation and evoked by oral intake of ferrous-spiked drinking water. Salivary electrolytes and metal constituents were also explored as potential biomarkers of TSA. Salivary markers in cancer patients were compared to a corresponding group of healthy subjects. The results indicated that impact of cancer treatment on chemosensory functions of patients treated for primary malignant gliomas can range from minimal to moderate impairment with maximum impairment developing during the 6-week CMT. When compared to healthy subjects, salivary oxidative stress response, total protein, Na, K, Cl, P, S, and Mg levels were significantly higher in cancer patients ($p < 0.05$), while salivary Zn, Fe, and oral pH levels were significantly lower in cancer patients ($p < 0.05$). Study of salivary constituents may provide clues on to the causes of taste and smell disorders.

On health aspects, this research explored the potential implications of individual sensitivity to metallic flavor, age, and beverage choices on overall consumption of drinking water as assessed through self-reported average daily beverage intake among 33 healthy adults. Plain water intake was notably lower among the elderly (60 – 84 year) and the average total beverage intake for all age groups was below the prescribed adequate intake level (AI). In the elderly group reduced intake of drinking water coincided with reduced sensitivity to metallic flavor. Sugar-sweetened beverages (SSB) constituted 46% of the beverage caloric intake for age group 19 – 39 compared to 28% and 21% in age groups 40 – 59 and 60 – 84, respectively. While at 79%, caloric intake from other beverages was highest among the elderly group. Increased consumption of plain water should be encouraged among all age groups to minimize caloric intake from SSB and other caloric beverages. The role of diminished taste sensitivity on reduced water intake among the elderly merits further exploration.

On environmental aspects, application of zerovalent iron nanoparticles (nZVI) in groundwater remediation and other treatment technologies raise concerns for human exposure to trace metal nanoparticles through drinking water. Iron nanoparticles have been found in drinking water systems in conjunction with other trace metal contaminants and are being considered for use in food fortification; therefore potential for human exposure through ingestion can become an issue of concern. This research took preliminary steps to assess whether ingestion of iron nanoparticles from drinking water could be detected through flavor perception, using in-vitro salivary lipid oxidation as an indirect indicator for metallic flavor perception by humans. Ten female subjects ages 29 to 59 years donated saliva samples for use in the in-vitro experiments. The preliminary findings indicate that oral exposure to ZVI nanoparticles may induce sensory properties different from that of ferrous salt, likely predictive of a diminished detection of metallic flavor by humans. Further research is warranted in this area.

Finally, this research exploration exemplifies the importance of interdisciplinary collaboration in solving the increasingly complex and multi-dimensional issues that impact human health and the environment. Assessment of human exposure risks to trace metals in drinking water and consumption patterns require collaborative insights from environmental engineers, sensory scientists, health and nutritionists; while in health compromised populations and cancer patients, understanding the causes of chemosensory dysfunctions require medical, diagnostic, as well as scientific insights.

Dedication

This dissertation is dedicated to my late mother, and living father whose first and last names translate into *Glory, Wisdom, Goodness, and Seeker of Knowledge*. I hope to honor their names as I continue through this life-long journey.

Acknowledgements

As no one travels alone on the path of scientific exploration, I must begin by acknowledging my advisor Dr. Andrea M. Dietrich who continued to travel with me on this path of exploration, always supportive, encouraging, positive, and tireless; as well as the diligent work of the immediate past scientists whose research efforts provided the framework and insights for this research: Dr. Andrea M. Dietrich at Virginia Tech and Dr. Pinar Ömur-Özbek at the Colorado State University. Additionally, I extend distinctive appreciations to my committee members: Dr. Susan Duncan, Dr. Brenda Davy, Dr. Dan Gallagher, and Dr. Greg Boardman for their suggestions, guidance, insights, and moral support. Special acknowledgements are also due to our research collaborators at Wake-Forest University School of Medicine, especially Dr. Glenn Lesser, Ms. Michelle Harmon, and Mr. Doug Case for their distinctive roles and contributions to the research studies associated with the brain tumor patients.

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Chapter I

Introduction

Characterization of flavor metals in drinking water is a multifaceted issue. Flavor producing metals, such as iron, copper, and zinc are essential nutrients, can be toxic, and are known to produce unpleasant tastes and flavor sensations in water as well as other food and/or beverage media. On the other hand, their occurrence in drinking water can add nutritional quality to water as a healthy beverage resource. Another important issue associated with metallic flavors is the variations in human perception. Oral intake of trace metal contaminants through drinking water is a probable source of exposure. The biochemical mechanism by which humans are able to detect the flavor of strongly metallic agents such as iron has been previously elucidated, but little is known about population variability in the ability to sense metallic flavors. Specifically, age-associated variations present a cause for concern as basic taste sensitivity in humans in known to decline by aging. Unlike in healthy adults, where human senses are often protective of overexposure to contaminants, and supportive of sensations of everyday life's pleasures, cancer patients often suffer from chemosensory (taste and smell) dysfunctions due to the disease itself and/or side effects of cancer treatment, especially those impacting the sensory organs; metallic phantom taste is a commonly experienced sensation, yet very little studied aspect of this debilitating disorder. This research explored the many aspects of metallic flavor in drinking water through applications of sensory science, medicine, health, and the environment.

The chapters of this dissertation were written in manuscript format. Chapter II presents the **literature review** relevant to the major aspects of this research.

Chapter III - **Age-associated Variation in Sensory Perception of Iron in Drinking Water and the Potential for Overexposure in the Human Population** presents findings on the research that evaluated sensory thresholds and biochemical indicators of metallic flavor perception in healthy adults for ferrous iron in drinking water, with a

focus of evaluating age implications. This paper was published in Environmental Science & Technology Journal.

Chapter IV - **Analysis of Salivary Fluid and Chemosensory Functions in Patients Treated for Primary Malignant Brain Tumors** – In collaboration with the Wake-Forest University Medical Center, this research assessed the impacts of cancer therapy on chemosensory functions of patients with malignant brain tumors undergoing combined modality (radiation & chemotherapy) treatment (CMT), using salivary biomarkers and self-reported taste and smell questionnaires.

Chapter V - **Water Consumption in Healthy Adults: Implications of Age, Flavor Perception, and Beverage Choices** explored the potential implications of individual sensitivity to metallic flavor, age, and beverage choices on overall consumption of drinking water and beverage intake as assessed through self-reported average daily beverage intake among healthy adults.

Chapter VI - **Role of Salivary Fluid in Metallic Flavor Production Associated with Iron and Copper Induced Lipid Oxidation (LO)** - Using salivary LO (SLO) as an indicator of metallic flavor intensity, this study compared levels of iron and copper induced SLO in artificial saliva and investigated the individual and interactive influences of fatty acids, proteins, and nitrite in the production and/or inhibition of metallic flavor.

Chapter VII – **In-vitro Evaluation of Iron-induced Salivary Lipid Oxidation Associated with Exposure to Zero-valent Iron Nanoparticles (nZVI)** - This research took preliminary steps to assess whether ingestion of iron nanoparticles from drinking water could be detected through flavor perception, using in-vitro SLO as an indirect indicator for metallic flavor perception by humans

Chapter VIII – **Perspectives on the current recent findings and insights for future research** presents insights on the research findings and perspectives for future research explorations.

Appendix A – **Institutional Review Board** approvals for the work involved human subjects are included in this section.

Appendix B – **Taste and Smell Questionnaire for Cancer Patients** this is the questionnaire utilized to assess taste and smell abnormalities in cancer patients as administered by the Wake-Forest University medical staff.

Appendix C – **Salivary Analysis Data on Cancer Patients and Healthy Subjects** this section includes more detailed summary of salivary data and additional plots of the data presented in Chapter IV.

Appendix D – **Beverage Consumption Data on Healthy Subjects** this section includes more detailed summary of salivary data presented in Chapter V.

Chapter II

Literature Review

2.1 Metallic Flavor and Aspects of Sensory Science

Consumers characterize foods and beverages through sensory evaluation. As defined by the Institute of Food Technologists, sensory evaluation is "*a scientific discipline used to evoke, measure, analyze and interpret reactions to those characteristics of foods and materials as they are perceived by the senses of sight, smell, taste, touch, and hearing*" (Stone and Sidel 1993). Sensory attributes of food items and beverages are typically perceived through appearance, odor/aroma/fragrance, consistency/texture/mouth feel, and flavor, including aromatics, chemical feelings, and taste (Meilgaard et al. 2006). With regard to beverages, appearance can be classified as color, turbidity/clarity, and the degree of effervescence or carbonation, while odors can be perceived externally through the nose, as aromas from food products, or fragrances from perfumes. On the other hand, flavor is an attribute of foods and beverages that consists of combined sensations of tastes (sour, sweet, salty, bitter, and umami), mouth feel, and odors produced inside the mouth upon ingestion of foods and/or beverages (Meilgaard et al. 2006). Variations in human sensory perceptions can be attributed to psychological factors such as memory and personal experience and physiological factors, such as age and health status (Taylor and Roberts 2004).

Metal salts of iron and copper are characterized as having a metallic taste or flavor. These metallic flavors are widely encountered as associated with off-flavors in foods and beverages or used as dietary supplements and fortifying agents. However, their sensory profiles are complex and not well understood. This section of the literature review provides an overview on the aspects of sensory science associated with metallic flavors.

2.1.1 Human sensory perception of taste and flavor

Our senses of taste and flavor perception guide us to identify and consume foods and nutrients that please us while avoiding toxins and/or spoiled material that may harm us.

Human taste perception is generally described by five basic qualities: salty, sour, sweet, bitter, and umami or savory, which is a sensation derived from glutamate, one of the 20 amino acids that make up the proteins in meat, fish and legumes. Glutamate is also a flavor enhancer used as a food additive in the form of monosodium glutamate (MSG). Other taste qualities such as fatty and metallic are sometimes regarded as basic taste qualities (Chaudhari and Roper 2010). Although there is increasing evidence in the literature recognizing the sensory perception of metals such iron and copper as flavor rather than basic taste qualities (Epke and Lawless 2007; Ömur-Özbek and Dietrich 2011). Fascinatingly, human flavor perception has been described as the "most complex of human behaviors" as it involves multiple senses, namely, smell, taste, texture, vision, and motor functions associated with salivary glands and mouth movements (Shepherd 2006). This complex functioning of human flavor perception has been actually demonstrated by neuroscientists using brain images (Shepherd 2006). When humans were subjected to taste stimuli alone, only primary taste centers on the tongue and brain regions associated with taste were activated, whereas odor stimuli by themselves activated the olfactory regions. Finally, when subjects were simultaneously presented with taste and odor stimuli, many regions of the brain were activated (Shepherd 2006; Small et al. 2005).

Within the oral cavity, components responsible for the taste perception include taste molecules, receptor cells, and an aqueous environment such as saliva, which helps to dissolve and carry the taste molecules (Spielman 1990). Special sense organs on the tongue and soft palate contain our taste receptors, which are housed in clusters within our oral epithelial cells membranes and referred to as taste buds. The taste buds located on the tip or front, side, and back of the tongue have been respectively classified as fungiform ("mushroom like), foliate ("leaf like"), and circumvallate ("wall like") papillae (Figure 2.1) (Breslin and Spector 2008). The oral epithelial cell membranes consist of a phospholipid bilayer, with proteins and carbohydrates linked to the lipid bilayer. Phospholipid bilayer consists of a polar head and a non-polar tail (Figure 2.2). The hydrophobic tails interact with one another and form sheet structures that act as permeability barriers, while the hydrophilic heads interact with the aqueous

medium on each side of the bilayer (Tymoczko et al. 2010). Phospholipids are composed of fatty acids attached to a glycerol backbone that also carries a phosphoryl group, thus forming a macromolecular structure with a polar head and a nonpolar tail (Figure 2.2). Phospholipids consist of unsaturated fatty acids (Tymoczko et al. 2010).

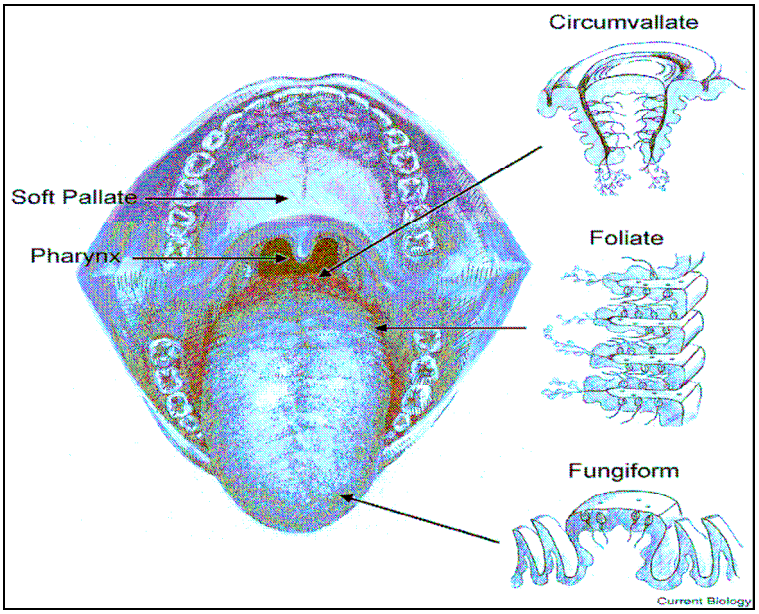

Figure 2.1. Schematic of human oral cavity, taste receptor cells, and taste buds. (Breslin and Spector 2008). Used with permission from Copyrights Clearance, Inc.

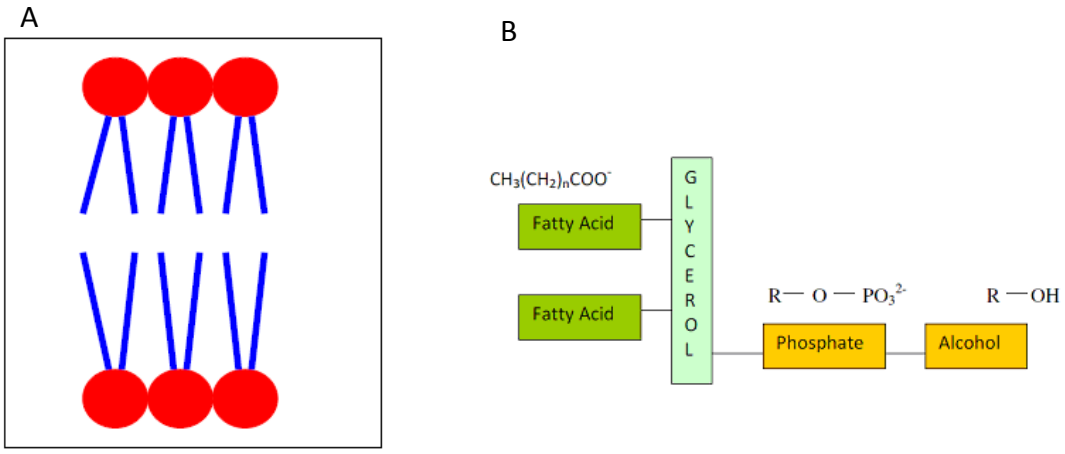

Figure 2.2. Illustration of membrane lipid bilayer (A) and Schematic structure of a phospholipid (B). The red circle designates hydrophilic heads.

In human epithelial cells, including skin and the oral cavity, arachidonic, linoleic, and oleic acids are among the major unsaturated fatty acids associated with phospholipids (Lekholm and Svennerholm 1977). In biological systems, fatty acids typically contain an even number of carbon atoms, usually between 14 to 24 carbon atoms, with the most common ones being 16- and 18- carbon fatty acids. Both linoleic (C18:2) and oleic (C18:1) acid consist of 18 carbons, while arachidonic acid (C20:4) consists of 20 carbon atoms (Tymoczko et al. 2010). Unsaturated fatty acids contain one or more carbon-carbon double bonds; the number of double bonds is typically designated following the number of carbons, such as C18:2, indicative of 18 carbons with 2 carbon-carbon double bonds (Tymoczko et al 2010). Fatty acids with more double bonds, as well as trans- rather than cis- positioning of the double bonds, are more susceptible to oxidation. Variety of proteins and glycoproteins are embedded within and/or are positioned on the surface of the membrane lipid bilayer (Tymoczko et al. 2010); these serve as receptor sites when taste and/or flavor substances are encountered within the oral cavity (Breslin and Spector 2008).

Since flavor perception results from the combined sensations of taste, smell, and mouth feel, understanding the odor detection and oral processing of flavor stimuli is important. With regard to odor detection, airborne odor stimuli are sensed by the olfactory epithelium structure that is located in the roof of the nasal cavity (Dietrich 2009; Meilgaard et al. 2007) (Figure 2.3). Odor molecules are captured by the millions of small, hair-like projections covering the olfactory epithelium, translated into signals through receptor proteins, and the signals sent to the olfactory bulb and brain for odor perception and recognition (Meilgaard et al. 2007). Air containing odor molecules can reach the olfactory bulb, through two pathways; one is through the nose (orthonasal) from inhaling external odors, and the other is through the back of the oral cavity (retronasal) from odors released in the mouth through ingested food or beverages (Meilgaard et al 2007). Retronasal odors reach the olfactory epithelium through the nasopharyngeal passage, which connects the nasal and oral cavities (Dietrich 2009; Meilgaard et al. 2007) (Figure 2.3).

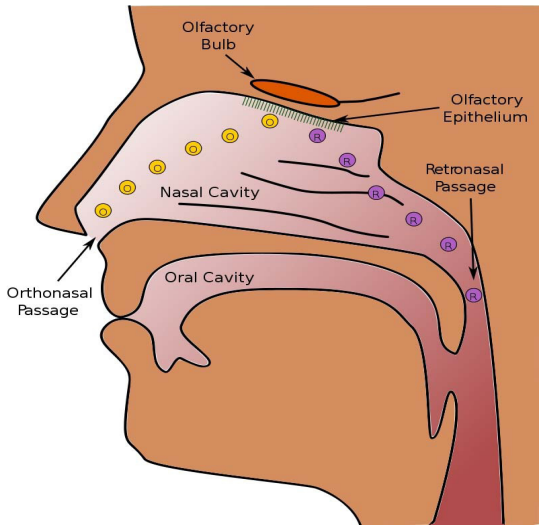

Figure 2.3. Schematic of human olfactory system. Shown in the figure are the orthonasal (O) and retronasal (R) paths of flavor compounds and odorants (Dietrich 2009). Used with permission from the copyright holder, IWA Publishing.

Since flavor perception results from the combined sensations of taste, smell, and mouth feel, understanding the odor detection and oral processing of flavor stimuli is important. With regard to odor detection, airborne odor stimuli are sensed by the olfactory epithelium structure that is located in the roof of the nasal cavity (Dietrich 2009; Meilgaard et al. 2007) (Figure 2.3). Odor molecules are captured by the millions of small, hair-like projections covering the olfactory epithelium, translated into signals through membrane-bound receptor proteins, and the signals sent to the olfactory bulb and brain for odor (Deibler and Delwiche 2004).

In addition to the above discussed mechanisms of taste and odor sensations that influence how humans perceive flavors, the oral processing, such as the action of chewing and swallowing influence perception of taste and/or flavor stimuli (Taylor and Roberts 2004). This includes interaction of flavor stimuli with salivary fluid, which is the subject of discussion in the proceeding section.

2.1.2 Salivary fluid composition and its role on taste and flavor perception

Saliva is the principal fluid component of the external environment of the taste receptor cells and, as such, plays an important role in taste sensitivity. Its main role includes

transport of taste substances to and protection of the taste receptor. In the initial process of taste perception, saliva acts as a solvent for taste substances; composed of about, 99% water, salivary fluid dissolves taste substances, and the latter diffuse to the taste receptor sites where a signal is generated and transmitted to the brain (Plattig 1988). Saliva is composed of fluids from three major and multiple minor salivary glands. The major glands include, the parotid, the sublingual, and the submandibular, also called submaxillary (Pedersen et al. 2002) (Figure 2.4). Each fluid has varying composition. The parotid saliva contains high levels of alpha-amylase, proline-rich proteins (PRPs) and bicarbonate; the sublingual gland contains high levels of mucins, while the submandibular glands produce a mixture of three sections (Humphrey and Williamson 2001). Whole saliva is more commonly studied due to the difficulty of isolating each oral fluid. In addition to the gland secretions, whole saliva consists of other components such as microbes, enzymes, anti-microbial fluid, leukocytes, sloughed epithelial cells, and secretions from nasal and lung fluids (Tenovuo 1989).

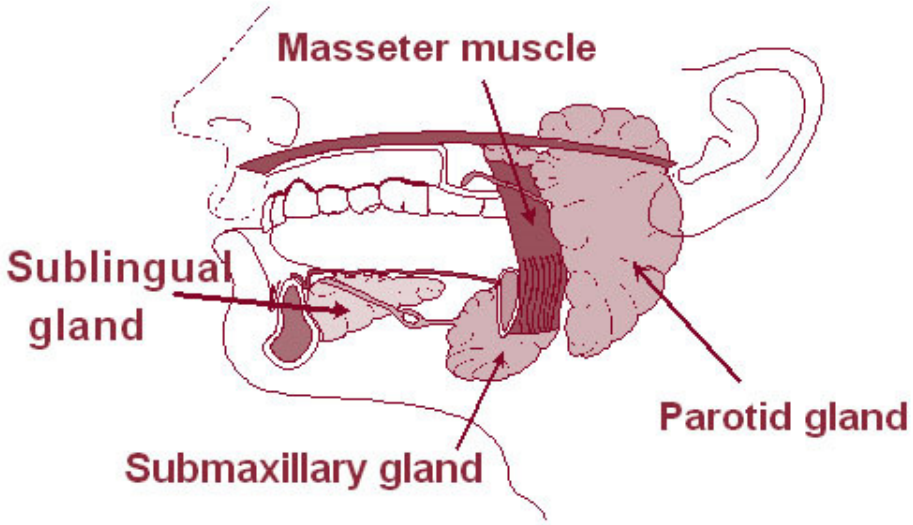

Figure 2.4. Illustration of the major human salivary glands. (http://training.seer.cancer.gov/head-neck/anatomy/salivary.html), used with permission: "*Public Domain*"

Salivary flow and composition can vary based on flavor stimuli. For example, sour tastes have the greatest effect on increasing salivary flow rate, while caffeine intake can reduce the flow rate (Taylor and Roberts 2004). Additionally, salivary amylase plays a key role in the perception of starch-based foods, while, mucins have been known to bind with certain volatile compounds, thus influencing the degree of perception (Taylor and Roberts 2004; Hong et al. 2010). Salivary proteins, PRPs are known for their binding interactions with tannins, associated with food polyphenols, and their influence on astringency of beverages such as wine and tea (Spielman 1990; Soares et al. 2011).

Variations in salivary flow can also influence the pH, buffering capacity, and electrolytes composition of saliva (Spielman 1990; Tenovuo 1989). Variety of factors, such as disease conditions, age, gender, emotional disorder, radiation therapy of the neck and head region, and many drugs can influence salivary flow rate and composition (Spielman 1990; Tenovuo 1989). Among disease conditions, Sjörgen's syndrome is best known for its negative effect on salivary flow rate. Average inorganic and organic constituents of human saliva is available from multiple sources giving varying concentrations as summarized in Table 2.1 (Actis et al. 2005; M. Behuliak et al. 2009; Hong 2006; Mirlohi et al. 2011; Ömur-Özbek 2008).

Table 2.1. Average constituents in whole saliva as reported in the literature.

Constituent	Mean ± s.d.	Range
Sodium (mmol/L)	12.03 ± 8.9	0.93 - 31.15
Potassium (mmol/L)	21 ± 4	0.02 - 40
Calcium (mmol/L)	2.16 ± 1.11	0.001 - 2.8
Magnesium (mmol/L)	0.83 ± 0.33	0.13 - 1.78
Copper (µg/L)	19.5	6.3 - 460
Zinc (µg/L)	49.2	46.9 - 1627
Phosphate (mmol/L)	-	1.5 - 25
Sulfate	5.8 ± 0.25	-
Chloride	-	10 - 56
Carbonate	20 ± 8	-
pH (S.U.)	6.5 ± 0.37	5.5 - 7.5
Nitrite (µmol/L)	178 ± 11	-
Chromium (µg/L)	230 ± 30	-
Total Protein (g/L)	0.62 ± 0.25	0.25 - 1.2
Salivary TBARS	0.25 ± 0.11	0.1 - 2.0
Free Fatty Acids (mg/100ml)	-	1.3 – 5.7

Salivary constituents in humans have been extensively studied in oral/dental research. Saliva composition and secretion rates are influenced by multiple factors, such as age, gender, diet, and general health; for example, salivary flow rate and buffering capacity have been shown to be significantly lower in females than males (Tenovuo 1989).

Changes in saliva composition have been primarily linked to salivary flow rate. In one study, it was shown that in unstimulated saliva, salivary flow rate decreased by age when 21 individuals in each age group of 18-35 years old and 65-83 years old were compared (Navazesh *et al.* 1992). Another study investigated the change in protein/peptide composition in saliva of 67 individuals in age range of 6 to 44 years old, and found that level and types of proteins varied significantly among different age groups (Cabras *et al.* 2009). Age-related change in salivary flow and total protein content has also been demonstrated in a large study with 1006 subjects ranging in age from 35 to greater than 75 years old, divided into different age categories. In this study it was shown that the salivary flow rate significantly decreased by age, whereas, the total protein content increased by age (Yeh *et al.* 1998). High variability in TBARS content of saliva has also been noted for repeated daily measurements taken on a given individual as well as among 38 individuals (M Behuliak et al. 2009).

Documented data in literature point to the variability in saliva composition based on age; however, the results are also conflicting; some indicating no age effect depending on the type of salivary flow, such as stimulated or unstimulated, submandibular or sublingual (Dodds *et al.* 2005). It has been noted that minimizing variability in sampling of saliva as well as some type of data normalizing measure would aid in controlling the inherent variability in saliva components (Tenovuo 1989; M. Behuliak et al. 2009).

2.1.3 Metallic flavor characteristics and oral sensation

The flavor generated by ferrous solutions has been described as metallic, sweet, bitter and astringent (Ömur-Özbek and Dietrich 2011; Cohen 1960; J Lim and H Lawless 2006). One study showed that the metallic salts cause a metallic taste as they create a surface electrical potential in the mouth (Plattig 1988). Another described high concentrations of divalent cations, such as zinc, as having bitter, salty, metallic,

astringent, sour, and sweet tastes (Lawless 2003), but another study (R Keast 2003) reported zinc had little taste.

Various studies have been conducted to determine the human threshold for ferrous iron. A study done by Lawless determined the geometric mean thresholds for three different iron salts: ferrous sulfate 28 mg/L, ferrous chloride 13 mg/L, and ferrous gluconate 10 mg/L; while the means were not significantly different, the variability between the individual responses were considerably high (J Lim and HT Lawless 2006). Others have found threshold values for ferrous sulfate ranging from 0.04-256 mg/L (Cohen *et al.* 1960). A more recent study had the threshold of ferrous iron as 0.003 to greater than 5 mg/L (Ömur-Özbek and Dietrich 2011); however, the data from this study were mostly represented by individuals less than 25 years old. Metallic flavor threshold concentrations were also found to be considerably varied depending on the quality of the water used for preparing samples (Hoehl *et al.* 2009).

The wide range of threshold values indicated a level of complexity in the causes of metallic flavors and lead to additional studies. The initial studies looked at the effect of nasal occlusion on threshold values (Hettinger et al. 1990). Theses studies showed an increase in the threshold values for ferrous iron when the nose was closed, indicating that perception from retronasal odor was blocked, but this was not the case for copper or zinc sulfate (Lawless *et al.* 2004). The values showed an increase ranging from 3.5 to 5.3 times for ferrous chloride and sulfate respectively in individual thresholds (Epke and Lawless 2007). Another study reported that copper speciation significantly affected taste such that soluble species were more readily tasted than particulate species (Cuppett *et al.* 2006). Retronasal component of metallic flavor was also demonstrated in a more recent study where individuals tasting water samples spiked with ferrous, ferric, cuprous, and cupric, with their nose occluded, could not taste the metallic; however with their nose open, metallic sensation was perceived strongest for ferrous, followed by cupric and cuprous salts (Ömur-Özbek and Dietrich 2011). No metallic taste was perceived for ferric chloride salt.

The effect of retronasal occlusion on reducing metallic flavor perception led to attempts to isolate and identify specific volatile compounds linked to metallic flavor. In this attempt, a study was conducted by placing copper or iron on the skin and collecting the volatilized compounds produced in a small headspace. Solid Phase microextraction (SPME) and GC/MS analysis was then performed on the headspace samples. The samples showed the production of aldehydes and ketones, including 1-octen-3-one. The carbonyl compounds were the lipid peroxides created by the metal oxidized lipids within the skin (Glindemann et al. 2006). Metallic flavor producing compounds have also been isolated from food matrices. For example, the metallic and fishy aftertaste associated with the consumption of red wine with fish meat has been attributed to the release of volatile compounds of fishy aftertaste, such as hexanal, heptanal, 1-octen-3-one, (E,Z)-2,4-heptadienal, nonanal, and decanal, as determined by gas chromatography-olfactometry and gas chromatography-mass spectrometry in dried scallop soaked in red wine (Tamura et al. 2009).

2.1.4 Taste and smell functions and aging

There have been numerous studies on the effect of age on taste sensitivity in humans, mostly focusing on the five basic tastes of salty, sweet, bitter, sour, and umami (Mojet *et al.* 2005). Researchers have documented evidence of diminished basic taste functions due to normal aging process; however, other factors associated with aging such as reduced saliva production, reduced sense of smell, use of prescription drugs, tooth loss, and interference with chewing function from the use of dentures, can contribute to altered taste functions (Boyce J M 2006). The study by Mojet et al. reported that age effects on taste threshold for the four basic tastes varied for the different tastants. For example, the difference in thresholds between old and young for salty taste was larger than sweet taste ($F_{1,38}$= 9.29, $P < 0.005$). The older group consisted of 21 healthy, adults in age range of 60-75 years old; whereas, the younger group included 22 healthy adults in the age range of 19-33 years olds. Additionally, there was a large difference in thresholds for sour taste ($F_{1,38}$ = 5.44, $P < 0.05$) (Mojet *et al.* 2001). In all cases, the taste sensitivity declined in the older age group.

With regard to the five basic tastes, evidence of age effects on taste sensitivity is well documented in the literature; however, data on the effect of age on metallic flavor threshold is lacking. Reported threshold studies on metallic taste and specifically for iron and cupper sulfate in water, have included individuals in age range of 17 to 50 years old (Zacarias *et al.* 2001) or 18 – 65 years old (Epke and Lawless 2007); however, no specific age effects have been noted. Additionally, it is not always clear how the proportions of study participants are distributed by age. Further gains in the knowledge of age-related changes in human taste sensitivity to metallic flavors would be valuable in relation to examination and management of taste disorders associated with drug use, disease, and/or normal aging process.

Human smell functions are also known to decline by aging, or impair due to other factors, such as disease conditions like Alzheimer's and Parkinson's, respiratory infections, side effects of some drugs, and environmental exposure (Ackerman and Kasbekar 1997; Boyce and Shone 2006; Calderon-Garciduenas et al. 2010; Doty 2007; Doty et al. 2008). In the United States, the National Health Interview Survey (NHIS) in 1994 had estimated that approximately 2.7 million American had self-reported smell impairments with frequency of occurrence being higher among adults ages 55 years and older (Murphy et al. 2002). While the loss or diminished smell functions is typically associated with retronasal perception, there have been a number studies examining the influence of orthonasal smell impairment on retronasal smell functions (Gudziol et al. 2007; Hummel et al. 2001; Stinton et al. 2010). Although the results of these studies have been conflicting; some showed that loss of orthonasal functions impacted the ability to identify basic taste stimuli (Hummel et al 2001; Gudziol et al 2007), while another study with larger populations showed that when controlling for subject age and sex, smell losses did not influence basic taste sensitivity (Stinton et al 2010). Recognizing this association of taste and smell functions is important since impairment of retronasal smell functions in turn influences the ability to taste the flavors of foods and beverages, and can ultimately impact the quality of life, in additions to the serious danger of exposure to potential toxicants with impaired or lost sensation. Additionally,

further explorations are warranted on the association of taste and smell functions specific to metallic stimuli, since metallic sensation has a considerable retronasal smell.

2.1.5 Lipid oxidation and metallic flavor perception

A mechanism responsible for the generation of metallic flavor is lipid oxidation; on the human skin surface, lipid oxidation has been linked to the production of "metallic" odor compounds produced when a metallic object such as a key or a penny is held in the hand (Glindemann et al. 2006). Occurrence of metal-induced lipid oxidation has also been demonstrated in the oral cavity of healthy human subjects when drinking water spiked with copper and iron was consumed (Ömur-Özbek 2008). Lipid oxidation is a measure of oxidative stress in the cellular environment. Oxidative stress is a result of imbalance between oxidants and antioxidants in the body; oxidative stress results in damage to macromolecular components of cells such as nucleic acids, proteins, and lipids. Specifically, lipid oxidation causes damage to cells by formation of lipid peroxides through generation of free radical species (Armstrong and Browne 1994). Free radical species are formed when covalent bonds between atoms are broken leaving unpaired electrons on the broken ends. Free radicals react with membrane lipids, consisting of mostly polyunsaturated fatty acids (PUFA); the resulting lipid hydroperoxides are highly reactive and unstable (Catala 2009). Metals act as catalysts in the free radical processes that breakdown polyunsaturated fats (Spanier 1991). Metals promote decomposition of these hydroperoxides by the production of reactive free radicals as shown below for iron:

$$Fe^{2+} + LOOH \rightarrow Fe^{3+} + LO^{\cdot} + OH^{\cdot}$$

$$Fe^{3+} + LOOH \rightarrow Fe^{2+} + LOO^{\cdot} + H^{\cdot}$$

In addition to being catalyzed by metals, such as iron and copper, free radical mediated oxidation of PUFA can be initiated by a number of reactive oxygen species (ROS) abundantly found in biological membranes (Frankel 1998). Typical ROS include superoxide radical ($O_2^{\cdot -}$) singlet oxygen (O_2^1), triplet oxygen (O_2^3), hydroxyl radical ($^{\cdot}OH$), alkoxyl radical ($ROO^{\cdot}$). There are numerous pathways and products that are produced in the lipid oxidation process (Frankel 1998; Shibamoto 2006). Two of the

common pathways are mediated by enzymes and result in the production of prostaglandin or leukotriene compounds (Figure 2.5).

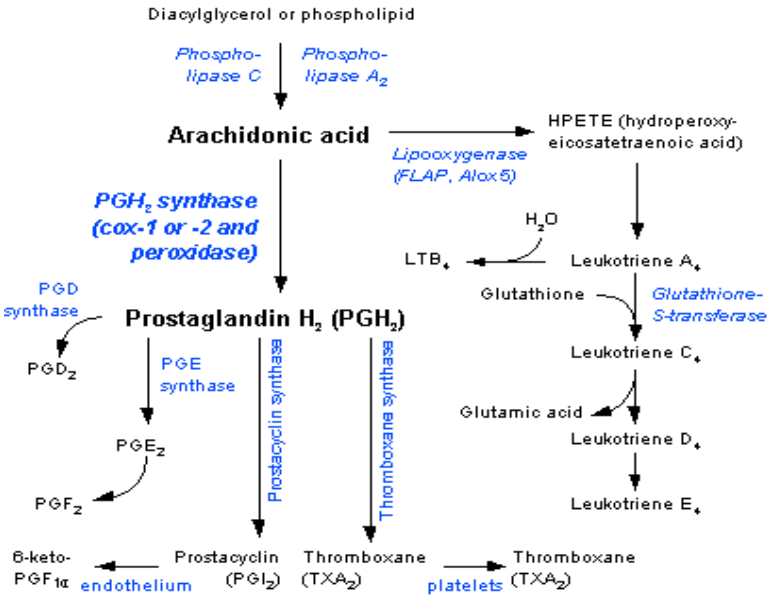

Figure 2.5. Enzyme mediated pathways of lipid oxidation.
[Source: http://en.wikipedia.org/wiki/File:Eicosanoid_synthesis.svg]; used with permission: "*Public Domain*"

The non-enzymatic pathway involves free radical reactions occurring at one of the double bonds in PUFA. The reaction proceeds through three steps known as initiation, propagation, and termination as highlighted in Figure 2.6.

(1) Initiation:	***(2) Propagation:***
$RH \rightarrow R^{\cdot} + H^{\cdot}$	$R^{\cdot} + O_2 \rightarrow ROO^{\cdot}$
(Catalyzed with heat, light, and/or metals)	$ROO^{\cdot} + RH \rightarrow ROOH + R^{\cdot}$ (slow)
$ROOH + M^{3+} \rightarrow ROO^{\cdot} + H^{\cdot} + M^{2+}$ (slow)	$RO^{\cdot} + RH \rightarrow ROH + R^{\cdot}$
$ROOH + M^{2+} \rightarrow RO^{\cdot} + OH^{\cdot} + M^{3+}$ (very fast)	
$2\ ROOH \rightarrow RO^{\cdot} + ROO^{\cdot} + H_2O$	***(3) Termination (occurs when two radicals react):***
	$R^{\cdot} + R^{\cdot} \rightarrow RR$
	$ROO^{\cdot} + ROO^{\cdot} \rightarrow ROOR + O_2$

Figure 2.6. Free radical directed lipid oxidation reactions.

Once free radicals are formed and lipid oxidation reactions are initiated, many intermediates and secondary by-products are formed (Shibamoto 2006) (Figure 2.7). There are several products of the non-enzymatic reaction, including conjugated dienes, isoprostanes, lipid hydroperoxides, and the hydroperoxide breakdown products 4-hydroxynonenal and malondialdehyde (MDA). These products have been used to monitor lipid peroxidation, and involve analysis by GC-MS, LC-MS, enzyme-linked immunoassays (ELISA), and fluorometric and spectrophotometric methods (Shibamoto 2006). Some of the secondary lipid oxidation products, such as MDA, 4-hydroxy-2-nonenal (HNE) have been used as biomarkers of oxidative damage in in-vitro and in-vivo studies (Dmitriev and Titov 2010). As the most commonly measured lipid oxidation products, MDA has been regarded as mutagenic and carcinogenic due to its ability to form DNA adducts (Marnett 1998).

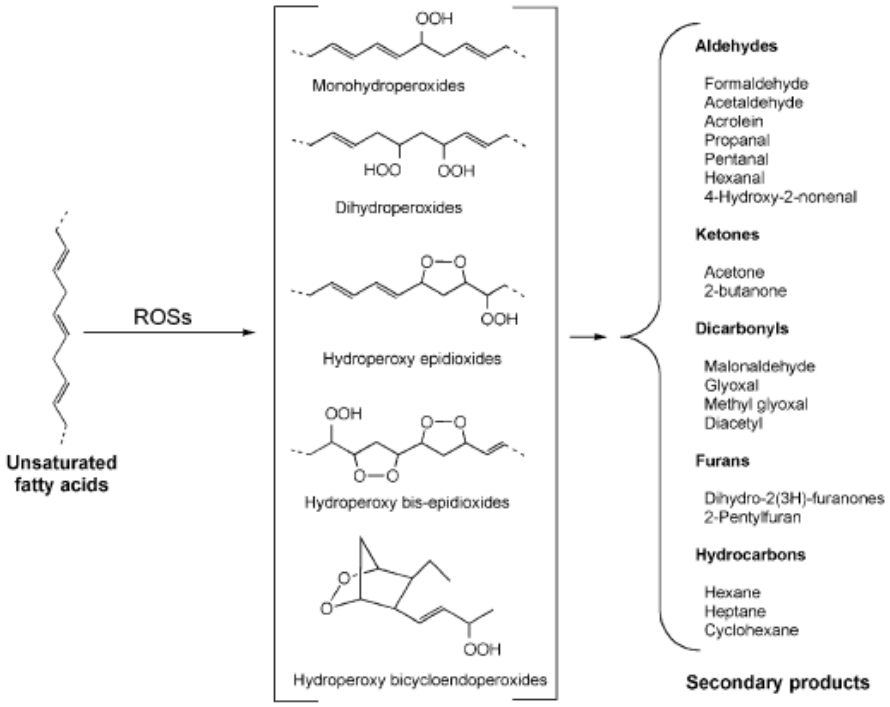

Figure 2.7. Proposed intermediates and secondary by-products of lipid oxidation. Figure used with permission from Copyrights Clearance Center, Inc. (Reference: Shibamoto 2006).

Many low molecular weight secondary by-products of lipid oxidation, such as the carbonyl compounds are highly reactive, water soluble, and volatile, as such their isolation and measurement is often challenging (Shibamoto 2006). Common measurement methods for lipid oxidation products are discussed in the proceeding section.

2.1.6 Measurement methods for salivary lipid oxidation

Lipid oxidation has been extensively studied using the thiobarbituric acid reactive substances (TBARS) assay, which involves derivatization of malondialdhyde with thiobarbituric acid to produce a pink product that is quantified in a UV-VIS spectrophotometer (Yagi 1998). This assay, while quick, is not always an accurate measure of MDA, since other aldehydes may react with the reagent (Figure 2.8).

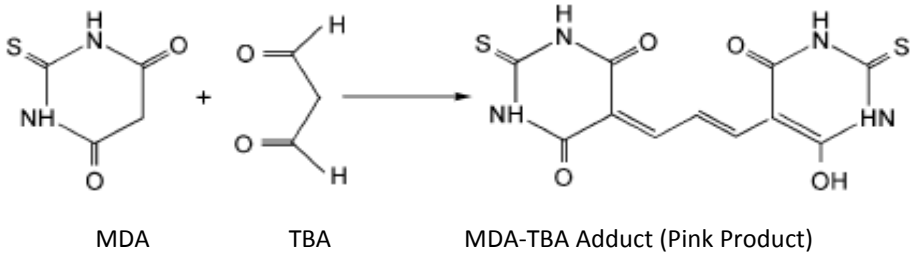

Figure 2.8. Reaction of thiobarbituric acid (TBA) with malondialdehyde. Figure used with permission from Copyrights Clearance Center, Inc. (Reference: Shibamoto 2006).

The measurement of TBARS has been a widely used method for screening and monitoring lipid peroxidation since the early 80's. The TBARS method has been used to evaluate samples that include human and animal tissues and fluids, drugs and foods. TBARS is a good indicator of oxidative stress and may be used to study the effect of nutrition on free radical formation, oxidative stress and ultimately on the health of the subjects (Yagi 1998). With more recent method developments, the use of TBARS method is not recommended for use in complex matrices, such as human plasma, since this method does not directly measure MDA

Recently, measurement of TBARS has been used to determine the lipid oxidation caused by metal salts in the oral cavity (Mirlohi et al. 2011; Ömur-Özbek 2008). In

these studies, the TBARS concentration in saliva was measured before and after treatment with a metal salt solution in eleven volunteers (who had not consumed food or beverages and had not smoked for at least thirty minutes prior to testing). Participants donated saliva five to eight times over several months. In these normal volunteers, there was a significant increase in TBARS concentration in saliva after rinsing the mouth with the ferrous iron solution. The concentration of MDA in control and metal saliva samples varied among participants but was always greater after ferrous ion treatment. This analysis demonstrated the occurrence of metal-induced lipid oxidation in the oral cavity.

While the TBARS methods continues to be utilized as a measure of lipid oxidation in food and biological systems, there are other commonly used methods of measuring the extent of lipid oxidation. For example, sensory methods in combination with instrumental analysis have been used to identify off-flavors in food matrices (Tamura et al. 2009; Frankel 1998). For instrumental analysis, low molecular weight carbonyls, such as MDA are first converted to a stable product through derivatization of carbonyls with 2,4-dinitrophenylhydrazine (DNPH). This method has been widely used due to its high reactivity and selectivity. During derivatization, the carbonyl loses its oxygen and forms a stable complex with the DNPH as shown in Figure 2.9. Once stabilized, DNPH-carbonyl product can be analyzed by high pressure liquid chromatography (HPLC) or gas chromatography-mass spectrometry (GC-MS) after being extracted with an appropriate organic solvent (Shibamoto 2006). Other derivatizing agents such as 0-(2,3,4,5,6-pentafluorobenzyl)-hydroxylamine (PFBHA) have also been used (Sugaya et al. 2001).

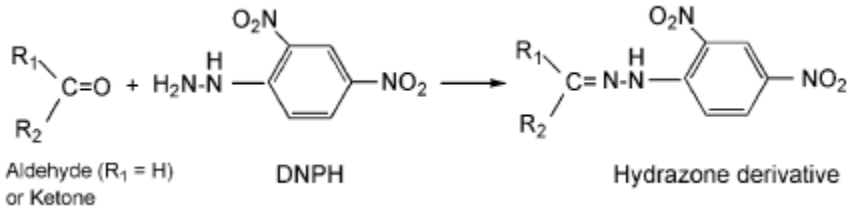

Figure 2.9. Reaction of carbonyls with DNPH to form a stable hydrazone derivative. Figure used with permission from Copyrights Clearance Center, Inc. (Reference: Shibamoto 2006).

Other commonly measured biomarkers of lipid oxidation are F_2-isoprostanes, protein carbonyls, and 4-hydroxy-2-nonenal (HNE) (Catala 2009; Moore and Roberts 1998). F_2-isoprostanes are formed through non-enzymatic reaction of free radical with membrane lipids, specifically arachidonic acid (Bevan et al. 2003; Nourooz-Zadeh 2008). Sensitive and accurate measurement using GC-MS has been established for measuring isoprostanes in human urine and plasma samples (Bevan et al. 2003; Li et al. 1999a).

In addition to lipid oxidation, protein oxidation has also been used as a measure for oxidative stress in vivo. Since oxidative stress can alter proteins as well as membrane lipids, measuring by-products of protein oxidation, such as protein carbonyls has been used in conjunction with lipid oxidation (Dotan et al. 2004). Protein carbonyls can be measured through derivatization by DNPH makes, followed by detection of the hydrazone derivative by spectroscopy or Western Blotting (Bevan et al. 2003; Dotan et al. 2004). For in-vivo studies, multiple measures of oxidative stress, such as lipid oxidation, protein oxidation, and DNA damage may need to be assessed to accurately define the level of oxidative stress (Dotan et al. 2004; Dalle-Donne et al. 2006).

2.1.7 Sensory methods in assessing taste sensitivity and smell functions

Human variation in taste perception has been recognized for more than 120 years (Hayes and Keast 2011). The first discovery on the now known genetic variation on human perception of the bitter compounds, phenylthiocarbamide and thiourea was reported in 1932 (Hayes and Keast 2011). In 2003, genetic variation in the bitter receptor gene TAS2R38 was linked to the molecular basis of differences in phenylthiocarbamide (PTC) detection thresholds (Kim et al. 2003). Since then, numerous researchers have explored the variations of human perception of taste and flavor using a variety of techniques. This brief review presents commonly used methods of assessing taste and smell functions in clinical and research applications.

A commonly used measure of variations in individual taste sensitivities is sensory threshold. Thresholds are defined as limits of sensory abilities and can be categorized

as absolute threshold, recognition threshold, the difference threshold, and the terminal threshold (Meilgaard et al 2007). The absolute or detection threshold is the lowest amount of a stimulus capable of producing a sensation, while recognition threshold is the level of stimulus at which the invoked sensation is recognized, such as recognizing a metallic flavor in water. The difference threshold, or the term just noticeable difference (JND) basically aims to fine tune the threshold by defining at what amount above or below a given stimulus would a sensation be felt. On the other hand, the terminal threshold is the threshold above, which there is no increase in the intensity of a perceived stimulus (Meilgaard et al 2007). A good threshold determination requires hundreds of samples and results do not always reproduce well. Published thresholds have been shown to vary by a factor of 100 or more (Meilgaard et al. 2007). Various data analysis and test design methods are used to deal with the high variability in threshold testing. A commonly used standardized method of human taste and/or odor threshold determination is the ascending concentration forced choice (AFC) procedure (American Standards for Testing Materials (ASTM) 1997; Lawless and Heymann 1998). The typical AFC method utilizes 3-sample presentation scenario where human subjects are presented with three taste samples, two of which are control samples and one is the taste stimulus. With samples presented in random order, the subjects are asked to identify the odd (i.e., the different) sample (ASTM 1997; Meilgaard et al 2007). A range of concentrations of the stimulus, spaced by a factor of 1.5 to 2, is often presented in an ascending order. The threshold for a given stimulus is typically confirmed when subjects consistently identify the odd sample several times in a row. A group of 25 subjects or more is recommended when estimating population thresholds for taste or odor stimuli (Lawless and Heymann 1998). In addition to the commonly used 3-sample AFC test, also called one-of-three test, a 5-sample AFC test has been used (Ömur-Özbek and Dietrich 2011; Cuppett 2006). In this test scenario, the chance for a subject to guess a correct identification is reduced from 33% (1/3) to 20% (1/5), thus improving the test performance accuracy.

Two common methods of estimating a population threshold are geometric mean and logistic regression. The geometric, rather than arithmetic mean is used in this case in

order to reduce the effect of outliers in estimating the threshold value. Individual thresholds are calculated by taking the geometric of the lowest concentration panelists can detect and the highest concentration they cannot. The lowest concentration they can detect is classified as the first of at least 3 correct choices in a row. The overall or population threshold concentration for a given stimulus is calculated as the geometric mean of the individual subjects threshold values. Logistic regression is another threshold determination analysis method. Logistic regression estimated the population threshold at the concentration of the stimulus at which 50% of subjects correctly identified. Both methods of calculation typically result in comparable estimates of population threshold (Ömur-Özbek and Dietrich 2011; Cuppett 2006).

Based on sensory principles similar to that of the ascending forced-choice procedure, other methods have been used to assess human taste and smell functions in clinical or diagnostic applications. A widely used method is a scratch-and-sniff test consisting of a booklet with 10–40 individual sheets that have microencapsulated beads impregnated with specific odors. As an example, the University of Pennsylvania Smell Identification Test (UPSIT) is widely used in clinical, research, and industrial applications (Doty 2008). In its most comprehensive variation, the odor test booklet consists of 40 different odors which subjects will smell in a given order by scratching the odor-impregnated surface by a pencil tip to release the odor, sniff the odor, make a forced-choice identification from a given selection of four answers (Doty 2008). The scratch and sniff test does not determine an odor threshold level. For that purpose, procedures similar to the above-discussed AFC sensory threshold method may be used to assess sensitivity to specific odorants. Another olfactory assessment method involves the use of an olfactometer, an instrument that allows the examiner to pass specific concentrations of a known odor to the nose of the patient; the patient would name the odor at given concentration that would be representative of their individual recognition threshold (Tsukatani et al. 2005). Other diagnostics measures of olfactory functions include physical examination of ears, nose, and throat, oral hygiene, personal history of diseases and medication, and the use of self-reported questionnaires (Hoffman et al. 2009).

Clinical methods for assessing taste functions typically involve sensitivity or threshold test using the five basic taste stimuli (sweet, sour, salty, bitter, and umami). In this method, known concentrations of a given taste stimulus are presented to patients and they are asked to sip the solution, identify the taste, and discard upon tasting (Hoffman et al. 2009; Wrobel and Leopold 2004). Alternatively, taste strips impregnated with a known concentration of a basic taste stimuli may be used; in this case the paper strip can be placed on the tongue and the patient would be asked to identify the taste substance (Hoffman et al 2009). This method has been used in assessing taste function in patients with Parkinson's disease (Kim et al. 2011).

2.2 Metallic Flavor and Aspects of Medicine

In the preceding sections, discussion of the sensory aspects of metallic flavors presented the complexities and fascinating functioning of human senses, as well as our current understanding on the mechanisms of how metallic off-flavors are produced and measured in foods, beverages, and biological systems. In the proceeding sections, we will discuss medical aspects associated with metallic flavor, namely, taste disorders, medication side-effect, disease conditions, and cancer therapies. Additionally, in this context, the role of salivary fluid in medical diagnostics will be discussed.

2.2.1 Metallic taste disorders, medications, and human disease

Taste and smell functions are critical aspects of human physiology. They provide us with the pleasures of experiencing flavors and aromas in foods and beverages and the smells of fragrances. They also protect us from the dangers of ingested or inhaled toxins. Thus, disorders of taste and smell functions can have important health implications as well as debilitating effects on our quality of life. Many factors have been associated with taste and smell loss and/or disorders, namely, normal aging, environmental exposure, nasal congestion and allergies, prescription medications, head trauma, neurological diseases such as Alzheimer's, and cancer and its treatment (Ackerman and Kasbekar 1997; Boyce and Shone 2006; Bernhardson et al. 2007; Deems et al. 1991; Doty 2009; Schiffman 1997).

Taste disorders are classified into three categories: *ageusia,* total loss of taste; *hypogeusia*, reduced ability to taste; *and dysgeusia*, distortion of taste (Schiffman 2009). Taste disorders, such as dysgeusia and hypogeusia are most common among the elderly, age 65 years or older, while ageusia is rare, but it can occur at any age (Schiffman 2009). An unpleasant or altered taste in the mouth, such as metallic, is classified as dysgeusia, and it can occur with or without intake of food or beverages (Schiffman 2009). The most common contributor to taste disorders among the elderly is medication effect (Schiffman 2009; Doty et al 2009). There are many medications that alter the ability to taste. Among these are antimicrobial, antihypertensive, and antihyperlipidemic agents, including atorvastatin (Lipitor®), losartan (Cozar®), pravastatin (Pravachol®), lisinopril (Zestril®), simvastatin (Zocor®) and terbinafine (Lamisil®) (Doty et al. 2008). In most cases, the frequency of taste disorders related to medications is relatively small as reported in 5% of patients; however, the frequency could be much higher depending on the medication (Doty et al 2009). For example, for subjects taking the sleep aid medication, eszopiclone (ESZ; Lunesta®), the frequency of bitter/metallic taste disorder was reported at 26.1% in one study that included 593 subjects (Krystal et al., 2003), while another study reported 66% and 53% taste disorders among 24 female and 15 male subjects, respectively (Doty et al. 2009). A listing of some common drugs that have been known to cause taste and/or smell disorders is summarized in Table 2.2 (Doty et al. 2009).

Numerous disease conditions are also associated with taste and smell disorders, as compiled by Naik et al and other researchers (Harris and Griffin 2003; Heald et al. 1998; Lang et al. 2006; Naik et al. 2010) is presented in Table 2.3. In some cases the onset of taste disorders can lead to further health complications as in one reported case, where loss of taste induced hypertension in a stroke patient (Czupryniak and Loba 2007). In this case, taste impairment led the patients to higher salt intake to make food more palatable, which ultimately resulted in developing hypertension. Taste and smell disorders associated with occupational exposure also merit important consideration as long-term olfactory impairment can also lead to impairment of taste functions (Landis et al. 2010). Landis and colleagues demonstrated that long-term olfactory impairment can

lead to significant decline of taste functions ($p < 0.001$), while short-term olfactory dysfunction did not influence taste functions in a group of 210 subjects whose olfactory and taste functions was assessed using Sniffin' Sticks and taste strips (Landis et al. 2010). Some occupational may involve long-term exposure to olfactory toxicants. Thus, impacts on smell and taste functions may be anticipated as a result of chronic exposure. In a 2006 review article, Gobba examined published epidemiological studies on olfactory dysfunctions associated with exposure to industrial chemicals and concluded that the frequency of disorders associated with occupational exposure is largely uncovered and most likely underestimated (Gobba 2006). Reported cases of olfactory toxicity include exposure to airborne agents in industrial setting, namely metals such as cadmium, chromium, arsenic, copper, manganese, mercury, nickel, and zinc, and numerous organic solvents (Gobba 2006).

Table 2.2. Medications that have been known to later taste ad/or smell functions. (Reference: Doty et al 2008) [1].

Drug Class	Agent
Antianxiety agents	Alprazolam, buspirone, flurazepam
Antibacterials	Ampicillin, azithromycin, ciprofloxacin, clarithromycin, enoxacin, ethambutol, metronidazole, ofloxacin, sulfamethoxazole, ticarcillin, tetracycline
Antidepressants	Amitriptyline, clomipramine, desipramine, doxepin, imipramine, nortriptyline
Antiepileptic drugs	Carbamazepine, phenytoin, topiramate
Antifungals	Griseofulvin, terbinafine
Antihistamines and decongestants	Chlorphenamine, loratadine, pseudoephedrine
Antihypertensives and cardiac medications	Acetazolamide, amiodarone, amiloride, amiodarone, bepridil, betaxolol, captopril, diltiazem, enalapril, hydrochlorothiazide, losartan, nifedipine, nisoldipine, nitroglycerin, propafenone, propranolol, spironolactone, tocainide
Anti-inflammatory agents	Auranofin, beclometasone, budesonide, olchicines, dexamethasone, flunisolide,
Antimanic drugs	fluticasone propionate, gold, penicillamine, Lithium
Antimigraine agents	Dihydroergotamine mesilate, naratriptan, rizatriptan, sumatriptan
Antineoplastics	Cisplatin, carboplatin, cyclophosphamide, doxorubicin, fluorouracil, levamisole, methotrexate, tegafur, vincristine
Antiparkinsonian agents	Anticholinergics, levodopa
Antipsychotics	Clozapine, trifluoperazine
Antiviral agents	Aciclovir, amantadine, ganciclovir, interferon, pirodavir, oseltamivir, zaicitabine
Bronchodilators	Bitolterol, pirbuterol
CNS stimulants	Amfetamine, dexamfetamine, methylphenidate
Hypnotics	Eszopiclone, zolpidem
Lipid-lowering agents	Atorvastatin, fluvastatln, lovastatin, pravastatin
Muscle relaxants	Baclofen, dantrolene
Smoking cessation aids	Nicotine
Thyroid drugs	Carbimazole, levothyroxine sodium and related compounds, propylthiouracil, thiamazole

1. Authors listing as evidenced from the Physician's desk reference [Physicians' Desk Reference. Montvale (NJ): Medical Economics Company, Inc., 2005].

Table 2.3. Medical conditions associated with taste disorders. Used with permission from Copyrights Clearance Center, Inc. (Reference: Naik et al 2010)

Condition	Details
Congenital/genetic Cleft palate	Down's syndrome, Turner's syndrome
Endocrine/metabolic	Hypoadrenalism, diabetes mellitus, hypothyroidism, pseudohypoparathyroidism
Medical procedures/therapies	Sinus surgery, radiotherapy, rhinoplasty, tonsillectomy etc.
Infections	Herpes simplex meningoencephalitis, HIV infection, upper respiratory infections, acute viral hepatitis, cirrhosis, Hansen's disease, nasal and paranasal sinus infections
Neurological	Alzheimer's disease, head trauma, multiple sclerosis, Parkinson's disease, temporal lobe, tumours, meningiomas and stroke
Occupational diseases	Exposure to acrylate, ammonia, benzene, cadmium dust etc.
Psychiatric illness	Hypochondrias, major depression, schizophrenia
Drugs	Many drugs
Organ failure	Renal failure, liver cell failure
Miscellaneous	Cystic fibrosis, giant cell arteritis, sarcoidosis

2.2.2 Taste and smell disorders and cancer therapies

Recognizing the debilitating effects of taste and smell impairments on human quality of life is not easily felt without personal experience, as for most of us, taste and smell functions are often taken for granted. Sadly, for many cancer patients, loss and/or impaired taste and smell functions are routinely encountered as so vividly described in the quotation by E.M. MacCarthy-Leventhal, a physician and a patient whom died of head and neck cancer in 1959 (MacCarthy-Leventhal 1959).

> "*What is it like to lose your sense of taste? To know that the most luscious fruit is a cinder, and its juice flavored with copper and bicarbonate, or that a Whitstable oyster is no more appetizing than a slug? If, by a might of effort, these 'cinders' are forced down with copious fluid, the consequences are acute indigestion and vomiting. The patient is not hungry anyway, and it is easier to starve*."

It is widely known that many cancer patients experience loss and/or altered taste and smell functions due to the disease itself or the effects of the chemo and radiation therapies. As early as 1970s, unpleasant changes in taste function have been reported in cancer patients (Dewys and Walters 1975); such taste alterations have been associated with occurrence of anorexia in cancer patients (DeWys 1977) as well as declining short-term and long-term impacts on their quality of life in general (de Graeff et al. 2000; Hong et al. 2009). While the occurrence of taste alterations and oral

discomfort in cancer patients are well known, the frequency, severity, and duration of these issues are not well understood (Epstein *et al.* 1999). In a 1999 survey of 100 neck and head cancer patients, dry mouth was reported as the highest symptom by 92% of the patients, followed by change in taste in 75%, and swallowing difficulties in 60% of the surveyed patients (Epstein et al 1999). Depressive mood changes were also prevalent among these patients at 50%. The frequency of the reported oral side effects was also correlated with the radiation treatment field and doses (Epstein *et al.* 1999). It has also been reported that the most prevailing taste alteration experienced by cancer patients is perception of metallic and bitter taste or aftertaste (McGowan 2008). Taste qualities also described as “nauseating” or “unpleasant” are often noted by patients (McDaniel 1998).

Metallic taste and bitter taste sensations in cancer patients have been associated with low levels of irradiation and chemotherapy using several tested chemotherapeutic drugs: cyclophosphamide, doxorubicin, 5-fluorouracil, methotrexate, and cisplatin (McDaniel 1998; Capra 2001 ; Comeau et al. 2001). Seventy-seven percent of patients treated with cisplatin alone or in combination with other chemotherapeutic agents reported metallic taste perception (Ravasco 2005). Upon treatment, release of chemotherapy drugs into the saliva fluid and interaction with taste buds can cause impairments by direct contact with taste receptor cells. Also, damage to sensory nerves such as chorda tympani can impact the olfactory system and consequently cause disturbances in taste and smell functions (Epstein and Barasch 2010). Additionally, in light of the recent research reporting concerning iron-induced lipid oxidation of human epithelial cells (Ömur-Özbek 2008; Glindemann et al. 2006), perception of metallic sensation in the mouth could also be associated with lipid oxidation of oral epithelial cells (Hovan et al. 2010). Other possible causes of unpleasant taste alterations that cancer patients experience may be associated with poor oral hygiene and/or infections within the oral cavity. It has been observed that good oral hygiene practices significantly diminish taste alteration. Others have noted that taste alteration may in part, be due to psychological causes when patients complain about taste (Comeau *et al.* 2001; Epstein 2002).

There are several types of therapies that can differently impact taste and smell functions in cancer patients. These include chemotherapy, radiation therapy, and a combined chemo and radiation therapy, typically referred to as common modality treatment (CMT). The most studied type of cancer involving taste and smell disorders is the cancer of head and neck region, since the treatments can more directly impact sensory organs. In this type of cancer, radiation treatment at 60-70 Gy for 6-8 weeks has been shown to cause taste impairment during the first week of treatment and becomes worse during the second week (Hovan et al 2010). Partial recovery of taste functions may occur 20-30 days after the radiation therapy, although in some patients, although in some patience, taste alterations may persist up to 7 years post- therapy (Hovan et al. 2010; Ripamonti and Fulfaro 1998). When chemotherapy is used alone, studies have shown varying rates of reported taste abnormalities ranging from mild to moderate (Hovan et al 2010). In three different reported studies, the incidence rates of dysgeusia in the studied groups of patients were 17%, 67%, and 100% (Beale et al. 2003; Macquart-Moulin et al. 2000; Maisano et al. 2003); in one study, altered taste functions returned to pre- treatment level after 1 year (Macquart-Moulin et al. 2000). In another study of patients with breast and gynecologic malignancies, chemosensory functions significantly decreased during chemotherapy, but recovered 3 month after therapy (Steinbach et al. 2009). In cases where CMT treatment has been utilized, the effects of cancer therapies on chemosensory functions have been varied. Some studies have reported higher incidence of taste dysfunctions among patients subjects to radiotherapy alone when compared to CMT; while other reports that severity of CMT effects of taste and smell function is influenced by the level of radiation treatment combined with the chemotherapy (Hovan et al. 2010; Öhrn et al. 2001).

As discussed, the effects of cancer therapies on taste and smell disorders and the debilitating impacts on the patients' quality of life are well known. Consequently, efforts to manage and/or treat taste disorders have also been widely examined. Taste disorder management and treatment strategies typically include intake of zinc supplementation, as zinc deficiency has been associated with taste disorder, as an enzyme cofactor, zinc is believed to have a role in human taste functions (Hovan et al. 2010; Ripamonti and

Fulfaro 1998; Öhrn et al. 2001). Clinical studies have shown that taste functions dramatically improved in cancer and dysguesia patients given oral zinc supplements in the form of zinc sulfate or zinc gluconate (Halyard et al. 2007; Heckmann et al. 2005; Ripamonti et al. 1998). Another study using a zinc compound, polaprezinc, was shown to improve taste functions in a clinical study of dysguesia patients (Sakagami et al. 2009). Another nutritional supplement, alpha lipoic acid (ALA), has been shown effective in treating taste disorders (Femiano et al. 2002). ALA has also been used in the treatment of burning mouth syndrome and neurological diseases, such as Alzheimer's (Holmquist et al. 2007; Lopez-Jornet et al. 2009; Steele et al. 2008). Aside from nutritional supplementation, other strategies for managing taste disorders include nutritional counseling, attention to oral hygiene and intake saliva fluid substitute in the case of dray mouth, food flavoring and enhancement, reduced intake of meat products that could induce metallic sensation in the mouth (Hong et al. 2009).

As reported widely in the literature, metallic and related off-flavors are the most commonly experienced taste alterations in cancer patients. Oral pain and metallic taste phantom phenomenon have been found to be significantly higher in cancer survivors than in healthy individuals. These issues have broad impacts on the quality of life for cancer patients and often lead to other implications such as reduced oral intake and nutritional deficiency, weight loss, and depression (Epstein and Barasch 2010). In head and neck cancer patients, loss of taste functions has been reported as the major cause of morbidity (Fernando *et al.* 1995). While taste and smell abnormalities are significant and have been reported in other types of cancers, including brain cancer or glioma patients, the majority of chemosensory research has been focused on head and neck cancers. Future research should aim at better understanding of the causes and persistence of taste disorders among various types of cancer patients in conjunction with reliable biomarkers to study the effects of the cancer therapies. Ultimately, development of therapy products and/or management strategies is desired to prevent or treat the debilitating taste and smell disturbances in cancer patients as well as other affected populations.

2.2.3 Role of salivary fluid in medical diagnostics

Among biological fluids used in medical diagnostics, human blood is the most commonly used (Kaufman and Lamster 2002); while other biological fluids and media have also been used in various applications. Samples of human breath condensate, urine, saliva, and lung fluid have been used in numerous diagnostic and research applications to assess biomarkers of oxidative stress associated with various disease conditions (Li et al. 1999b; Suma et al. 2010; Wood et al. 2003). Biological samples of human saliva, serum, and hair have also been used to assess environmental and/or occupational exposure (Barbosa et al. 2006; Gil et al. 2011).

Due to the ease and non-invasive method of collections, the use of saliva in medical diagnostics and research application is increasingly being recognized (Pfaffe et al. 2011). Many systematic diseases affect salivary gland functions and consequently influence the production of saliva as well its constituents; therefore, use of biomarkers in salivary fluid can serve as biomarkers for early detection of diseases (Kaufman and Lamster 2002). For example, in cystic fibrosis (CF), a hereditary chronic lung disease, the saliva of patients is known to have higher calcium and protein contents than healthy subjects (Livnat et al. 2010; Mangos and Donnelly 1981); likewise, the lipid compositions of CF patients' saliva has been shown to be different from that of healthy individuals (Slomiany et al. 1983). Sjögren's syndrome (SS), an autoimmune disease with an estimated prevalence of more than 1 million in the United States also leads to reduction in salivary secretions (Kaufman and Lamster 2002). Some studies showed that patients with SS had higher levels of salivary IgA and IgB, latoferrin, albumin, and sodium (Benaryeh et al. 1983; Tishler et al. 1997). Salivary fluid has also been used in diagnosis of viral and infectious diseases, such as HIV and infections of the oral cavity. Other diagnostic applications include drug monitoring and hormonal levels monitoring (Kaufman and Lamster 2002). Salivary analysis have also been used for early detection of malignancies; such diagnosis is associated with the detection of a elevated levels of inactivated tumor suppressing protein in saliva as well as serum of cancer patients (Kaufman and Lamster 2002; Tavassoli et al. 1998). In brain cancer patients, salivary parameters, such as flow, pH, osmolality, and antioxidant activity were found to

be significantly reduced compared to healthy subjects, while protein-thiols were significantly increased (Suma et al. 2010). In women diagnosed with breast cancer, higher level of a cancer antigen was found in their saliva compared to healthy subject; additionally, a tumor marker was present in their saliva, but absent in the saliva of healthy subjects (Farnaud et al. 2010; Streckfus et al. 2000). Salivary biomarkers have also been extensively used in the study of dental caries. For example, reduced salivary pH and buffering capacity has been associated with higher incidence of dental caries (Tenovuo 1989). Reduced levels of salivary mucins, have also been associated with higher incidences of dental problems, such as decaying, missing, or filled teeth in healthy adults (Banderas-Tarabay et al. 2002); other salivary proteins, such as statherin and cystatin have also been used as biomarkers for the risks of developing teeth caries and other oral diseases (Rudney et al. 2009).

A Review of the literature on diagnostic potentials of saliva suggests many promising applications as this biological fluid has often been referred to as “mirror of the body” (Farnaud et al. 2010; Lee et al. 2009; Segal and Wong 2008). Prominent researchers in the field of salivary diagnostics compare the potentials of salivary fluid as a rich source of biomarkers of human conditions and diseases, powered by technological application comparable to scientific breakthroughs such as the discovery of antibiotics and invention of anesthesia in the field of medicine (Segal and Wong 2008). Among the challenges associated with the use of saliva as a diagnostic fluid, is the low level concentration of biomarkers as compared to human serum, as such more sensitive instrumentation and quantitative techniques are required to identify specific biomarkers (Segal and Wang 2008). However, many technological progresses have been made, namely, development of microfluidics and microelectromechanical systems (MEMS) for saliva diagnostics and sensitive measurement of salivary constituents, salivary proteomics and genomics aimed at identifying the numerous salivary proteins mRNA expressions as biomarkers of human diseases as well as in developmental indicators in newborn infants (Segal and Wong 2008; Maron 2011). Finally, the use of nanotechnology in the study of salivary fluid offers many potential applications as indicated by the intriguing discovery of unique nanosized cellular components, secreted

by salivary glands and called “exosomes” as biomarkers of cancer and other diseases (Sharma et al. 2011; Simpson et al. 2008).

2.3 Flavor Producing Metals in Drinking Water and Aspects of Health and Environment

Presence of flavor producing metals in drinking water can impact the aesthetic qualities, and palatability of drinking water. The perception qualities can in turn influence water consumption. This review exclusively pertains to the metallic flavor of water associated with iron, copper, and zinc, although major electrolytes, such as calcium and magnesium add flavor to water, but they are not characterized as metallic. The most common culprits of metallic flavor in drinking water are iron, copper, and zinc; they are also considered to be essential nutrients in human diet (WHO 2011) as well as potentially toxic at excessive levels (USEPA 2009). Iron has the strongest metallic flavor, while copper and zinc have strong bitterness and astringency attributes in addition to their metallic flavors (Ömur-Özbek and Dietrich 2011; Zacarias et al. 2001; Cuppett 2006; Hong and Kim 2011; RSJ Keast 2003). In the proceeding sections, we will discuss the chemistry, health, and environmental aspects of these flavor metals in drinking water.

2.3.1 Chemistry and occurrence of flavor metals in drinking water

Iron

Iron is the sixth most abundant element in the universe and the most abundant metal. In the earth’s crust, iron is the second most common metal after aluminum (Silver 1993). In the nature, iron is typically found in mineral ores as its oxide forms, magnetite and hematite, or bound to sulfur (pyrites) and carbonate, as siderite (Silver 1993). In anaerobic groundwater, iron is mainly present in its ferrous, Fe(II), form. Total iron concentrations in groundwater can range from 0.5 – 10 mg/L, although concentrations as high as 50 mg/L have been reported depending on geographical region (WHO 2011). The median concentration of iron in surface waters has been reported to be 0.7 mg/L (WHO 2011). Iron complexes and solid forms that can occur in aqueous systems include hydroxide complexes and solid substances as listed in Table 2.4 (Freeze and Cherry 1979). Additionally, in the presence of common electrolytes, other complexes

can be formed (Freeze and Cherry 1979; Tang 2010). The dominance of various species in solution is pH dependent. For example, with a solubility constant of 5×10^{-15}, $Fe(OH)_2$ and a solution pH of 7.0, the concentration of ferrous species in pure water is about 0.5 mole/L or 28 g/L Fe(II) (Sawyer et al. 1994).

Table 2.4. Speciation of common flavor metals in aqueous systems.

Soluble Metal Complexes	Solid Substances / Precipitates
Iron	
$Fe(OH)^{2+}$	Fe(0)
$Fe(OH)^{2+}$	FeO, $Fe(OH)_2$, $FeCO_3$, $Fe_3(PO_4)_2$
$HFeO_2^-$	Fe_3O_4, Fe_2O_3, $Fe(OH)_3$, FeOOH, $FeSO_4$
Copper	
$Cu(OH)^+$	$[Cu_2(OH)_2(CO_3)]$, $CuSO_4$, CuO
$Cu(OH)_3^-$, $Cu(OH)_4^{2-}$	$Cu(OH)_2$, $CuCO_3$, $Cu_3(PO_4)_2$
Zinc	
$ZnOH^+$, $Zn(OH)_3^-$, $Zn(OH)_4^{2-}$	$Zn_3(PO_4)_2$, $ZnHPO_4$, $ZnSO_4$, ZnO

In most drinking water systems, the concentration of iron is typically below 0.3 mg/L, but this level could be exceeded in cases where various iron salts are used as coagulating agents in water-treatment plants and where cast iron, steel, and galvanized iron pipes are used for water distribution (WHO 2011).

Copper

Like iron, copper is an important element to humans, both in terms of its overall abundance on earth, as well as its role in industrial applications, manufacturing, annual consumption, and a nutrient source necessary for life. For these reasons, copper has been ranked as 7th in terms of its overall importance to humans (Silver 1993). Copper is found is surface water, groundwater, seawater and drinking water (WHO 2011). In aqueous environmnet, copper exists as free as well as complexes of ionic and/or particulate forms (Table 2.4). In pure water, copper exists in its free ionic form, Cu(II) at pH levels below 6, while at higher pH levels (6.5 to 12), it precipitates in its complexed form of cupric hydroxide. In surface and well waters and in the absence of pollution from run-off, copper concentrations are relatively low (typically below 1 mg/L), while in drinking water distribution systems, copper concentrations can vary widely (WHO 2011). The wide variations is typically due to water characteristics, such as pH, hardness and copper availability in the distribution system (WHO 2011; ASTDR 2004). As reported in

the WHO guidance document, results from numerous studies from Europe, Canada and the USA indicate that copper levels in drinking-water can range from ≤0.005 to >30 mg/L with the main source often being the corrosion of interior copper plumbing (ASTDR 2004; WHO 2011). In a most recent study, elevated levels of copper in tap water, associated high alkalinity could be controlled through the use of the anti-corrosive agent, orthophosphates (Grace et al. 2012).

Zinc

Zinc and its compounds are found in the earth's crust and are present in most rocks, minerals, and sediments; therefore, soluble compounds of zinc can be released to natural waters due to weathering of these materials (ASTDR 2005). Like iron and copper, in water zinc can be present in its free ionic form or form complexes with hydroxide or other anions depending on the pH and presence or absence of other electrolytes (Table 2.4) (Sawyer et al 1993). In drinking water systems, zinc is used in the production of corrosion-resistant alloys and brass, and for galvanizing steel and iron Products (WHO 2011). In natural water systems, the concentration of zinc is typically below 0.01 mg/L (WHO 2011), while in drinking water distribution systems, considerably higher levels could be present due to selective corrosion of brass, consisting of copper-zinc alloy (Sarver et al. 2010). In galvanized pipe plumbing systems zinc concentrations have been reported to range from 0.13–1.28 mg/L; these levels were >10 times higher than those in homes with copper pipe plumbing systems (ASTDR 2005; Sharrett et al. 1982)

2.3.2 Implications on health and water consumption

Characterization of flavor metals in drinking water is a multifaceted issue. Flavor producing metals, namely iron, copper, and zinc are essential nutrients, can be toxic, and are known to produce unpleasant tastes and flavor sensations in water as well other food and/or beverage media. On the other hand, their occurrence in drinking water can add nutritional quality to water as a healthy beverage resource. Therefore understanding of the health implications of flavor producing metals as well their aesthetic related impacts on water consumption merits consideration.

Health and nutritional aspects

Among the three discussed flavor-producing metals, only copper is currently being regulated in the United States as a health based standard in drinking water, while zinc and iron are considered to be secondary standards, (guidelines by recommendation rather than regulatory requirement) based on their aesthetic, not health based impacts on the quality of drinking water. As compared, drinking water regulatory guidelines for these flavor-producing metals is relatively uniform in USA and many European countries (Table 2.5) (WHO 2011; Claes et al. 1997).

Table 2.5. Drinking water quality guidelines for iron, copper, and zinc in USA and Europe.

Metal (mg/L)	WHO	EU	UK	Germany	Belgium	Austria	Switzerland	Poland	Russia	USA
Fe	0.3	0.2	0.2	0.2	0.2	0.1	0.3	0.5	0.3	0.3
Cu	2	3	3	3	2	0.1	1.5	0.5	1	1 (1.3)*
Zn	5	5	5	5	5	3	5	5	3	5

***** In the USA, there are two standards for copper: health-based at 1.3 mg/L and aesthetic based at 1 mg/L.

Excessive intake of metals, such as copper (as well as iron), through drinking water can be particularly harmful as inorganic copper can directly contribute to the free copper pool in the blood by partially bypassing the liver (Brewer 2010a). Likewise, intake of copper from nutritional supplements can be harmful as copper deficiency is typically rare; multivitamin supplements typically contain inorganic copper that can directly contribute to the free copper pool in the blood that can lead to toxicity (Brewer 2010). Copper uptake is inhibited by zinc; as such, zinc therapy is often used to treat copper overload disease, called Wilson disease (Brewer et al. 1998). According to the Institute of Medicine Food and Nutrition Board, the median intake of copper from food in the United States is approximately 1.0 to 1.6 mg/day for adult men and women. The Tolerable Upper Intake Level (UL) for adults is 10 mg/day, which is based on protection from liver damage as the critical adverse effect (IOM 2011). While, the current recommended daily intake of dietary copper for adults (Table 2.6) is 0.9 mg/day (IOM 2011). It is interesting to note that this level is less than the health-based standard for copper in drinking water (USEPA 2009).

Iron and zinc are also considered as essential elements in human diet as vital nutrients necessary for bodily functions; as such, there are dietary recommendations for their regular intake (Table 2.6). The median dietary intake of iron is approximately 16 to 18 mg/day for men and 12 mg/day for women (IOM 2011). The Tolerable Upper Intake Level (UL) for adults is 45 mg/day of iron, based on gastrointestinal distress as an adverse health effect (IOM 2011). For zinc, the median intake from food in the United States was approximately 9 mg/day for women and 14 mg/day for men, while the UL for adults is 40 mg/day, a value based on reduction in erythrocyte copper-zinc superoxide dismutase activity (IOM 2011). The absorption of iron and zinc by the body is influenced by the type of food that is taken thus making them more or less bioavailable upon intake. For example, iron from meat sources is absorbed by the body more efficiently than iron from vegetable source (IOM 2011).

Table 2.6. Recommended daily intake levels for iron, copper, and zinc.

Group	Age (years)	Intake (mg/day)		
		Iron	Copper	Zinc
Infants/children	0-6 month	0.27	0.27	2
	7-12 month	11	0.22	3
	1-3	7	0.34	3
	4-8	10	0.44	5
Females	9-13	8	0.7	8
	14-18	15	0.89	9
	19-50	18	0.9	8
	> 50	8	0.9	8
Males	9-13	8	0.7	8
	14-18	11	0.89	11
	19-70	8	0.9	11
	> 70	8	0.9	11
Pregnant	≤ 18	27	1	12
	19-50	27	1	11
Lactating	≤ 18	10	1.3	13
	19-50	9	1.3	12

Source: Food and Nutrition Board, Institute of Medicine–National Academy of Sciences

With an estimated 1.6 billion people affected globally, anemia is the most widely occurring nutrient deficiency worldwide, with highest prevalence among pregnant women and school aged children (WHO 2008). For this reasons, toxicity concerns are not widely recognized except in the cases of genetic iron overload disease, hemochromatosis (HH), highly prevalent in Western countries (Halliwell and Gutteridge

1990). Consequently, the prevalence of anemia is among the lowest in Western countries, such as Europe and USA (WHO 2008). In recognition of these facts, in some cases excessive exposure to iron through fortification of food products, beverages, and supplement intake has been linked to risks of numerous diseases associated with iron toxicity, such as heart disease, cancer, and Alzheimer's (Brewer 2010; Schumann et al. 2007).

Drinking water has been regarded as a minor source of nutrients for elements such as iron and zinc, while copper is considered as a significant nutrient source (WHO 2008; Deveau 2010). In addition to drinking, intake of other beverages can significantly contribute to the overall intake of metals from beverage sources. In a recent study, total concentrations of iron in variety of fruit juices ranged from 0.5 to 10 mg/L, total copper ranged from 0.02 to 0.3 mg/L, and total zinc ranged from 0.05 to 6 mg/L (Tormen et al. 2011). In alcoholic beverages, metal concentration can vary widely. One study that reviewed the literature on various types of alcoholic drinks reported that total iron levels ranged from 0.01 to 25 mg/L, total copper ranged from 0.04 to 15 mg/L, and total zinc levels ranged f from 0.1 to 68 mg/L (Ibanez et al. 2008). In most cases, the higher metals levels were associated with wine and Scotch whisky. The wide variations in metals contents of alcoholic beverages have been associated with the crop source, region, and beverage production stages (Ibanez et al 2008).

Implications on water consumption

The issue of water consumption among human populations is important in many aspects, in terms of both environmental sustainability as well as global health concerns. In addition to the global concerns for safety and sustainability, water consumption is relevant to public health as it has been promoted as an obesity control measure to replace calorie containing beverages. Numerous studies have linked obesity to the increased intake of sugar-sweetened beverages (SBB) (Bleich et al. 2009; Daniels and Popkin 2010; Davy et al. 2008; Dennis et al. 2009). Additionally, a large number of studies have investigated the role of water consumption on the total energy intake and its ultimate effect as a weight loss/control measure in the human population (Daniels and Popkin 2010; Davy et al. 2008; Dennis et al. 2009; Dubnov-Raz et al. 2011).

Epidemiological studies conducted by the US National Health and Nutrition Examination Survey (NHANES) have indicated a positive correlation between total energy intake and consumption of beverages other than water (IOM 2004). Other studies have reported reduced caloric intake when water replaced other beverages (Kant et al. 2009; Popkin et al. 2005; Stookey et al. 2007). According to the NHANES, in the period of 2005-2006, American adults consumed 3.18 L of total water in a given day, with plain water and beverages representing 33% and 48% of the total beverage intake, respectively (Kant et al. 2009). The United States Institute of Medicine (IOM), Food and Nutrition Board recommends an Adequate Intake (AI) of daily total water in healthy adult males and females at 3.7 and 2.7 liters, respectively. In spite of this recommendation, the amount of water consumed in a day varies considerably among different age groups. For example, for the most recent period of 2005 – 2006, U.S. adults in age groups 19 - 39 and 40 – 59 consumed 1.1 liters of plain water per day, while those greater than age 60 consumed 0.8 liters. Likewise, the daily total beverage intake declined in the elderly (i.e., age > 60) to 2.1 L compared to 2.7 L and 2.9 L for the 19 – 39 and 40 – 59 age groups, respectively (Popkin 2010).

With regard to health, intake of adequate fluid and/ water is vital for the maintenance of human body functions, such as cellular metabolism, regulation of body heat, and maintaining the osmolarity of various bodily fluids (Jequier and Constant 2010; Popkin et al. 2010). Dehydration has been associated with increased risk of developing kidney stones, urinary tract infections and bladder cancer, while mild day-to-day dehydration has been associated with fatigue and impaired cognitive performance (Popkin et al. 2010; Ritz and Berrut 2005). Elderly and infants are typically at greater risk of dehydration (IOM 2004; Jequier and Constant 2010). A reason for this increased risk of dehydration is a higher surface-to-body-weight ratio in infants as compared to adults (IOM 2004; Jequier and Constant 2010). While in the elderly, diminished sensation of thirst as well as behavioral reasons such as fear of incontinence and/or disabling conditions associated with aging can contribute to dehydration risk. Age-associated decline in taste functions as well as swallowing disorders have also been recognized to contribute to dehydration risks among the elderly (Asai 2004; Bratlund et al. 2010).

Mouth dryness has also been shown to influence drinking water consumption and beverage acceptability in elderly adults, resulting in voluntary dehydration and preference for cold and/or acidic thirst quenching beverages (Brunstrom 2002).

Since water consumption is so vital to bodily functions and plain drinking water is being increasingly encouraged as a healthy beverage in place of calorie containing beverages, factors that influence human consumption of drinking water should be taken into consideration when examining consumers' behavior. These factors may include consumer preference for a certain taste or flavor, such as tap, bottled, well, or mineral water as well as the availability of a safe and clean source of water. Consumers' choice of drinking water may also be driven by their perception of risks associated with a given water source (Doria 2010; Doria et al. 2009). For example, in public water systems, when water utilities experience problems with a certain contaminant of concern, consumers may resort to drinking alternative source, such as bottled and/or filtered water due to safety concerns. In fact, this was shown to be the case in a study on Canadian consumers (Dupont et al. 2010). In a recent survey of local household water quality in Virginia localities, 11% of the 47 households reported concerns about unpleasant taste in their waters with the top two taste complaints being metallic and sulfur (Benham et al. 2010); however, whether these taste quality concerns impacted the household drinking water consumption was not reported. As presented in earlier discussions, taste or flavor thresholds vary widely among individuals for iron and copper and less known for zinc; however, in most cases, the aesthetic based standards of water quality provide protection against unpleasant taste sensation.

2.3.3 Nanoparticles of flavor metals and environmental exposure potentials

Environmental Nanoparticles (NPs), sized 1 to 100 nanometers, are classified as naturally occurring or engineered; naturally occurring NPs are ubiquitous in nature and exist in all environmental media: air, water, and the subsurface (Wigginton et al. 2007). On the other hand, engineered NPs are intentionally produced for various applications in science, technology, medicine, industries, and daily consumer products (Sahu and Casciano 2009). In this review segment, we will discuss environmental aspects of metal NPs of iron, copper, and zinc, as these metals have been the subject of our

discussion with regard to their flavor producing properties and toxicity potentials and nutritional benefits to humans.

Due to their microscopic sizes, the physical and chemical properties of NPs are often much different from that of larger particles of the same material, as such their characterization is essential in assessing environmental and toxicity impacts. In nanoparticle characterization, properties such as particle size, shape, surface area, and dispersion properties are often measured in toxicological studies (Sahu and Casciano 2009). In exposure studies, nanoparticle size is an important factor, as it would govern particle transport and deposition within the body as well as within the environment (Wiggington et al 2007; Sahu and Casciano 2009). Particle size as well as properties, such as surface area and shape also influence the reactivity of metals NPs, thus determining their potentials for selective uses in treatment technologies as well as their toxicity implications (Sahu and Casciano 2009; Karlsson et al. 2009). In addition to surface properties, particle aggregation has also been shown to affect the reactivity of metal NPs as in the study of hematite reactivity towards carbon tetrachloride by decreasing with particle aggregation (Vikesland et al. 2007).

Among classes of naturally occurring NPs, oxides of iron have been the most studied in environmental research. Metal NPs of iron exist in minerals as iron oxide forms, goethite and hematite goethite, akaganeite, ferrihydrite and schwertmannite as important constituents of soils, sediments and mines drainage outflows (Wigginton et al. 2007). Unlike iron oxides, elemental iron, in its reduced zerovalent form does not exist stably in the aquatic environment, as it is highly reactive and gets readily oxidized in the presence of oxygen (Keenan et al. 2009); however, stabilized forms of zerovalent iron nanoparticles (nZVI), have been manufactured as engineered NPs for use in numerous environmental applications. These include removal of toxic contaminanats such as nitrate, perchlorates, arsenic, hexavalent chromium, uranium, and antibiotics from drinking water, and the use of magnetic iron oxides for targeted drug delivery and food fortification (Cao et al. 2005; Fang et al. 2011; Ghauch et al. 2009; Hilty et al. 2010; Tanboonchuy et al. 2011).

Few studies have investigated toxicity impacts of iron nanoparticles in the aquatic environment due to the ubiquitous nature of iron in the form of oxides as well as the limited mobility of iron nanoparticles once oxidized to form ferrous, ferric, and iron oxides. Although based on these characteristics, exposure potential to humans and higher organisms is believed to be unlikely, toxicity concerns are increasing as more engineered nanoparticles are being developed to enhance the mobility of nZVI through the use of particle stabilizing agents, such as surfactants, polymers, and polyelectrolytes. Additionally, the highly reductive property of nZVI that makes it so appealing in terms of removal of toxic contaminants, has been recognized as a mean by which surface and/or ground water contaminants can be effectively captured on iron oxide surfaces and transported through drinking water systems (Waychunas et al. 2005; Hochella et al. 2008). For example, nanoscale iron oxides have been found bounded to copper in surface waters many kilometers downstream from mining sites (Hochella et al. 2005; Plathe 2010). In a drinking water system, a sample of 20 nm particles was found to contain lead and iron, the iron was tentatively identified as its oxide form, hematite (Wigginton et al. 2007; Zhao et al. 2011).

Another recent study on copper release in water distribution systems demonstrated that flow of water within distribution systems can result in mechanical release of 0.05 to 0.2 nm copper particles attached to corroded pipe surfaces (Vargas et al. 2010). Nanosized copper particles are considered to be more toxic than micro size particles, especially in vitro as demonstrated by in vitro toxicity studies with mice (Meng et al. 2007; Chen et al. 2006). These studies demonstrated that copper nanoparticles of 24 nm in size were highly toxic to mice with affected organs being kidney, liver, and spleen on kidney. In contrast, the 17 micron copper particles were classified as non-toxic (Chen et al 2005). In a follow-up study, Meng et al showed that the acute toxicity of copper nanoparticles corresponded with higher levels of cupric ions present in kidney of mice treated with nano copper than those of micro copper particles (Meng et al 2006). In the presence of gastric juice, nano copper particles were shown to be highly reactive, consuming more hydrogen ions and forming cupric ion, and thus increasing the pH from 1.7 to 6.0 within 100 minutes after exposure, while in the case of micro copper particles,

the cupric ion concentration was significantly less and the pH change was insignificant (Meng et al 2006). Other researcher have hypothesized that such toxicity studies demonstrate that upon ingestion, smaller copper nanoparticles (< 0.1 nm) would likely pass through the oral fluid to reach the digestive systems (Vargas et al 2010) as opposed to the findings of Hong et al which indicated that soluble copper binds to salivary proteins and retained in the mouth (Hong et al. 2010).

Like iron and copper, zinc nanoparticles are also persistent in the aquatic environment, typically bounded in sulfide, oxide, and other mineral forms as well as interacting with natural humic substances (Wigginton et al. 2007; Lau and Hsu-Kim 2008). Additionally, other environmental nanoparticles are known to exhibit high sorption tendencies for zinc, namely iron oxides and sulfides (Wiggington et al 2007). While the dissolution of zinc and other metal nanoparticles in the aquatic environment is dependent on water chemistry and solubility of complexed metal hydroxides, for nanoparticles, size and surface area are also important factors (Auffan et al. 2009).

With regard to nutrition and sensory properties, iron and zinc nanoparticles have been recognized for their promising applications as food fortifying agents (Hilty et al. 2010). Concerning human nutrition, iron and zin deficiency are global problems, affecting nearly 2 million people worldwide, mostly in developing countries and among children and pregnant women (WHO 2008; Miller 2010). In this regard, fortification of food products with iron oxide nanoparticles is believed to increase their bioavailability and absorption efficiency under digestive conditions, while at the same time exhibiting oxidative stress comparable to that of ferrous sulfate salt (Zimmermann and Hilty 2011). Additionally, the use of iron oxide nanoparticles enhances the sensory properties by inherently lighter color and low solubility at neutral pH, thus minimizing the potential for altered coloration of food as often seen when using inorganic iron salts (Zimmerman and Hilty 2011).

This literature review highlighted occurrence of iron, copper, and zinc nanoparticles in the aquatic environment and oral exposure potentials; however, human exposure potential through inhalation cannot be underestimated. Inhalation can also be an

important route of exposure through intake of aerosols and atmospheric dust (Keenan et al. 2009; Guo et al. 2009; Shi et al. 2011). Nanoparticles of zerovalent iron have exhibited high toxicity to human bronchial epithelial cells (Keenan et al 2009). Metal nanoparticles and are believed to play an important role in neurodegenerative diseases due to their efficient abilities to reach the brain by way of the olfactory epithelium and cause damage to proteins responsible for regulating brain functions, such as in Alzheimer's disease (Bondy 2011).

2.4 References

Ackerman BH, Kasbekar N. **1997**. Disturbances of taste and smell induced by drugs. Pharmacotherapy 17(3): 482-496.

Actis AB, Perovic NR, Defago D, Beccacece C, Eynard AR. **2005**. Fatty acid profile of human saliva: a possible indicator of dietary fat intake. Archives of Oral Biology 50(1): 1-6.

American Standards for Testing Materials (ASTM). **1997**. Standard Practice for Determination of Odor and Taste Thresholds by a Forced Choice Ascending Concentration series Method of Limits. E-679-79. (Annual Book of Standards). Philadelphia, PA:American Society for Testing of Materials (ASTM).

Armstrong D, Browne R. **1994**. The analysis of free radicals, lipid peroxides, antioxidant enzymes and compounds to oxidative stress as applied to the clinical chemistry laboratory. Free Rad Diag Med 366.

Asai JL. **2004**. Nutrition and the geriatric rehabilitation patient - Challenges and solutions. Top Geriatr Rehabil 20(1): 34-45.

ASTDR. **2004**. Toxicological Profile for Copper. U.S. Department of Health and Human Services-Public Health Service-Agency for Toxic Substances and Disease Registry (ASTDR). Atlanta, GA.

ASTDR. **2005**. Toxicological Profile for Zinc. U.S. Department of Health and Human Services-Public Health Service-Agency for Toxic Substances and Disease Registry (ASTDR). Atlanta, GA.

Auffan M, Rose J, Wiesner M, Bottero J. **2009**. Chemical stability of metallic nanoparticles: A parameter controlling their potential cellular toxicity in vitro. Environmental Pollution 157 (2009) 1127–1133(157): 1127-1133.

Banderas-Tarabay JA, Zacarias-D'Oleire IG, Garduno-Estrada R, Aceves-Luna E, Gonzalez-Begne M. **2002**. Electrophoretic analysis of whole saliva and prevalence of dental caries. A study in Mexican dental students. Arch Med Res 33(5): 499-505.

Barbosa F, Rodrigues M, Buzalaf M, Krug F, Gerlach R, Tanus-Santos J. **2006**. Evaluation of the use of salivary lead levels as a surrogate of blood lead or plasma lead levels in lead exposed subjects Arch Toxicol 80(10): 633-637.

Beale P, Judson I, O'Donnell A, Trigo J, Rees C, Raynaud F, et al. **2003**. A phase I clinical and pharmacological study of cis-diamminedichloro (2-methylpuridine) platinum II (AMD473). British Journal of Cancer 88: 1128-1134.

Behuliak M, Pálffy R, Gardlík R, Hodosy J, Halcák L, Celec P. **2009**. Variability of thiobarbituric acid reacting substances in saliva. Dis Markers 26(2): 49-53.

Behuliak M, Palffy R, Gardlik R, Hodosy J, Halcak L, Celec P. **2009**. Variability of thiobarbituric acid reacting substances in saliva. Disease Markers 26(2): 49-53.

Benaryeh H, Szargel R, Gutman D. **1983**. Salivary IgA in Sjorgen Patients. International Journal of Oral Surgery 12(2): 120-123.

Benham B, Ling EJ, Scott JP, Lutz J, Thompson J, Thompson J. **2010**. Evaluation of Household Water Quality in Fluvanna and Louisa Counties, Virginia. October 2010, Virginia Household Water Quality Program. Virginia Cooperative Extension.

Bernhardson B, Tishelman C, Rutqvist L. **2007**. Self-reported taste and smell changes during cancer chemotherapy. Support Care Cancer DOI 10.1007/s00520-007-0319-7.

Bevan J, Durand F, Hickenbotham P, Kitas G, Patel P, Podmore I, et al. **2003**. Validation of a novel ELISA for measurement of MDA-LDL in human plasma. Free Radical Biology & Medicine 35(5): 517-527.

Bleich SN, Wang YC, Wang Y, Gortmaker SL. **2009**. Increasing consumption of sugar-sweetened beverages among US adults: 1988-1994 to 1999-2004. Am J Clin Nutr 89(1): 372-381.

Bondy SC. **2011**. Nanoparticles and Colloids as Contributing Factors in Neurodegenerative Disease. Int J Environ Res Public Health 8(6): 2200-2211.

Boyce J M SGR. **2006**. Effects of aging on smell and taste. Postgraduate Medical Journal 82: 3.

Boyce JM, Shone GR. **2006**. Effects of ageing on smell and taste. Postgraduate Medical Journal 82(966): 239-241.

Bratlund CV, O'Donoghue CR, Rocchiccioli JT. **2010**. Dehydration and Dysphagia: Challenges in the Older Adult. J Med Speech-Lang Pathol 18(3): 1-10.

Breslin PAS, Spector AC. **2008**. Mammalian taste perception. Curr Biol 18(4): R148-R155.

Brewer GJ, Dick RD, Johnson VD, Branberg JA, Kluin KJ, Fink JK. **1998**. The treatment of Wilson's disease with zinc: XV Long term follow-up studies. Journal of Laboratory and Clinical Medicine(132): 264-278.

Brewer GJ. **2010**. Copper toxicity in the general population. Clin Neurophysiol 121(4): 459-460.

Brunstrom JM. **2002**. Effects of mouth dryness on drinking behavior and beverage acceptability. Physiology & Behavior 76(3): 423-429.

Cabras T, Pisano E, Boi R, Olianas A, Manconi B, Inzitari R, et al. **2009**. Age-Dependent Modifications of the Human Salivary Secretory Protein Complex. J Proteome Res 8(8): 4126-4134.

Calderon-Garciduenas L, Franco-Lira M, Henriquez-Roldan C, Osnaya N, Gonzalez-Maciel A, Reynoso-Robles R, et al. **2010**. Urban air pollution: Influences on olfactory function and pathology in exposed children and young adults. Exp Toxicol Pathol 62(1): 91-102.

Cao JS, Elliott D, Zhang WX. **2005**. Perchlorate reduction by nanoscale iron particles. J Nanopart Res 7(4-5): 499-506.

Capra S, Ferguson M, Ried K. **2001** Cancer: impact of nutrition intervention outcome - nutrition issues for patients. Nutrition 17: 769-772.

Catala A. **2009**. Lipid peroxidation of membrane phospholipids generates hydroxy-alkenals and oxidized phospholipids active in physiological and/or pathological conditions. Chemistry and Physics of Lipids 157(1): 1-11.

Chaudhari N, Roper SD. **2010**. The cell biology of taste. J Cell Biol 190(3): 285-296.

Chen Z, Menga H, Xing G, Chena C, Zhaoa Y, Jia G, et al. **2006**. Acute toxicological effects of copper nanoparticles in vivo. Toxicology Letters 163: 109-120.

Claes M, Bagar-Povse M, Drinovec J, Holynska B, Kump P, Ostachowicz B, et al. **1997**. Inventory of regulations and analysis techniques for trace elements in some water supply companies in Eastern and Western Europe. Sci Total Environ 207(2-3): 141-148.

Cohen J. **1960**. Taste threshold concentrations of metals in drinking water. Journal of American Water Works Association 52(5): 10.

Cohen JM, Kamphake LJ, Harris EK, Woodward RL. **1960**. Taste Threshold Concentrations of Metals in Drinking Water. Journal Of The American Water Works Association 52(5): 660-670.

Comeau TB, Epstein JB, Migas C. **2001**. Taste and smell dysfunction in patients receiving chemotherapy: a review of current knowledge. Support Care Cancer 9(8): 575-580.

Cuppett JD, Duncan S, Dietrich AM. **2006**. Evaluation of copper speciation and water quality factors that affect aqueous copper tasting response.: submitted to Chemical Senses, also 2005. J.D. Cuppett Master's thesis, Virginia Tech.

Cuppett JD. **2006**. Evaluation of copper speciation and water quality factors that affect aqueous copper tasting response. Chemical Senses(31): 8

Czupryniak L, Loba J. **2007**. Loss of taste-induced hypertension - caveat for taste modulation as a therapeutic option in obesity. Eat Weight Disord-Stud Anorex 12(1): E11-E13.

Dalle-Donne I, Rossi R, Colombo R, Giustarini D, Milzani A. **2006**. Biomarkers of oxidative damage in human disease. Clin Chem 52(4): 601-623.

Daniels MC, Popkin BM. **2010**. Impact of water intake on energy intake and weight status: a systematic review. Nutr Rev 68(9): 505-521.

Davy BM, Dennis EA, Dengo AL, Wilson KL, Davy KP. **2008**. Water consumption reduces energy intake at a breakfast meal in obese older adults. J Am Diet Assoc 108(7): 1236-1239.

de Graeff A, de Leeuw JRJ, Ros WJG, Hordijk GJ, Blijham GH, Winnubst JAM. **2000**. Long-term quality of life of patients with head and neck cancer. Laryngoscope 110(1): 98-106.

Deems DA, Doty RL, Settle G, Mooregillon V, Shaman P, Mester AF, et al. **1991**. Smell and Tatse Disorders, A Study of 750 Patients from the University-of-Pennsylvania Smell and Taste Center. Arch Otolaryngol Head Neck Surg 117(5): 519-528.

Deibler K, Delwiche J, eds. **2004**. Handbook of flavor characterization: sensory analysis, chemistry, and physiology.

Dennis EA, Flack KD, Davy BM. **2009**. Beverage consumption and adult weight management: A review. Eat Behav 10(4): 237-246.

Deveau M. **2010**. Contribution of drinking water to dietary requirments of essential metals Journal of Toxicology and Environmental Health Part A(73): 235-241.

Dewys WD, Walters K. **1975**. Abnormalities of Taste Sensations in Cancer Patients. Cancer 36(5): 1888-1896.

DeWys WD. **1977**. Anorexia in cancer patients. Cancer Res 37(7 Pt 2): 2354-2358.

Dietrich AM. **2009**. The sense of smell: contributions of orthonasal and retronasal perception applied to metallic flavor of drinking water. J Water Supply Res Technol-Aqua 58(8): 562-570.

Dmitriev LF, Titov VN. **2010**. Lipid peroxidation in relation to ageing and the role of endogenous aldehydes in diabetes and other age-related diseases. Ageing Res Rev 9(2): 200-210.

Dodds MWJ, Johnson DA, Yeh CK. **2005**. Health benefits of saliva: a review. J Dent 33(3): 223-233.

Doria MD, Pidgeon N, Hunter PR. **2009**. Perceptions of drinking water quality and risk and its effect on behaviour: A cross-national study. Sci Total Environ 407(21): 5455-5464.

Doria MD. **2010**. Factors influencing public perception of drinking water quality. Water Policy 12(1): 1-19.

Dotan Y, Lichtenberg D, Pinchuk I. **2004**. Lipid peroxidation cannot be used as a universal criterion of oxidative stress. Progress in Lipid Research 43: 200-227.

Doty RL, Shah M, Bromley SM. **2008**. Drug-induced taste disorders. Drug Saf 31(3): 199-215.

Doty RL, Treem J, Tourbier I, Mirza N. **2009**. A double-blind study of the influences of eszopiclone on dysgeusia and taste function. Pharmacol Biochem Behav 94(2): 312-318.

Doty RL. **2007**. Olfaction in Parkinson's disease. Parkinsonism Relat Disord 13: S225-S228.

Doty RL. **2008**. The smell identification Test™ administration manual: 3rd Edition. Haddon Heights, New Jersey.

Doty RL. **2009**. The Olfactory System and Its Disorders. Semin Neurol 29(1): 74-81.

Dupont D, Adamowicz WL, Krupnick A. **2010**. Differences in water consumption choices in Canada: the role of socio-demographics, experiences, and perceptions of health risks. J Water Health 8(4): 671-686.

Epke EM, Lawless HT. **2007**. Retronasal smell and detection thresholds of iron and copper salts. Physiol Behav 92(3): 487-491.

Epstein J, Phillips N, Parry J, et al. **2002**. Quality of life, taste, olfactory and oral function following high-dose chemotherapy and allogeneic hematopoietic cell transplantation. Bone Marrow Transplant 30: 785-792.

Epstein JB, Barasch A. **2010**. Taste disorders in cancer patients: Pathogenesis, and approach to assessment and management. Oral Oncol 46(2): 77-81.

Epstein JB, Emerton S, Kolbinson DA, Le ND, Phillips N, Stevenson-Moore P, et al. **1999**. Quality of life and oral function following radiotherapy for head and neck cancer. Head Neck 21(1): 1-11.

Fang ZQ, Qiu XQ, Huang RX, Qiu XH, Li MY. **2011**. Removal of chromium in electroplating wastewater by nanoscale zero-valent metal with synergistic effect of reduction and immobilization. Desalination 280(1-3): 224-231.

Farnaud SJC, Kosti O, Getting SJ, Renshaw D. **2010**. Saliva: Physiology and Diagnostic Potential in Health and Disease. The scientific World Iournal 10: 434-456.

Femiano F, Scully C, Gombos F. **2002**. Idiopathic dysgeusia; an open trial of alpha lipoic acid (ALA) therapy. Int J Oral Maxillofac Surg 31(6): 625-628.

Fernando IN, Patel T, Billingham L, Hammond C, Hallmark S, Glaholm J, et al. **1995**. The effect of head and neck irradiation on taste dysfunction: a prospective study. Clin Oncol (R Coll Radiol) 7(3): 173-178.

Frankel E. **1998**. Lipid Oxidation. Dundee: The Oily Press.

Freeze R, Cherry J. **1979**. Groundwater: Prentice Hall. Geneva, Switzerland.

Ghauch A, Tuqan A, Assia HA. **2009**. Antibiotic removal from water: Elimination of amoxicillin and ampicillin by microscale and nanoscale iron particles. Environ Pollut 157(5): 1626-1635.

Gil F, Hernandez AF, Marquez C, Femia P, Olmedo P, Lopez-Guarnido O, et al. **2011**. Biomonitorization of cadmium, chromium, manganese, nickel and lead in whole blood, urine, axillary hair and saliva in an occupationally exposed population. Sci Total Environ 409(6): 1172-1180.

Glindemann D, Dietrich AM, Staerk HJ, Kuschk P. **2006**. The two smells of touched or pickled iron (skin) carbonyl-hydrocarbons and organophosphines. . Angewandte Chemie Intl Ed (European Journal "Applied Chemistry") 45: 7006-7009.

Gobba F. **2006**. Olfactory toxicity: long-term effects of occupational exposures. Int Arch Occup Environ Health 79(4): 322-331.

Grace S, Lytle DA, Goltz MN. **2012**. Control of new copper corrosion in high-alkalinity drinking water. J Am Water Work Assoc 104(1): 39-40.

Gudziol V, Hummel C, Negoias S, Ishimaru T, Hummel T. **2007**. Lateralized differences in olfactory function. Laryngoscope 117(5): 808-811.

Guo B, Zebda R, Drake SJ, Sayes CM. **2009**. Synergistic effect of co-exposure to carbon black and Fe(2)O(3) nanoparticles on oxidative stress in cultured lung epithelial cells. Part Fibre Toxicol 6.

Halliwell B, Gutteridge JMC. **1990**. Role of Free-Radicals and Catalytic Metal-ions in Human Disease – An Overview. Method Enzymol 186: 1-85.

Halyard MY, Jatoi A, Sloan JA, Bearden JD, Vora SA, Atherton PJ, et al. **2007**. Does zinc sulfate prevent therapy-induced taste alterations in head and neck cancer patients? Results of phase III double-blind, placebo-controlled trial from the north central cancer treatment group (N01C4). Int J Radiat Oncol Biol Phys 67(5): 1318-1322.

Harris AM, Griffin SM. **2003**. Postoperative taste and smell deficit after upper gastrointestinal cancer surgery - An unreported complication. J Surg Oncol 82(3): 147-150.

Hayes JE, Keast RSJ. **2011**. Two decades of supertasting: Where do we stand? Physiol Behav 104(5): 1072-1074.

Heald AE, Pieper CF, Schiffman SS. **1998**. Taste and smell complaints in HIV-infected patients. Aids 12(13): 1667-1674.

Heckmann SM, Hujoel P, Habiger S, Friess W, Wichmann M, Heckmann JG, et al. **2005**. Zinc gluconate in the treatment of dysgeusia - a randomized clinical trial. J Dent Res 84(1): 35-38.

Hettinger TP, Myers WE, Frank ME. **1990**. Role of olfaction in perception of non - traditional taste stimuli. Chemical Senses 15(6): 755-760.

Hilty FM, Arnold M, Hilbe M, Teleki A, Knijnenburg JTN, Ehrensperger F, et al. **2010.** Iron from nanocompounds containing iron and zinc is highly bioavailable in rats without tissue accumulation. Nat Nanotechnol 5(5): 374-380.

Hochella MF, Kasama T, Putnis A, Putnis CV, Moore JN. **2005**. Environmentally important, poorly crystalline Fe/Mn hydrous oxides: Ferrihydrite and a possibly new vernadite-like mineral from the Clark Fork River Superfund Complex. American Mineralogist 90: 718-724.

Hochella MF, Lower SK, Maurice PA, Penn RL, Sahai N, Sparks DL, et al. **2008**. Nanominerals, mineral nanoparticles, and Earth systems. Science 319: 1631-1635.

Hoehl K, Schoenberger GU, Busch-Stockfisch M. **2009**. Water quality and taste sensitivity for basic tastes and metallic sensation. Food Qual Prefer 21(2): 243-249.

Hoffman HJ, Cruickshanks KJ, Davis B. **2009**. Perspectives on Population-based Epidemiological Studies of Olfactory and Taste Impairment. In: International Symposium on Olfaction and Taste, Vol. 1170, (Finger TE, ed). Oxford:Blackwell Publishing, 514-530.

Holmquist L, Stuchbury G, Berbaum K, Muscat S, Young S, Hager K, et al. **2007**. Lipoic acid as a novel treatment for Alzheimer's disease and related dementias. Pharmacol Ther 113(1): 154-164.

Hong JH, Duncan SE, Dietrich AM, O'Keefe SF. **2010**. Evaluation of the operationally defined soluble, insoluble, and complexing copper consumed through drinking water in human saliva. Eur Food Res Technol 231(6): 977-984.

Hong JH, Kim KO. **2011**. Operationally defined solubilization of copper and iron in human saliva and implications for metallic flavor perception. Eur Food Res Technol 233(6): 973-983.

Hong JH, Omur-Ozbeck P, Stanek B, Dietrich AM, Duncan S, Lee YW, et al. **2009**. Taste and Odor Abnormalities in Cancer Patients. Journal of Supportive Oncology 7.

Hong JHD, S.E.; Dietrich, A.M. and O'Keefe, S.F. **2006**. Effect of Copper on Volatile Flavor Compounds in a Model Mouth System. . Journal of Agricultural and Food Chemistry 54(24): 9168.

Hovan AJ, Williams PM, Stevenson-Moore P, Wahlin YB, Ohrn KEO, Elting LS, et al. **2010**. A systematic review of dysgeusia induced by cancer therapies. Support Care Cancer 18(8): 1081-1087.

Hummel T, Konnerth CG, Rosenheim K, Kobal G. **2001**. Screening of olfactory function with a four-minute odor identification test: Reliability, normative data, and investigations in patients with olfactory loss. Ann Otol Rhinol Laryngol 110(10): 976-981.

Humphrey SP, Williamson RT. **2001**. A review of saliva: Normal composition, flow, and function. J Prosthet Dent 85(2): 162-169.

Ibanez JG, Carreon-Alvarez A, Barcena-Soto M, Casillas N. **2008**. Metals in alcoholic beverages: A review of sources, effects, concentrations, removal, speciation, and analysis. J Food Compos Anal 21(8): 672-683.

IOM. **2004**. Dietary reference intakes for water, potassium, sodium, chloride, and sulfate / Panel on Dietary Reference Intakes for Electrolytes and Water, Standing Committee on the Scientific Evaluation of Dietary Reference Intakes, Food and Nutrition Board. Institute of Medicine (U.S.), Panel on Dietary Reference Intakes for Electrolytes and Water. Washington D.C.

IOM. **2011**. Dietary reference intakes for vitamin A, vitamin K, arsenic, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium and zinc.

Institute of Medicine, Food and Nutrition Board. Washington, DC:National Academy Press.

Jequier E, Constant F. **2010**. Water as an essential nutrient: the physiological basis of hydration. Eur J Clin Nutr 64(2): 115-123.

Kant AK, Graubard BI, Atchison EA. **2009**. Intakes of plain water, moisture in foods and beverages, and total water in the adult US population-nutritional, meal pattern, and body weight correlates: National Health and Nutrition Examination Surveys 1999-2006. Am J Clin Nutr 90(3): 655-663.

Karlsson H, Gustafsson J, Cronholm P, Möller L. **2009**. Size-dependent toxicity of metal oxide particles—A comparison between nano- and micrometer size. Toxicol Lett(188): 112-118.

Kaufman E, Lamster I. **2002**. The diagnostic applications of saliva-a review. Critical Reviews in Oral Biology and Medicine 13(2): 197-212.

Keast RSJ. **2003**. The effect of zinc on human taste perception. Journal of Food Science 68(5): 1871-1877.

Keenan CR, Goth-Goldstein R, Lucas D, Sedlak DL. **2009**. Oxidative Stress Induced by Zero-Valent Iron Nanoparticles and Fe(II) in Human Bronchial Epithelial Cells. Environ Sci Technol 43(12): 4555-4560.

Kim HJ, Jeon BS, Lee JY, Cho YJ, Hong KS, Cho JY. **2011**. Taste function in patients with Parkinson disease. J Neurol 258(6): 1076-1079.

Kim UK, Jorgenson E, Coon H, Leppert M, Risch N, Drayna D. **2003**. Positional cloning of the human quantitative trait locus underlying taste sensitivity to phenylthiocarbamide. Science 299(5610): 1221-1225.

Landis BN, Scheibe M, Weber C, Berger R, Bramerson A, Bende M, et al. **2010**. Chemosensory interaction: acquired olfactory impairment is associated with decreased taste function. J Neurol 257(8): 1303-1308.

Lang CJG, Leuschner T, Ulrich K, Stobbel C, Heckmann JG, Hummel T. **2006**. Taste in dementing diseases and Parkinsonism. J Neurol Sci 248(1-2): 177-184.

Lau BLT, Hsu-Kim H. **2008**. Precipitation and growth of zinc sulfide nanoparticles in the presence of thiol-containing natural organic ligands. Environ Sci Technol 42(19): 7236-7241.

Lawless HT, Heymann H. **1998**. Sensory Evaluation of Food: Principles and Practices. NY: Chapman & Hall.

Lawless HT, Schlake S, Smythe J, Lim J, Yang H, Chapman K, et al. **2004**. Metallic Taste and Retronasal Smell. Chemical Senses [Chem Senses] 29(1).

Lawless HTR, F.; Horne, J.; Hayes, A. **2003**. The taste of calcium and magnesium salts and anionic modifications. Food Quality and Preference 14: 319.

Lee JM, Garon E, Wong DT. **2009**. Salivary diagnostics. Orthod Craniofac Res 12(3): 206-211.

Lekholm U, Svennerholm L. **1977**. Lipid pattern and fatty acid composition of human palatal oral epithelium. Scandinavian Journal of Dental Research 85(4): 279-290.

Li H, Lawson JA, Reilly M, Adiyaman M, Hwang SW, Rokach J, et al. **1999**. Quantitative high performanceliquid chromatography/tandem mass spectrometric analysis of the four classes of F2-isoprostanes in human urine. Proc Natl Acad Sci USA 96: 13381-13386.

Lim J, Lawless H. **2006**. Detection thresholds and taste qualities of iron salts. Food Qual Prefer 17(6): 513-521.

Lim J, Lawless HT. **2006**. Detection thresholds and taste qualities of iron salts. Food quality and preference 17(6): 513-521.

Livnat G, Bentur L, Kuzmisnsky E, Nagler RM. **2010**. Salivary profile and oxidative stress in children and adolescents with cystic fibrosis. J Oral Pathol Med 39(1): 16-21.

Lopez-Jornet P, Camacho-Alonso F, Leon-Espinosa S. **2009**. Efficacy of alpha lipoic acid in burning mouth syndrome: a randomized, placebo-treatment study. J Oral Rehabil 36(1): 52-57.

MacCarthy-Leventhal EM. **1959**. Post-radiation mouth blindness. Lancet Dec 19;2(7112): 1138-1139.

Macquart-Moulin G, Viens P, Palangié T, Bouscary M, Delozier T, Roché H, et al. **2000**. High-dose sequential chemotherapy with recombinant granulocyte colony-stimulating factor and repeated stem-cell support for inflammatory breast cancer patients: does impact on quality of life jeopardize feasibility and acceptability of treatment? J Clin Oncol 18(4): 754-764.

Maisano R, Mare M, Zavettieri M, Caristi N, Mesiti M, Scisca C, et al. **2003**. Is weekly docetaxel an active and gentle chemotherapy in the treatment of metastatic breast cancer? Anticancer Research 23: 1923-1930.

Mangos JA, Donnelly WH. **1981**. Isolated parotid acinar-cells from patients with cystic-fibrosis – Morphology and composition. J Dent Res 60(1): 19-25.

Marnett L. **1998**. Lipid peroxidation—DNA damage by malondialdehyde. Mutation Research 424(1999): 83-95.

Maron JL. **2011**. Exploring the neonatal salivary transcriptome: Technical optimization and clinical applications. Clin Biochem 44(7): 467-468.

McDaniel RRV. **1998**. Development of a preparatory sensory information videotape for women receiving chemotherapy for breast cancer. Cancer Nurs 21: 143-148.

McGowan D. **2008**. Chemotherapy-induced oral dysfunction: a literature review. Br J Nurs 17(22): 1422-1426.

Meilgaard M, Carr T, Civille G. **2006**. Sensory Evaluation Techniques.

Meilgaard M, Carr T, Civille G. **2007**. Sensory Evaluation Techniques.

Meng H, Chen Z, Xing G, Yuan H, Chen C, Zhao F, et al. **2007**. Ultrahigh reactivity and grave nanotoxicity of copper nanoparticles. Journal of Radioanalytical and Nuclear Chemistry 272(3): 595-598.

Miller D. **2010**. New leverage against iron deficiency. Nature Nanotechnology 5: 318-319.

Mirlohi S, Dietrich AM, Duncan SE. **2011**. Age-Associated Variation in Sensory Perception of Iron in Drinking Water and the Potential for Overexposure in the Human Population. Environ Sci Technol 45(15): 6575-6583.

Mojet J, Christ-Hazelhof E, Heidema J. **2001**. Taste perception with age: generic or specific losses in threshold sensitivity to the five basic tastes? Chemical Senses 26(7): 845-860.

Mojet J, Christ-Hazelhof E, Heidema J. **2005**. Taste perception with age: pleasantness and its relationships with threshold sensitivity and supra-threshold intensity of five taste qualities. Food Qual Prefer 16(5): 413-423.

Moore K, Roberts LJ. **1998**. Measurement of lipid peroxidation. Free Radic Res 28(6): 659-671.

Murphy C, Schubert CR, Cruickshanks KJ, Klein BEK, Klein R, Nondahl DM. **2002**. Prevalence of olfactory impairment in older adults. JAMA-J Am Med Assoc 288(18): 2307-2312.

Naik BS, Shetty N, Maben EVS. **2010**. Drug-induced taste disorders. European Journal of Internal Medicine 21(2010): 240-243.

Navazesh M, Mulligan RA, Kipnis V, Denny PA, Denny PC. **1992**. Comparison of whole saliva flow-rates and mucin concentrations in healthy Caucasian young and aged adults. J Dent Res 71(6): 1275-1278.

Nourooz-Zadeh J. **2008**. Key issues in F2-isoprostane analysis. Biochemical Society Transactions 36(5): 1060-1065.

Öhrn KE, Wahlin Y, Sjödén P. **2001**. Oral status during radiotherapy and chemotherapy: a descriptive study of patient experiences and the occurrence of oral complications. Support Care Cancer 9(4): 247-257.

Ömur-Özbek P, Dietrich AM. **2011**. Retronasal perception and flavour thresholds of iron and copper in drinking water. J Water Health 9(1): 1-9.

Ömur-Özbek P. **2008**. Macromolecular Reactions and Sensory Perceptions at the Air-Water-Human Interface. [PhD Dissertation]. Blacksburg, VA: Virginia Tech.

Pedersen AM, Bardow A, Jensen SB, Nauntofte B. **2002**. Saliva and gastrointestinal functions of taste, mastication, swallowing and digestion. Oral Diseases 8(3): 117-129.

Pfaffe T, Cooper-White J, Beyerlein P, Kostner K, Punyadeera C. **2011**. Diagnostic Potential of Saliva: Current State and Future Applications. Clin Chem 57(5): 675-687.

Plathe KL. **2010**. Nanoparticle - Heavy Metal Associations in River Sediments [PhD Dissertation]. Blacksburg: Virginia Tech.

Plattig K. **1988**. The Senses of Taste. Chap.1. In: Sensory Analysis of Foods. . 2nd ed. Piggot, J.R. (Ed.), Elsevier Applied Scientific Publishers, Ltd., London p 1-23.

Popkin BM, Barclay DV, Nielsen SJ. **2005**. Water and food consumption patterns of US adults from 1999 to 2001. Obes Res 13(12): 2146-2152.

Popkin BM, D'Anci KE, Rosenberg IH. **2010**. Water, hydration, and health. Nutr Rev 68(8): 439-458.

Popkin BM. **2010**. Patterns of beverage use across the lifecycle. Physiology & Behavior 100(1): 4-9.

Ravasco P. **2005**. Aspects of taste and compliance in patients with cancer. European Journal of Oncology Nursing 9: 84-91.

Ripamonti C, Fulfaro F. **1998**. Taste alterations in cancer patients. J Pain Symptom Manage 16(6): 349-351.

Ripamonti C, Zecca E, Brunelli C, Fulfaro F, Villa S, Balzarini A, et al. **1998**. A randomized, controlled clinical trial to evaluate the effects of zinc sulfate on cancer patients with taste alterations caused by head and neck irradiation. Cancer 82(10): 1938-1945.

Ritz P, Berrut G. **2005**. The importance of good hydration for day-to-day health. Nutr Rev 63(6): S6-S13.

Rudney JD, Staikov RK, Johnson JD. **2009**. Potential biomarkers of human salivary function: A modified proteomic approach. Archives of Oral Biology 54(1): 91-100.

Sahu SC, Casciano DA, eds. **2009**. Nanotoxicity: From In Vivo to In Vitro Models to Health Risks. John Wiley & Sons, Ltd.

Sakagami M, Ikeda M, Tomita H, Ikui A, Aiba T, Takeda N, et al. **2009**. A zinc-containing compound, Polaprezinc, is effective for patients with taste disorders: randomized, double-blind, placebo-controlled, multi-center study. Acta Oto-Laryngol 129(10): 1115-1120.

Sarver E, Zhang YF, Edwards M. **2010**. Review of Brass Dezincification Corrosion in Potable Water Systems. Corros Rev 28(3-4): 155-196.

Sawyer C, McCarty P, Parkin G. **1994**. Chemistry for Environmental Engineering, 4th Edition: McGraw-Hill, Inc.

Schiffman SS. **1997**. Taste and smell losses in normal aging and disease. JAMA-J Am Med Assoc 278(16): 1357-1362.

Schiffman SS. **2009**. Effects of Aging on the Human Taste System. In: International Symposium on Olfaction and Taste, Vol. 1170, (Finger TE, ed). Oxford:Blackwell Publishing, 725-729.

Schumann K, Ettle T, Szegner B, Elsenhans B, Solomons NW. **2007**. On risks and benefits of iron supplementation recommendations for iron intake revisited. J Trace Elem Med Biol 21(3): 147-168.

Segal A, Wong DT. **2008**. Salivary diagnostics: enhancing disease detection and making medicine better. Eur J Dent Educ 12: 22-29.

Sharma PK, Mittal N, Deswal S, Roy N. **2011**. Calorie restriction up-regulates iron and copper transport genes in Saccharomyces cerevisiae. Mol Biosyst 7(2): 394-402.

Sharrett AR, Orheim RM, Carter AP, Hyde JE, Feinleib M. **1982**. Components of variations in lead, cadmium, copper, and zinc Concentration in home drinking water - The Seattle study of trace metal exposure. Environ Res 28(2): 476-498.

Shepherd M. **2006**. Smell images and the flavour system in the human brain. Nature 444: 316-321.

Shi ZB, Woodhouse MT, Carslaw KS, Krom MD, Mann GW, Baker AR, et al. **2011**. Minor effect of physical size sorting on iron solubility of transported mineral dust. Atmos Chem Phys 11(16): 8459-8469.

Shibamoto T. **2006**. Analytical methods for trace levels of reactive carbonyl compounds formed in lipid peroxidation systems. Journal of Pharmaceutical and Biomedical Analysis 41: 12-25.

Silver J, ed. **1993**. Chemistry of Iron. Chapman & Hall.

Simpson RJ, Jensen SS, Lim JWE. **2008**. Proteomic profiling of exosomes: Current perspectives. Proteomics 8(19): 4083-4099.

Slomiany B, Zdebska E, Murty V, Slomiany A. **1983**. Lipid composition of human labial salivary gland secretions. Archives of Oral Biology 28: 711-714.

Small DM, Gerber JC, Mak YE, Hummel T. **2005**. Differential neural responses evoked by orthonasal versus retronasal odorant perception in humans. Neuron 47: 593-605.

Soares S, Vitorino R, Osorio H, Fernandes A, Venancio A, Mateus N, et al. **2011**. Reactivity of Human Salivary Proteins Families Toward Food Polyphenols. J Agric Food Chem 59: 5535-5547.

Spanier AM. **1991**. A rapid, direct chemical assay for the quantitive determination of thiobarbituric acid reactive substances in raw, cooked, and cooked/stored muscle foods. Journal of Muscle Foods 2(3): 165-176.

Spielman AI. **1990**. Interaction of Saliva and Taste. J Dent Res 69(3): 838-843.

Steele JC, Bruce AJ, Drage LA, Rogers RS. **2008**. alpha-Lipoic acid treatment of 31 patients with sore, burning mouth. Oral Diseases 14(6): 529-532.

Steinbach S, Hummel T, Bohner C, Berktold S, Hundt W, Kriner M, et al. **2009**. Qualitative and Quantitative Assessment of Taste and Smell Changes in Patients Undergoing Chemotherapy for Breast Cancer or Gynecologic Malignancies. J Clin Oncol 27(11): 1899-1905.

Stinton N, Atif MA, Barkat N, Doty RL. **2010**. Influence of Smell Loss on Taste Function. Behav Neurosci 124(2): 256-264.

Stone H, Sidel J. **1993**. Sensory Evaluation Practices. San Diego: Academic Press.

Stookey JD, Constant F, Gardner CD, Popkin BM. **2007**. Replacing sweetened caloric beverages with drinking water is associated with lower energy intake. Obesity 15(12): 3013-3022.

Streckfus C, Bigler L, Tucci M, Thigpen J. **2000**. A preliminary study of CA15-3, c-erB-2, epidermal growth factor receptor, cathespin-D, and p53 in saliva among women with breast carcinoma. Cancer Investigation 18(2): 101-109.

Sugaya N, Nakagawa T, Morita M, Onodera S. **2001**. Analysis of aldehydes in water by headspace-GC/MS. Journal of Health Sciences 47(1): 21-27.

Suma HR, Prabhu K, Shenoy RP, Annaswamy R, Rao S, Rao A. **2010**. Estimation of salivary protein thiols and total antioxidant power of saliva in brain tumor patients. J Canc Res Ther 6(3): 278-281.

Tamura T, Taniguchi T, Suzuki Y, Okubo T, Takata R, Konno T. **2009**. Iron Is an Essential Cause of Fishy Aftertaste Formation in Wine and Seafood Pairing. J Agric Food Chem 57: 8850-8856.

Tanboonchuy V, Hsu JC, Grisdanurak N, Liao CH. **2011**. Impact of selected solution factors on arsenate and arsenite removal by nanoiron particles. Environ Sci Pollut Res 18(6): 857-864.

Tang J. **2010**. Modeling contaminant transport in polyethylene and metal speciation in saliva [Masters Thesis]. Blacksburg: Virginia Tech.

Tavassoli M, Brunel N, Maher R, Johnson NW, Soussi T. **1998**. P53 antibodies in the saliva of patients with squamous cell carcinoma of the oral cavity. International Journal of Cancer 78: 390-391.

Taylor J, Roberts D, eds. **2004**. Flavor Perception. Blackwell Publishing.

Tenovuo J. **1989**. Human Saliva: Clinical Chemistry and Micriobiology, Volume I. 205.

Tishler M, Yaron I, Shirazi I, Yaron M. **1997**. Saliva: An additional diagnostic tool in Sjogren's syndrome. Semin Arthritis Rheum 27(3): 173-179.

Tormen L, Torres DP, Dittert IM, Araujo RGO, Frescura VLA, Curtius AJ. **2011**. Rapid assessment of metal contamination in commercial fruit juices by inductively coupled mass spectrometry after a simple dilution. J Food Compos Anal 24(1): 95-102.

Tsukatani T, Reiter ER, Miwa T, Costanzo RM. **2005**. Comparison of diagnostic findings using different olfactory test methods. Laryngoscope 115(6): 1114-1117.

Tymoczko J, Berg J, Stryer L. **2010**. Biochemistry: A Short Course.

USEPA. **2009**. 2009 Drinking Water Standards and Health Advisories Tables. EPA-822-R09-011. Washington D.C.

Vargas IT, Pavissich JP, Olivares TE, Jeria GA, Cienfuegos RA, Pasten PA, et al. **2010**. Increase of the concentration of dissolved copper in drinking water systems due to flow-induced nanoparticle release from surface corrosion by-products. Corrosion Sci 52(10): 3492-3503.

Vikesland P, Heathcock A, Rebodos R, Makus E. **2007**. Particle Size and Aggregation Effects on Magnetite Reactivity toward Carbon Tetrachloride. Environmental Science & Technology 41(15): 5277-5283.

Waychunas GA, Kim CS, Banfield JF. **2005**. Nanoparticulate iron oxide minerals in soils and sediments: unique properties and contaminant scavenging mechanisms. Journal of Nanoparticle Research (2005) 7: (7): 409–433.

WHO. **2008**. Worldwide prevalence of anaemia1993–2005 WHO Global Database on Anaemia. World Health Organization (WHO)

WHO. **2011**. Guidelines for Drinking Water Quality, 4th Edition, World Health Organization Geneva, Switzerland.

Wigginton NS, Haus KL, Hochella MF. **2007**. Aquatic environmental nanoparticles. J Environ Monit 9(12): 1306-1316.

Wood LG, Gibson PG, Garg ML. **2003**. Biomarkers of lipid peroxidation, airway inflammation and asthma. European Respiratory Journa 21: 177-186.

Wrobel BB, Leopold DA. **2004**. Clinical assessment of patients with smell and taste disorders. Otolaryngol Clin N Am 37(6): 1127-+.

Yagi K. **1998**. Simple assay for the level of total lipid peroxides in serum or plasma. Meth Mol Biol 108: 101-106.

Yeh CK, Johnson DA, Dodds MWJ. **1998**. Impact of aging on human salivary gland function: A community-based study. Aging-Clin Exp Res 10(5): 421-428.

Zacarias I, Yanez CG, Araya M, Oraka C, Olivares M, Uauy R. **2001**. Determination of the taste threshold of copper in water. Chemical Senses 26(1): 85-89.

Zhao DL, Chen CL, Sheng GD, Wang XK. **2011**. Effect of environmental conditions on the retention behaviour of Pb(II) by hematite. J Chem Technol Biotechnol 86(8): 1099-1106.

Zimmermann MB, Hilty FM. **2011**. Nanocompounds of iron and zinc: their potential in nutrition. Nanoscale 3(6): 2390-2398.

Chapter III

Age-associated Variation in Sensory Perception of Iron in Drinking Water and the Potential for Overexposure in the Human Population

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Abstract

Humans interact with their environment through the five senses but, little is known about population variability in the ability to assess contaminants. Sensory thresholds and biochemical indicators of metallic flavor perception in humans were evaluated for ferrous (Fe^{2+}) iron in drinking water; 61 subjects aged 19 – 84 years, participated. Metallic flavor thresholds for individuals and sub-populations based on age were determined. Oral lipid oxidation and oral pH were measured in saliva as potential biochemical indicators of metallic flavor perception. Individual thresholds were 0.007 – 14.14 mg/L Fe^{2+} and the overall population threshold was 0.17 mg/L Fe^{2+} in reagent water. Average thresholds for individuals younger and older than 50 years of age (grouped by the daily recommended nutritional guidelines for iron intake) were significantly different ($p = 0.013$); the population thresholds for each group were 0.045 mg/L Fe^{2+} and 0.499 mg/L Fe^{2+}, respectively. Few subjects <50 years were also insensitive to metallic flavor. There was no correlation between age, oral lipid oxidation, and oral pH. Standardize olfactory assessment found poor sensitivity for Fe^{2+} corresponded with conditions of mild, moderate, and total anosmia. The findings demonstrate an age-dependent sensitivity to iron indicating as people age they are less sensitive to metallic perception.

Key Words: Exposure, Odors, Metals, Iron, Drinking water quality, Aging.

3.1 Introduction

Humans assess the environment through their five senses and sensory assessment is the first line of defense for health protection and survival. Recent evidence of a potential environmental threat uncovered by human senses is the 2010 widely publicized earthy-musty contamination of Tylenol® reportedly due to the presence of tribromoanisole, which is odorous at pg amounts and which can be produced by biomethylation of the pesticide tribromophenol (Kroll 2010; Malleret and Bruchet 2002). Human sensory assessment was instrumental in discovering the source of the 2007 Wuxi City, water crisis in China. Two million residents of Wuxi City received odorous tap water as a result of the massive die-off of cyanobacteria in the source water (Zhang et al. 2011). Although taste and odor episodes are concurrent with many drinking water illness outbreaks in developed countries, complaints of off-tastes, odors, or colors reported by consumers are often overlooked by health officials and water utility personnel (Hrudey and Hrudey 2004). As a glaring example, the deadly 1993 *Cryptosporidium* outbreak in Milwaukee, WI was preceded by unheeded consumer complaints of increased turbidity in the drinking water (Hrudey and Hrudey 2004). Metal contamination of drinking water by copper, which produces a distinct metallic flavor in water (Cuppett et al. 2006; Ömur-Özbek and Dietrich 2011), was identified as the source of 27 illness outbreaks in the US from 1971 to 2006 (Craun et al. 2010). Lead poisoning from drinking water also occurs as recently documented by strongly positive correlation between lead concentrations in drinking water and elevated lead blood levels in children ≤1.3 years, as well as those ≤30 months of age in Washington DC (Edwards et al. 2009). Unlike lead, flavor-producing metals such as iron and copper can be tasted by humans at levels well below their regulatory limits. In such cases, human senses can be an important first line of defense against metal poisoning; for the same reason, inability to taste, presents a serious cause for concern.

While humans do use their senses to assess their environment, historically little environmental research has focused on sensory characteristics of contaminants. One reason for this lack of research is that sensory characteristics of environmental contaminants are not always known. Even if sensory data are reported on a material

safety data sheet (MSDS), they are not always observed. For environmental contaminants that have tastes and/or odors, the variability in the human population to detect the contaminants is frequently unknown as only threshold values are usually reported (Young et al. 1996). Notable exceptions in drinking water are the well-studied aqueous cyanobacteria metabolites geosmin (earthy) and 2-methylisoborneol (musty). The literature reports that the typical odor recognition thresholds for these two compounds range from 1-10 ng/L at 45 ℃ (Piriou et al. 2009). The odor threshold values are inversely correlated with temperature and some humans either possess very high thresholds or specific anosmias (Whelton and Dietrich 2004) .

There is a natural variability in the human senses, which is widely acknowledged for sight and hearing because of routine screening and data collection. However, the variability is less studied and understood for the chemical senses of taste and smell. The most widely studied genetic variation for taste is responsiveness to the bitter compounds 6-n-propylthiouracil (PROP) and phenylthiocarbamide (PTC), which are used to distinguish super-taster, medium taster, and non-taster perception of bitterness based on variations in the expression of the TAS2R receptor genes (Bartoshuk 1994; Bartoshuk et al. 1994). The relationship of bitter taster-status to taste perception and hedonics is an active area of research. Likewise, advances in understanding the complexity of the sense of smell are fairly recent and resulted in awarding of the 2004 Nobel Prize for Medicine to Linda Buck and Richard Axel (Miller 2004). The ability and variability to smell is controlled by genetic factors associated with variations in odor receptor genes (Reed and Knaapila 2010). There are approximately 350 different odor receptors that assess thousands of odors and their intensities, with a single receptor capable of recognizing multiple odorants and individual odorants interacting with multiple receptors (Buck and Axel 1991; Buck 2004). Aging is also a contributing factor to variability as humans experience a gradual decline in smell functions, with the rate of increasing above 70 years of age (Doty et al. 1984; Murphy et al. 2002).

Worldwide, human of all ages are exposed to metals in drinking water(Asante et al. 2007; Chai et al. 2010; Dietrich et al. 2004). Metal pollution is especially common in groundwater due to dissolution of metals into the aquifers (Concha et al. 2010). One of

the most abundant metals in the earth's crust, iron occurs naturally in groundwater or comes from the corrosion of pipes in distribution systems (Volke et al. 2000; World Health Organization (WHO) 2003). Under low oxygen conditions in groundwater, iron is present in its reduced ferrous iron (II) form at concentrations up to several milligrams per liter. In the United States, iron levels in groundwater range from less than 0.001 to 30 mg/L depending on the geographical region, with higher values typical for groundwater in Southern and Midwest States (DeSimones et al. 2009). In these regions, the average groundwater iron levels often exceed the United States Environmental Protection Agency (USEPA) Secondary Maximum Contaminant Level (SMCL) of 0.3 mg/L Fe based on color and taste (United States Environmental Protection Agency) (Figure 3.1). Likewise, the World Health Organization (WHO) guideline is set at the same level.

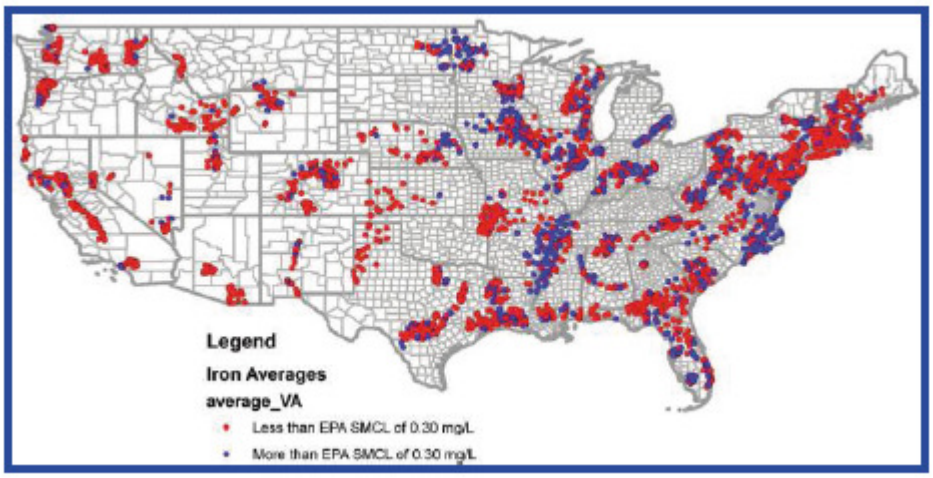

Figure 3.1: Average concentration of iron in U.S. groundwater as compiled from the U.S. Geological Survey's National Water-Quality Assessment Mapper for the years 1990-2010. Red dots represent areas with average iron concentrations below the USEPA secondary maximum contaminant limit (SMCL) of 0.3 mg/L. The blue dots correspond to areas with iron levels above that of the SMCL.

Iron is an essential element and not typically considered to be a toxic metal. However, recent reports indicate that high iron intake is associated with increased risk of cancer (Peto 2010; Zacharski et al. 2008); in fact, iron chelating drugs have been used for cancer therapy (Buss et al. 2004; Kovacevic et al. 2011). Excessive iron accumulation in the body has also been associated with aging as well as diseases of the heart, Alzheimer's disease, and Parkinson's disease. This has been mainly attributed to the

ability of iron (as well as copper) to participate in oxidative reactions that generate reactive oxygen species or free radicals that are responsible for many pathological conditions as well as natural aging (Papanikolaou and Pantopoulos 2005). Another aspect of iron toxicity is its role as a growth element in disease-causing microorganisms as well as in tumors (Weinberg 2010). As an example, iron supplementation has been associated with increased risk of placental malaria parasite in pregnant women (Senga et al. 2011). A recent review article by Brewer (Brewer 2010) succinctly presents the realistic and possibly alarming "story" of "copper and iron toxicity during aging in humans", identifying their unacknowledged toxicities as a "looming public health" problem for the aging population.

In the United States, population studies from the National Health and Nutrition Examination Survey (NHANES) III have indicated different levels of iron accumulation in the body, as measured by serum ferritin (a protein in the body that stores iron levels), based on age, race, and gender, with the levels increasing dramatically in women above 50 years of age (Zacharski et al. 2000). Due to its potential to cause gastrointestinal stress and increase oxidative damage to biological membranes, proteins, or DNA, the German Federal Institute for Risk Assessment classifies iron as a potential high risk human health hazard while recognizing its nutritional necessity at various stages of human development (Domke et al. 2005).

Metallic flavored drinking water complaints are common. In a survey of North American utilities, 14-20% of all taste complaints were described as metallic (Suffet et al. 1996). Scottish citizens used "metallic" to describe 12.4% of all taste and odor complaints for drinking water in 2006 (Scottish Government 2008). Although metallic flavor sensation in humans is common, its causes are not well understood. Metallic flavor is known to be a slight taste sensation coupled to a major retronasal-odor sensation from metal induced lipid-oxidation in the oral cavity. The sensation has been described as metallic, sweet, bitter and astringent (Ömur-Özbek and Dietrich 2011; Hong et al. 2010a; Lim and Lawless 2006). The odor component is perceived through the release of odorous flavor compounds in the back of the oral cavity where the oral and nasal passages meet; this is referred to as retronasal perception. The significance of retronasal

perception for Fe^{2+} is that it results in a decrease in the flavor threshold as can be demonstrated by examining the effect of nasal occlusion (Ömur-Özbek and Dietrich 2011; Hettinger et al. 1990; Lawless et al. 2004). Metallic sensation is typically perceived strongest for ferrous, followed by cupric and cuprous salts (Ömur-Özbek and Dietrich 2011). Previously determined threshold concentrations for iron have ranged from 0.04 to 256 ppm (Cohen 1960). A most recent and comprehensive measure of individual ferrous iron thresholds indicate a range of 0.003 to greater than 5 mg/L for human subjects primarily younger than 50 years of age (Ömur-Özbek and Dietrich 2011). No specific studies have examined the impact of age on metallic flavor threshold in water, although age associated effects on thresholds for the five basic tastes of salty, sweet, bitter, sour, and umami have been widely studied dating back to 1940, as comprehensively outlined in a 2001 review article by Mojet and colleagues (Mojet et al. 2001).

In this study, our objectives were to determine the effect of age on metallic flavor threshold in water; to examine whether flavor threshold variations among individuals can be attributed to physical/chemical factors such as oral pH and lipid oxidation; and assess the potential for individuals to use their chemical senses to detect iron in drinking water at the SMCL or higher concentrations. The impact of aging was of specific importance and interest due to the prevalence of taste and smell disorders among the elderly population.

3.2 Materials and Methods

3.2.1 Human Subjects. The study was approved by the Institutional Review Board (IRB) at Virginia Tech (IRB Project No. 06-395). Human subjects were recruited from the community, students, faculty and staff of Virginia Tech and Blacksburg, Virginia by means of paper and email flyers. Subjects were required to have no chronic oral or general health problems, be non-smokers, and not pregnant. All subjects read and signed an informed consent form in accordance with the approved IRB protocols. The subjects were untrained, but were aware from the informed consent that the focus was metallic flavor. A total of 61 (36 females) multinational subjects, ages 19 – 84 years, participated in the study. From this group of 61 subjects, subgroups participated in the

various experiments. Forty-one (41) subjects (26 females), ages 19-84 participated in the ferrous threshold study and 15 subjects (10 females), aged 52-84, of the 41 also participated in an assessment of their smell function using a standardized test (vide infra). Twenty-six of these 41 subjects participated in the oral lipid oxidation (OLO) experiments; another 20 subjects joined the OLO experiments for a total of 46 subjects (24 females), aged 19-84.

The sensory protocol for flavor threshold determination used in our current study was identical to that of Omur-Ozbek and Dietrich (Ömur-Özbek and Dietrich 2011). That previous study determined ferrous thresholds for 28 subjects (15 females) ages 19-65. Thus, the two data sets were combined to determine an overall population threshold for metallic flavor in drinking water based on 69 subjects (41 females) ages 19-84.

3.2.2 Flavor Recognition Threshold and Oral pH. Sensory Methods: this research utilized sensory methods and materials identical to those previously reported (Ömur-Özbek and Dietrich 2011). Briefly, for threshold determinations, an ascending concentration one-of-five forced choice test was used which had a 20% chance of guessing correctly (American Standards for Testing Materials (ASTM) 1997). Samples were served at 22 - 24 ºC in taste-and odor free 3-oz Solo plastic cups (Solo Cup Company, Lake Forest IL) filled with 20 ml of sample and/or control water. Previous studies (Cuppett et al. 2006; Ömur-Özbek and Dietrich 2011; Lim and Lawless 2006) demonstrated that this volume was sufficient to determine taste perception. Only one sensory session that tested a single ferrous concentration was conducted per day. A single test and concentration per day was necessary to avoid any effect of aftertaste, which is typical of metallic flavor. Subjects were instructed to avoid consuming food or beverages for at least one hour prior to each sensory testing session. Tests were conducted in a quiet room with no distracting odors or sounds. During each testing session, subjects' oral pH was measured using a pH indicator strip (Cen-Med/Fisher M95883).

To familiarize subjects with metallic flavor, they were given Nanopure® water with a high iron concentration before threshold testing began. At each session, subjects

received 5 cups each labeled with a different 3-digit number. Four cups contained Nanopure® water and one contained the ferrous solution. The cups were presented in a random order such that the ferrous solution could be in any of the five positions. Subjects were instructed to taste the samples from left to right without going back, wait 1 minute in between samples, and to select the metallic tasting sample and mark it on their score sheet. For a given subject, testing was complete when the subject correctly identified three sequential ferrous concentrations or reached the last and highest concentration approved by the IRB.

Sample Preparation: A 100 mg/L iron stock was prepared daily using food-grade Iron (II) Sulfate (Sigma-Aldrich, PA, CAS # 13463-43-g) and Nanopure® water which was taste and odor free. Ferrous solutions were prepared daily by diluting the stock solution with Nanopure® water, which also served as the control. The concentrations tested were 0.005, 0.01, 0.025, 0.05, 0.1, 0.25, 0.5, 1, 2, 4, 5, 10, and 20 mg/L Fe^{2+}. Solutions were monitored to prevent the oxidation of ferrous iron to ferric iron. The concentrations were verified using inductively coupled plasma – mass spectroscopy (Thermo Electronic Corporation, X-Series ICP-MS, Waltham, MA), following Standard Method 3120B (American Public Health Association (APHA); the American Water Works Association (AWWA); the Water Environment Federation (WEF) 2005).

3.2.3 Oral Lipid Oxidation and Oral pH. Taste samples of 10 mg/L Fe^{2+} were prepared from ferrous sulfate in Nanopure® water. Subjects were presented with two 3-oz Solo plastic cups, one filled with 2 mL of Nanopure® water and the other with 2 mL of 10 mg/L Fe^{2+}. Subjects were asked to sip 2 mL of Nanopure® water, as the control, then swish it around their mouth for 20 seconds, and without swallowing, expectorate until approximately 4 mL of saliva (control) was collected. After a short rest period, subjects sipped 2 mL Fe^{2+} solution, swished the sample around their mouth, and expectorate the second 4 mL saliva sample (ferrous). Saliva samples were frozen immediately and stored at -50 ºC for up to one month until analysis. Oral pH was measured, as previously described after sampling the control solution and again after sampling the metal salt solution. Saliva analysis included measurement of lipid oxidation by thiobarbituric acid reactive substances (TBARS) and total protein. The TBARS method

(Spanier 1991) was modified to work with liquid samples and to enhance readings at low concentrations (Wang 2009). Total protein content of saliva was analyzed using Bradford assay (Copeland 1994). Since the protein content of saliva can vary among individuals, the TBARS results were normalized by the total protein content of the individual's saliva, and the results were reported as μM of TBARS produced per gram of total protein.

3.2.4 Olfactory Functions Assessment. Based on the results of the flavor threshold testing, a subgroup was selected for assessment of their olfactory functions. The subgroup consisted of 15 subjects (10 females), aged >52 years and included subjects with low and high sensitivities to metallic flavor of iron. Smell function was assessed with the widely used standardized University of Pennsylvania Smell Identification Test (UPSIT) which is a "scratch-and-sniff" test that establishes both absolute (i.e., normosmia, anosmia, or mild, moderate or severe microsmia) and relative (percentile ranks) indices of olfactory function (Doty 1997, 2008).

3.2.5 Data Analyses. The best estimates of thresholds (BET) were calculated by the geometric mean method (American Standards for Testing Materials (ASTM) 1997; Lawless and Heymann 1998), which calculates the threshold as the geometric mean of the highest incorrect concentration and the lowest correct concentration. The group threshold was calculated as the geometric mean of all individual geometric mean threshold values (American Standards for Testing Materials (ASTM) 1997). Population threshold estimates also were determined separately for less than and greater than 50 years old age groups. The splitting of the threshold data between age groups at 50 years old was based on consideration of the WHO dietary guidelines that specifies reduced dietary intake of iron at 50 years of age and older.

Statistical software, JMP 8.0 (SAS, Cary, NC) was used for all data analyses. One-way analysis of variance (ANOVA) and comparison of the means, using Student's t-Test and Tukey HSD or Wilcoxon/Kruskal-Wallis Rank sum test, were performed on the individual flavor threshold data, segregated by age group (less than and greater than 50 years). For the oral lipid oxidation experiments, comparative analysis was performed on the

mean OLO responses between the control and treatment (ferrous) groups. Linear correlation analysis was performed to identify the degree of correlation, if any, between age and flavor threshold, age and OLO, and oral pH and threshold. For the olfactory functions assessment, subjects were grouped based on their olfactory assessment rating scores as categorized by the UPSIT protocol. The UPSIT scores were compared to the corresponding flavor threshold levels to identify any relationships between individual metallic flavor thresholds and olfactory functions.

3.3 Results and Discussion

3.3.1 Flavor Recognition Threshold and Age. The subjects recognized the metallic flavor in the samples. The best estimate threshold levels for individual flavor thresholds ranged from 0.007 to 14.14 mg/L Fe^{2+} (Table 3.1 and Figure 3.2) The highest thresholds (e.g., individuals most insensitive to the flavor of iron) were typically observed among those > 50 years of age, although low and high thresholds were noted among both younger and older subjects. The overall population threshold for all the 41 subjects in the current study was calculated at 0.17 mg/L Fe^{2+}. The population threshold estimate for ≤ 50 years old age group was 0.045 mg/L Fe^{2+}; this is nearly identical to recently previously reported value of 0.042 mg/L Fe^{2} from our laboratory (Ömur-Özbek and Dietrich 2011) (Table 3.1). This indicates that the threshold estimate for the comparable age group could be duplicated given the same sensory methods and conditions. For the age group > 50 years old, the geometric mean population threshold was estimated at 0.50 mg/L ferrous iron, which was statistically different from ($p = 0.013$) and approximately ten times greater than the ≤ 50 years old age group (Table 3.1). The combined flavor threshold data for the current and previous studies ($n = 69$) indicated a weak correlation ($R^2 = 0.28$) between age and flavor threshold among individuals > 50 years old, and no correlation ($R^2 = 0.003$) for those in the age range of 18 to 50 years old (Figure 3.2); the correlation ($R^2 = 0.23$) still existed when all ages were combined.

Table 3.1: Summary of flavor threshold data for ferrous iron in Nanopure® water using an ascending forced choice one-of-five sensory procedure.

Data Source	Age Range	Sample size (n)	Concentration, mg/L Fe^{2+}	
			Range Individual Threshold	Geometric Mean Population Threshold
Study 1 (previous[6])	All (19 - 65)	28 (F = 15)[c]	0.003 - > 5[b]	0.042 (SD[d] = 0.99)
	≤ 50 (19 – 50)	25 (F = 14)	0.003 - > 5[b]	0.050 (SD[d] = 0.99)
	> 50 (51 – 65)	3 (F = 1)	> 5	0.189 (SD = 2.87)
Study 2 (current research)	All (19 - 84)	41 (F = 26)	0.007 - 14.14	0.174 (SD = 4.41)
	≤ 50 (19 – 50)	18 (F = 11)	0.007 – 8.77	0.045 (SD = 1.99)
	> 50 (51 – 84)	23 (F = 15)	0.007 - 14.14	0.498 (SD = 5.34)
Overall [a]	All (19 – 84)	69 (F = 41)	0.003 – 14.14	0.105 (SD = 3.58)
	≤ 50 (19 – 50)	43 (F = 25)	0.003 – 8.77	0.044 (SD = 1.51)
	> 50 (51 – 84)	26 (F = 16)	0.007 – 14.14	0.445 (SD = 5.10)

[a] Combined data from Studies 1 and 2.
[b] For Study 1, the highest concentration of ferrous tested was 5 mg/L and the concentrations ranged from 0.003 to > 5 mg/L of ferrous; value of 5 was used in population threshold calculations, when the reported individual flavor threshold was greater than 5 mg/L of ferrous. For Study 2, range of ferrous concentrations tasted was 0.005 – 20 mg/L.
[c] F = The number of female subjects.
[d] SD = Standard deviation of the individual flavor thresholds.

3.3.2 Flavor Recognition Threshold and Oral pH. The pH of Nanopure® water used in preparation of the metallic taste samples ranged from 5.5 – 6.0, while the participants' oral pH levels varied from 5.5 – 7.5 (mean = 6.5, standard deviation = 0.37). There was no correlation (R^2 = 0.004) between a subject's flavor threshold and their median oral pH (Figure 3.3). Based on chemistry of the redox reactions of ferrous iron in aqueous systems, at the pH range encountered in the sample water and the oral environment, ferrous iron remains in solution as the dominant species (Benjamin 2002). With regard to metallic flavor perception, the dissolved metal form is the major flavor producing species, as it has been shown to be the case for both iron and copper (Cuppett et al. 2006; Ömur-Özbek and Dietrich 2011; Hong et al. 2010b)

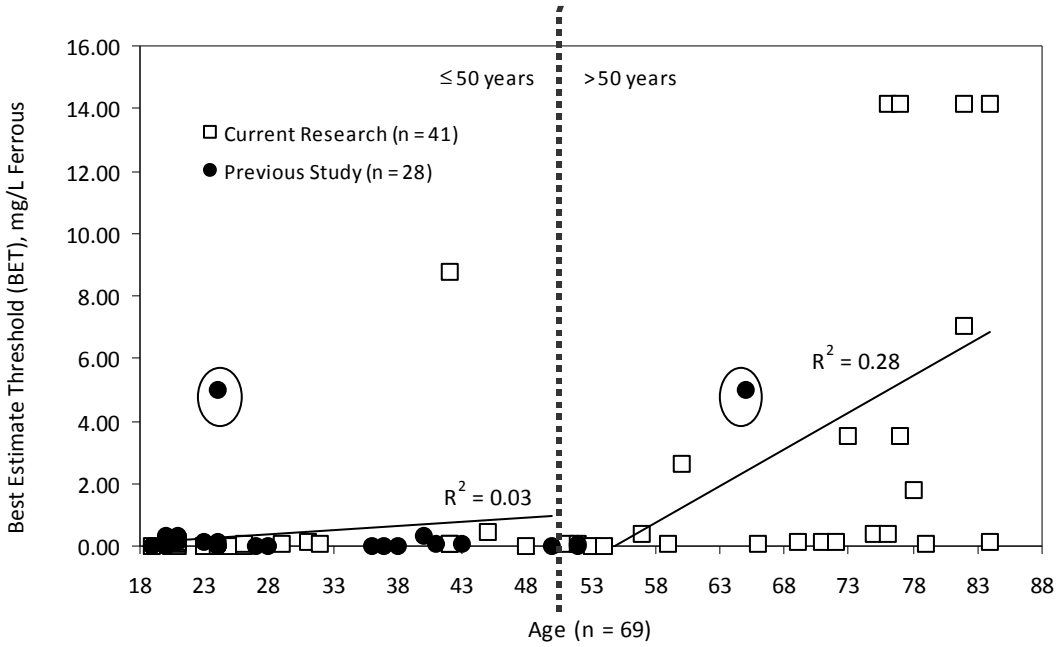

Figure 3.2: Relationship between individuals' flavor thresholds for ferrous-iron (Fe^{2+}) in Nanopure® water versus age, using an ascending forced-choice sensory procedure. The circled data points indicate that the maximum concentration tested was 5 mg/L Fe^{2+}, in these incidences, the individual threshold estimates were reported as greater than 5 mg/L Fe^{2+}.

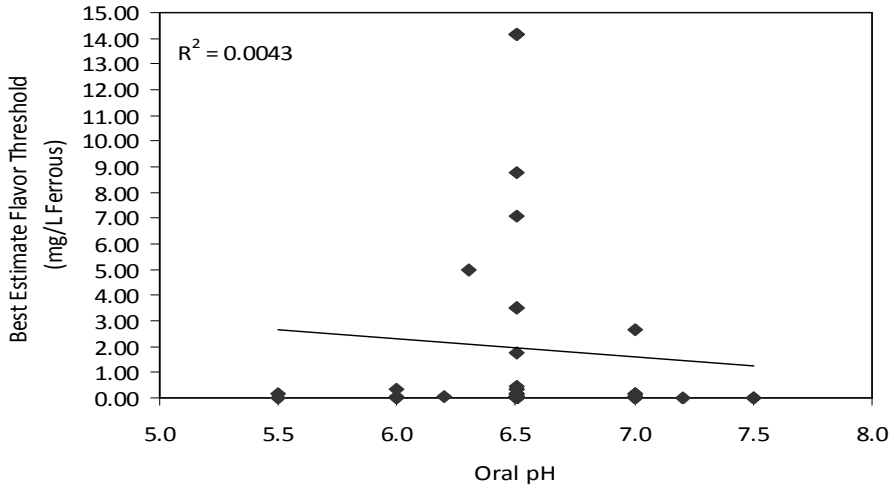

Figure 3.3: Relationship between individuals' flavor thresholds for ferrous-iron in Nanopure® water and their individual median oral pH. Measurements taken in the oral cavity following the completion of each sensory session, using the one-of-five forced choice sensory procedure.

3.3.3 Oral Lipid Oxidation, Age, and Oral pH. Reported values for salivary TBARS in the control samples of our healthy adults ranged from 0.011 – 1.24 µM which is compatible to the range of 0.01 - 2.0 µM for healthy adults as reported in the literature (Celec et al. 2005; Ömur-Özbek 2008). Oral lipid oxidation levels, as measured by µM TBARS/g protein, were considerably higher in the saliva sample collected after tasting of the ferrous containing water than the control saliva sample collected after tasting of Nanopure® water (Figure 3.4). In the control saliva samples, TBARS levels ranged from 0.013 – 1.73 µM/g total protein (mean = 0.451, SD = 0.395). In the ferrous saliva samples, TBARS levels ranged from 0.149 – 5.54 µM/g total protein (mean = 1.43, SD = 1.40). The average TBARS/g total protein for the control and "metal" saliva samples were significantly different ($p < 0.0001$). This observation is consistent with previous research findings that exposure to ferrous iron induces lipid oxidation in the oral cavity, resulting in the formation of volatile flavor compounds associated with retronasal perception of metallic flavor (Lawless et al. 2004; Ömur-Özbek 2008).

No direct correlation was found between oral lipid oxidation and age (Figure 3.4). Participants' oral pH levels showed no, or little, change after tasting the control and ferrous-containing water; this observation was consistent for all participants, regardless of their age.

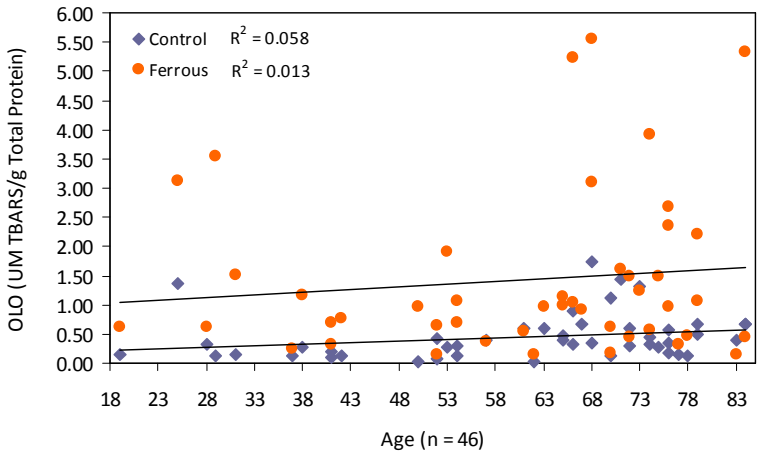

Figure 3.4: Relationship between oral lipid oxidation (OLO) and age. The "Control" data (open diamonds) represent OLO levels measured in the saliva of individuals after ingestion of 2 mL of Nanopure® water. The "Ferrous" data (closed circles) represent OLO levels measured in the saliva after ingestion of Nanopure® water spiked with 10 mg/L of ferrous iron.

3.3.4 Olfactory Functions Assessment. To investigate a possible explanation for the decreased metallic threshold in the >50 year olds, olfactory function was measured for 15 subjects aged 52-84. Individual UPSIT scores ranged from a low of 9 to a high of 38 out of the total possible 40 points. Based on their scores, the subjects were categorized into 4 groups: norosmia (n = 5), mild microsmia (n = 5), moderate microsmia (n = 2) and total anosmia (n = 3). The condition of norosmia is indicative of normal smell functions, while the remaining categories represent mild, moderate, or total impairment of smell functions. When compared with their individual flavor threshold data, reduced or poor sensitivity to the metallic flavor of ferrous iron corresponded with the conditions of mild, moderate, and total anosmia; these were observed among subjects with median ages of 52, 82, and 77 years old, respectively (Figure 3.4). Additionally, a weak linear correlation (R^2 = 0.11) was observed between the individual UPSIT scores and the flavor threshold data (Figure 3.5). Among the subjects, those characterized with "Total Anosmia" (n = 3) were all elderly males, 77-78 years old. These results are consistent with numerous published studies on the prevalence of olfactory impairment in elderly adults. One study on 2,941 residents of Beaver Dam, Wisconsin, USA, aged 53 to 97 years old, reported the prevalence of impaired olfactory functions at 24.5%. The extent of impairment increased dramatically to 62.5% prevalence among those aged 80 – 97 year. Olfactory impairment was also reported to be more prevalent among the male subjects (Murphy et al. 2002).

3.3.5 Discussions on findings. Assessing the potential for individuals sense excess iron in drinking water. In 84% of the test population, which consisted of 69 subjects for the combined current and previous (Ömur-Özbek and Dietrich 2011) studies (Figure 3.1, Table 3.1), individual ferrous flavor thresholds fell well below the USEPA and WHO aesthetic level of 0.3 mg/L, indicating that the aesthetic standard may not protect the majority of the population from experiencing the metallic flavor of iron (Figure 3.6). In such cases, consumer dissatisfaction with aesthetic qualities of water may lead to decreased consumption and tendency to utilize alternative sources, such as bottled water (Burlingame et al. 2007; Dietrich 2006). On the other hand, in this population, the ability to sense iron in drinking water would provide protection against overexposure to

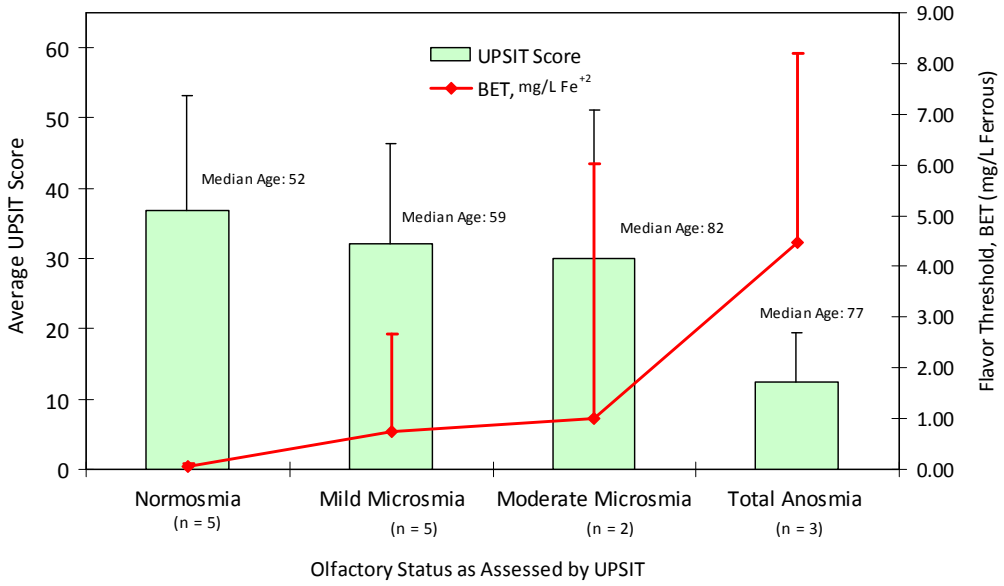

Figure 3.5: Relationship between olfactory functions as assessed by the University of Pennsylvania Smell Identification Test (UPSIT) and flavor thresholds for ferrous-iron (Fe^{2+}) in Nanopure® water.

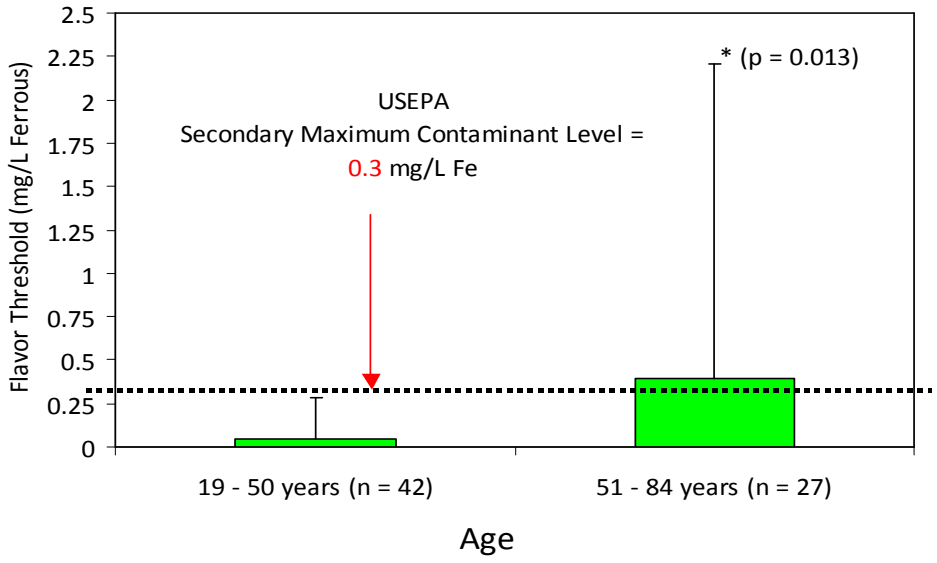

Figure 3.6: Comparison of the geometric mean population flavor thresholds of ferrous iron in Nanopure® water for above and below 50 years old age groups. The dotted line represents the current aesthetic based standard (SMCL) as established by the USEPA. Error bars are based on the standard error of the mean. Asterisk indicates a significantly higher population flavor threshold for the 51-84 years old age group as compared to that of the 19-50 years old age group (p = 0.013).

reduced iron. With regard to consumer ability to perceive the metallic flavor of iron in their drinking water, a recent study found no differences in the detection and recognition thresholds for the perception of ferrous sulfate in deionized water, tap water or spring water (Hoehl et al. 2010). Similarly, no difference was found for the flavor threshold of cupric ion in distilled or tap water as long as the copper remained soluble (Cuppett et al. 2006). These reports suggest that water quality does not impact metallic flavor perception in water.

Not all subjects could taste iron below the USEPA and WHO aesthetic level. Approximately 16% of the studied population (11 out of 69) could not taste 2 mg/L of ferrous iron; the majority (9 out of 11) of these individuals were > 50 years of age. Among this older group, 4 out of 9 could not taste ferrous iron at or above 10 mg/L Fe^{2+}. Iron concentrations above 2 mg/L are not common in oxidized drinking water. However, in anoxic groundwater, typical ferrous iron concentration can range from 0.5 – 10 mg/L Fe and concentrations as high 50 mg/L may be found (World Health Organization (WHO) 2003). At 0.3 – 10 mg/L Fe^{2+} concentration, exposure to iron from drinking water would be about 0.6 – 20 mg Fe, for an average adult consuming 2 liters of water per day. To protect against excessive iron storage in the body, the Joint Expert Committee on Food Additives (JEFCA) established a maximum dietary intake of 0.8 mg /kg of body weight for iron from all sources, excluding iron oxides from food additives and iron supplements for clinical purposes; based on this guideline, allocated dietary intake of iron from drinking water has been estimated at about 2 mg/L (World Health Organization (WHO) 2003). Thus, prolonged exposure to excess iron through consumption of drinking water alone or in water-prepared beverages like tea, coffee, diluted concentrates, along with loss and/or reduced sensitivity to metallic flavor, could pose a risk of overexposure to iron or other flavor producing metals in susceptible individuals.

With regard to toxicity impacts, one reported epidemiological study on 16,408 pregnant, Lithuanian women found that chronic exposure to elevated levels of iron and manganese in drinking water was associated with reduced birth weight in newborn infants. This association was found to be the case for each metal individually as well as

in combination; although the study could not exclude other environmental and/or genetic risk factors (Grazuleviciene et al. 2009). In groundwater, iron is often present in conjunction with manganese (Bouchard et al. 2007), which has an aesthetic threshold of 0.05 mg/L (United States Environmental Protection Agency). Thus, the ability to detect excess iron through sensory perception or the lack thereof could provide a protection against other potentially harmful and less flavorful metals or pose a serious risk of overexposure, respectively.

Examining the biochemical indicators of metallic flavor perception in humans. Oral lipid oxidation has been used as an indicator of oxidative stress as well as metallic flavor production in humans. Lipid oxidation is a measure of oxidative stress in the cellular environment due to an imbalance between oxidants and antioxidants in the body, which can damage macromolecular components of cells such as nucleic acids, proteins, and lipids (Repetto et al. 2010). Specifically, lipid oxidation causes damage to cell membranes by formation of lipid peroxides through generation of reactive free radical species (Armstrong and Browne 1994), including hydroxyl, superoxide anion, singlet oxygen, hydrogen peroxide, and organic compounds that are commonly referred to as reactive oxygen species (ROS). Transition metals such as iron, copper, manganese, and aluminum participate in free radical reactions and facilitate production of ROS in cells; toxicity effects of copper, manganese and iron have been associated with liver damage and neurological diseases (Repetto et al. 2010; Butterworth 2010).

The odor components of volatile secondary metabolites of lipid oxidation contribute significantly to metallic flavor perception. Our study confirms previous findings that drinking ferrous-containing water results in an increase in lipid oxidation (Ömur-Özbek 2008). Using only TBARS as an indicator, we observed no direct correlation between age and level of oral lipid oxidation among our study subjects; however, it is important to note that oral exposure to ferrous iron induced lipid oxidation in all subjects regardless of age and gender. Since the by-products of this biochemical reaction are associated with metallic flavor and retronasal perception, insensitivity to the flavor of iron, in spite of induced oral lipid oxidation, could be indicative of diminished olfactory functions.

Significance of olfactory functions in metallic flavor perception and potential health implications. The influence of olfactory functions on taste perception has been widely studied and reported in the literature (Doty 2009; Stinton et al. 2010). While with some human senses, impairment in one sense leads to strengthening of another, such as loss of vision leading to enhanced hearing and touching abilities, this is not necessarily the case with the senses of taste and smell, as the two are closely related (Landis et al. 2010). With regard to flavor perception, such as metallic, olfactory functions are important as demonstrated to some extent by our current study, as well as by previous studies that examined the impact of nose closure on flavor perception of metals in drinking water (Hettinger et al. 1990; Dietrich 2009; Epke and Lawless 2007). These findings have important health implications, as taste and smell disorders are especially common in aging adults, and the age distribution in the human population is changing. According to the World Health Organization the number of people over 60 years was reported at 650 million in 2007 and projected to reach 2 billion by the year 2025 (World Health Organization (WHO) 2007). The U.S. Census Bureau estimates that the U.S. population older than 65 will increase from 35 million in 2000 to 60 million by the year 2025 (United States Census Bureau 2010). The U.S. National Institute on Deafness and other Communication Disorders estimates that approximately 3 million adult Americans have taste and/or smell disorders (National Institute of Health - National Institute on Deafness and Other Communication Disorders (NIDCD) 2010). Specifically, taste and/or smell impairment is highly prevalent among elderly adults over age 53 and is notably higher among men than women (National Institute of Health - National Institute on Deafness and Other Communication Disorders (NIDCD) 2010). In fact, the risk factor for taste and smell impairment in this population is nearly doubled for every 5-year increase in age and almost tripled in males (Murphy et al. 2002).

Societal implications. This research comprehensively assessed the humans' ability to apply their chemical senses to detect iron contamination in drinking water. A key finding is that adults vary in their ability to apply sensory perception to detect environmental contaminants. Some adults cannot detect iron at concentrations 10- to 45-fold above the drinking water standard and may be susceptible to metal poisoning. This includes

individuals as young as in their 20's, but above age 50 a greater percentage of individuals cannot detect the flavor of reduced iron. While iron is an essential element for living organisms, excessive iron accumulation in the body causes cell and tissue damage by catalyzing reactions that generate free radical reactive oxygen species possibly leading to disease and cancer. Increased iron levels have also been associated with diseases commonly encountered during aging, such as atherosclerosis and Alzheimer's disease (Brewer 2010; Zecca et al. 2004). Considering the decreased recommendation for dietary iron intake for individuals above 50 years of age and the increased potential for excessive accumulation of iron in the body, this raises a concern about the increased risk of iron exposure for older adults from consumption of groundwater, water-prepared foods and beverages, and dietary sources.

3.4 Conclusions

Our findings are consistent with other research that demonstrates a decline in the chemical senses with age, but it is unique in that drinking water is the source of the environmental sensory contaminant and evidence is provided for wide variation in the human population. While our research focused on iron, there are implications for other metal of health concern, most notable copper from copper pipes as our previous research has demonstrated that copper is less flavorful than iron and it is known that copper is also more toxic than iron.

Over the past 40 years, advances in drinking water safety and quality, largely driven by environmental regulations and protection of public health, has dramatically decreased the risk of human exposure to microbiological and chemical contaminants (Roberson 2011). More recently and looking into the future, aesthetic/sensory qualities of drinking water are considered important factors to consumers as measures of preference and perception of safety (Dietrich 2006). In this regard, understanding the population variability of human senses in detecting drinking water contaminants is important both in terms of product acceptability as well as susceptibility of overexposure to potential toxicants.

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References

American Public Health Association (APHA); the American Water Works Association (AWWA); the Water Environment Federation (WEF), ed. **2005**. Standard Methods for Examination of Water and Wastewater. Washington DC.

American Standards for Testing Materials (ASTM). **1997**. Standard Practice for Determination of Odor and Taste Thresholds by a Forced Choice Ascending Concentration series Method of Limits. E-679-79. (Annual Book of Standards). Philadelphia, PA: American Society for Testing of Materials (ASTM).

Armstrong D, Browne R. **1994**. The analysis of free radicals, lipid peroxides, antioxidant enzymes and compounds to oxidative stress as applied to the clinical chemistry laboratory. Free Rad Diag Med 366.

Asante KA, Agusa T, Subramanian A, Ansa-Asare OD, Biney CA, Tanabe S. **2007**. Contamination status of arsenic and other trace elements in drinking water and residents from Tarkwa, a historic mining township in Ghana. Chemosphere 66(8): 1513-1522.

Bartoshuk LM, Duffy VB, Miller IJ. **1994**. PTC/PROP Tasting – Anatomy, Psychophysics, and sex effects. Physiol Behav 56(6): 1165-1171.

Bartoshuk LM. **1994**. Chemical Senses. Annu Rev Psychol 45: 419-449.

Benjamin MM. **2002**. Water Chemistry, 1st ed.: McGraw-Hill.

Bouchard M, Laforest F, Vandelac L, Bellinger D, Mergler D. **2007**. Hair manganese and hyperactive behaviors: Pilot study of school-age children exposed through tap water. Environ Health Perspect 115(1): 122-127.

Brewer GJ. **2010**. Risks of copper and iron toxicity during aging in humans. Chem Res Toxicol 23(2): 319-326.

Buck L, Axel R. **1991**. A Novel Multigene Family May Encode Odorant Receptors – A Molecular-Basis for Odor Recognition. Cell 65(1): 175-187.

Buck LB. **2004**. Olfactory receptors and odor coding in mammals. Nutr Rev 62(11): S184-S188.

Burlingame GA, Dietrich AM, Whelton AJ. **2007**. Understanding the basics of tap water taste. J Am Water Work Assoc 99(5): 100-+.

Buss JL, Greene BT, Turner J, Torti FM, Torti SV. **2004**. Iron chelators in cancer chemotherapy. Curr Top Med Chem 4(15): 1623-1635.

Butterworth RF. **2010**. Metal toxicity, liver disease and neurodegeneration. Neurotox Res 18(1): 100-105.

Celec P, Hodosy J, Celecova V, Vodrazka J, Cervenka T, Halcak L, et al. **2005**. Salivary thiobarbituric acid reacting substances and malondialdehyde – Their relationship to reported smoking and to parodontal status described by the papillary bleeding index. Disease Markers 21(3): 133-137.

Chai LY, Wang ZX, Wang YY, Yang ZH, Wang HY, Wu X. **2010**. Ingestion risks of metals in groundwater based on TIN model and dose-response assessment – A case study in the Xiangjiang watershed, central-south China. Sci Total Environ 408(16): 3118-3124.

Cohen J. **1960**. Taste threshold concentrations of metals in drinking water. Journal of American Water Works Association 52(5): 10.

Concha G, Broberg K, Grander M, Cardozo A, Palm B, Vahter M. **2010**. High-Level Exposure to Lithium, Boron, Cesium, and Arsenic via Drinking Water in the Andes of Northern Argentina. Environ Sci Technol 44(17): 6875-6880.

Copeland RA. **1994**. Methods for protein quantification.Chap.3.:Methods for Protein Analysis: A Practical Guide to Laboratory Protocols. In: Chapman and Hall (Ed), New York, NY, p 39-58.

Craun GF, Brunkard JM, Yoder JS, Roberts VA, Carpenter J, Wade T, et al. **2010**. Causes of Outbreaks Associated with Drinking Water in the United States from 1971 to 2006. Clin Microbiol Rev 23(3): 507-+.

Cuppett JD, Duncan SE, Dietrich AM. **2006**. Evaluation of copper speciation and water quality factors that affect aqueous copper tasting response. Chemical Senses 31(7): 689-697.

DeSimones LA, Hamilton PA, Gilliom RJ. **2009**. The quality of our nation's waters-Quality of water from domestic wells in principal aquifers of the United States, 1991–2004-Overview of major findings: U.S. Geological Survey Circular 1332 U.S. Department of Interior.

Dietrich AM, Glindemann D, Pizarro F, Gidi V, Olivares M, Araya M, et al. **2004**. Health and aesthetic impacts of copper corrosion on drinking water. Water Sci Technol 49(2): 55-62.

Dietrich AM. **2006**. Aesthetic issues for drinking water. Journal of Water Health 4(Suppl. 1): 11-16.

Dietrich AM. **2009**. The sense of smell: contributions of orthonasal and retronasal perception applied to metallic flavor of drinking water. J Water Supply Res Technol-Aqua 58(8): 562-570.

Domke A, Grossklaus R, Niemann H, Przyrenbel H, Richter K, Schmidt E, et al. **2005**. Use of Minerals in Foods – Toxicological and nutritional-physiological aspects. 1614-3795. Berlin:BfR-Hausdruckerei Dahlem.

Doty RL, Shaman P, Applebaum SL, Giberson R, Siksorski L, Rosenberg L. **1984**. Smell Identification Ability – Changes with Ages. Science 226(4681): 1441-1443.

Doty RL. **1997**. Studies of human olfaction from the University of Pennsylvania Smell and Taste Center. Chemical Senses 22(5): 565-586.

Doty RL. **2008**. The smell identification Test™ administration manual: 3rd Edition. Haddon Heights, New Jersey.

Doty RL. **2009**. The Olfactory System and Its Disorders. Semin Neurol 29(1): 74-81.

Edwards M, Triantafyllidou S, Best D. **2009**. Elevated Blood Lead in Young Children Due to Lead-Contaminated Drinking Water: Washington, DC, 2001-2004. Environ Sci Technol 43(5): 1618-1623.

Epke E, Lawless H. **2007**. Retronasal smell and detection thresholds of iron and copper salts. Physiology and Behavior 92: 487-491.

Grazuleviciene R, Nadisauskiene R, Buinauskiene J, Grazulevicius T. **2009**. Effects of Elevated Levels of Manganese and Iron in Drinking Water on Birth Outcomes. Pol J Environ Stud 18(5): 819-825.

Hettinger TP, Myers WE, Frank ME. **1990**. Role of olfaction in perception of non – traditional 'taste' stimuli. Chem Senses 15(6): 755-760.

Hoehl K, Schoenberger GU, Busch-Stockfisch M. **2010**. Water quality and taste sensitivity for basic tastes and metallic sensation. Food Qual Prefer 21(2): 243-249.

Hong JH, Duncan SE, Dietrich AM, O'Keefe SF. **2010b**. Evaluation of the operationally defined soluble, insoluble, and complexing copper consumed through drinking water in human saliva. Eur Food Res Technol 231(6): 977-984.

Hong JH, Duncan SE, Dietrich AM. **2010a**. Effect of copper speciation at different pH on temporal sensory attributes of copper. Food Qual Prefer 21(1): 132-139.

Hrudey SE, Hrudey EJ. **2004**. Safe Drinking Water: Lessons from Recent Outbreaks in Affluent Nations.: International Water Association.

Kovacevic Z, Yu Y, Richardson D. **2011**. Chelators to the Rescue: Different Horses for Different Courses! Chem Res Toxicol 24(3): 279-282.

Kroll D. year. "Wining" about Corked Tylenol. **2011**. Available: http://cenblog.org/terra-sigillata/2010/10/24/wining-about-corked-tylenol/ [Accessed January 24, 2011].

Landis BN, Scheibe M, Weber C, Berger R, Bramerson A, Bende M, et al. **2010**. Chemosensory interaction: acquired olfactory impairment is associated with decreased taste function. J Neurol 257(8): 1303-1308.

Lawless HT, Heymann H. **1998**. Sensory Evaluation of Food: Principles and Practices. NY: Chapman & Hall.

Lawless HT, Shlake S, Smythe J, Lim J, Yang H, Chapman K, et al. **2004**. Metallic taste and retronasal smell. Chemical Senses 29(1): 25-33.

Lim J, Lawless H. 2006. Detection thresholds and taste qualities of iron salts. Food Qual Prefer 17(6): 513-521.

Malleret L, Bruchet A. **2002**. A taste and odor episode caused by 2,4,6-tribromoanisole. Journal of American Water Works Association 94(7): 84-95.

Miller G. **2004**. 2004 Nobel Prizes – Axel buck share award for deciphering how the nose knows. Science 306(5694): 207-207.

Mojet J, Christ-Hazelhof E, Heidema J. **2001**. Taste perception with age: generic or specific losses in threshold sensitivity to the five basic tastes? Chemical Senses 26(7): 845-860.

Murphy C, Schubert CR, Cruickshanks KJ, Klein BEK, Klein R, Nondahl DM. **2002**. Prevalence of olfactory impairment in older adults. JAMA-J Am Med Assoc 288(18): 2307-2312.

National Institute of Health – National Institute on Deafness and Other Communication Disorders (NIDCD). **2011**. Smell and Taste. Available: http://www.nidcd.nih.gov/health/smelltaste/ [Accessed May 2011].

Ömur-Özbek P, Dietrich AM. **2011**. Retronasal perception and flavour thresholds of iron and copper in drinking water. J Water Health 9(1): 1-9.

Ömur-Özbek P. **2008**. Macromolecular Reactions and Sensory Perceptions at the Air-Water-Human Interface. [PhD Dissertation]. Blacksburg, VA: Virginia Tech.

Papanikolaou G, Pantopoulos K. **2005**. Iron metabolism and toxicity. Toxicol Appl Pharmacol 202(2): 199-211.

Peto MV. **2010**. Aluminium and Iron in Humans: Bioaccumulation, Pathology, and Removal. Rejuv Res 13(5): 589-598.

Piriou P, Devesa R, De Lalande M, Glucina K. **2009**. European reassessment of MIB and geosmin perception in drinking water. J Water Supply Res Technol-Aqua 58(8): 532-538.

Reed DR, Knaapila A. **2010**. Genetics of Taste and Smell: Poisons and Pleasures. In: Progress in Molecular Biology and Translational Science, Vol 94: Genes and Obesity, Vol. 94. San Diego:Elsevier Academic Press Inc, 213-240.

Repetto MG, Ferrarotti NF, Boveris A. **2010**. The involvement of transition metal ions on iron-dependent lipid peroxidation. Arch Toxicol 84(4): 255-262.

Roberson J. **2011**. What's Next after 40 Years of Drinking Water Regulations? Environ Sci Technol 45: 154-160.

Scottish Government. **2006**. Drinking Water Quality in Scotland 2006: Annual Report by the Drinking Water Quality Regulator. Available:

http://www.scotland.gov.uk/Publications/2008/03/05164641/9 [Accessed: February 2011].

Senga EL, Harper G, Koshy G, Kazembe PN, Brabin BJ. **2011**. Reduced risk for placental malaria in iron deficient women. Malar J 10 (Article Number 47).

Spanier AM. **1991**. A rapid, direct chemical assay for the quantitative determination of thiobarbituric acid reactive substances in raw, cooked, and cooked/stored muscle foods. Journal of Muscle Foods 2(3): 165-175.

Stinton N, Atif MA, Barkat N, Doty RL. **2010**. Influence of Smell Loss on Taste Function. Behav Neurosci 124(2): 256-264.

Suffet I, Corado A, Chou D, McGuire M, Butterworth S. **1996**. AWWA Taste and Odor Survey. J Am Water Work Assoc 88(4): 168-180.

United States Census Bureau. **2011**. Projections of the United States resident population by age, sex, race, and Hispanic origin. Available: http://www.census.gov/population/www/projections/natproj.html [Accessed March 2011.

United States Environmental Protection Agency. **2003**. National secondary drinking water regulations. Codes of Federal Register 40, Part 143, July 1, 2003. Available: http://www.access.gpo.gov/nara/cfr/waisidx_03/40cfr143_03.html [Accessed May 2011.

Volke C, Dundore E, Schiermann J, Lechevallier M. **2000**. Practical evaluation of iron corrosion control in a drinking water distribution system. Water Research 34(6): 1967-1974.

Wang H. **2009**. Determination of thiobarbituric acid reactive substances (TBARS) for water base liquid food with low oxidation. In: Unpublished: Virginia Tech.

Weinberg ED. **2010**. The hazards of iron loading. Metallomics 2(11): 732-740.

Whelton AJ, Dietrich AM. **2004**. Relationship between intensity, concentration, and temperature for drinking water odorants. Water Research 38(6): 1604-1614.

World Health Organization (WHO). **2003**. Iron in drinking-water. Background document for preparation of WHO Guidelines for drinking-water quality. WHO/SDE/WSH/03.04/8.

World Health Organization (WHO). **2011**. 10 facts on aging and the life course. Available: http://www.who.int/features/factfiles/ageing/en/index.html [Accessed May 2011].

Young WF, Horth H, Crane R, Ogden T, Arnott M. **1996**. Taste and odour threshold concentrations of potential potable water contaminants. Water Research 30(2): 331-340.

Zacharski LR, Chow BK, Howes PS, Shamayeva G, Baron JA, Dalman RL, et al. **2008**. Decreased cancer risk after iron reduction in patients with peripheral arterial disease: Results from a randomized trial. J Natl Cancer Inst 100(14): 996-1002.

Zacharski LR, Ornstein DL, Woloshin S, Schwartz LM. **2000**. Association of age, sex, and race with body iron stores in adults: Analysis of NHANES III data. Am Heart J 140(1): 98-104.

Zecca L, Youdim MBH, Riederer P, Connor JR, Crichton RR. **2004**. Iron, brain ageing and neurodegenerative disorders. Nat Rev Neurosci 5(11): 863-873.

Zhang X, Chen C, Lin P, Hou A, Niu Z, Wang J. **2011**. Emergency Drinking Water Treatment during Source Pollution Accidents in China: Origin, Analysis, Framework, and Technologies. Environ Sci Technol 45(1): 161-167.

Chapter IV

Analysis of Salivary Fluid and Chemosensory Functions in Patients Treated for Primary Malignant Brain Tumors

Abstract

Disorders of chemosensory (taste and smell) functions are common yet understudied side effects of cancer and its treatment. This study examined the impact of cancer therapy on chemosensory functions and salivary constituents. Twenty-two brain cancer patients (10 females), median age 60, newly diagnosed with primary malignant gliomas were monitored over a 30-week period; they underwent 6-weeks of combined modality treatment (CMT) with radiation and the alkylating agent temozolomide followed by an additional 6 monthly cycles of temozolomide. Of the 22 cancer patients, 17 patients (7 females) had baseline data and were included in the data analyses. Chemosensory functions were assessed at 0 (baseline), 3, 6, 10, 18, and 30 weeks, using a validated questionnaire that provided a chemosensory complaint score (CSCS) ranging from 0 (normal) to 16 (maximally impaired). At each time-point, paired samples of saliva were collected before and after an oral rinse with 10 ± 1 mg/L ferrous-iron containing water. Oxidative stress response was determined by oral lipid oxidation (OLO) using thiobarbituric acid reactive substances (TBARS) method; salivary electrolytes and metallic constituents were determined using inductively coupled plasma (ICP) spectroscopy. Parallel analyses were performed on a corresponding group of 22 healthy subjects (14 females), median age 58. Chemosensory assessment indicated that the mean CSCS in cancer patients increased significantly at all time-points ($p = 0.04$) exept at 30-week. This corresponded with a notable, but nonsignificant increase in the salivary oxidative responses at the 10- and 18- week timepoints ($p = 0.13$). When compared to the healthy subjects, salivary oxidative stress response, total protein, Na, K, Cl, P, S, and Mg serum levels, as averaged across all times, were significantly higher in cancer patients ($p < 0.05$), while salivary Zn, Fe, and oral pH levels were significantly lower in cancer patients ($p < 0.05$). Neither time nor treatment had a significant impact on the total salivary protein, metals, and electrolytes ($p > 0.05$).

The findings indicates that the impact of cancer treatment on chemosensory functions of patients treated for primary malignant gliomas can range from minimal to moderate impairment with maximum impairment developing during the 6-week CMT. Study of salivary constituents may provide clues to the causes of taste and smell disorders.

Key Words: metallic flavor, oral lipid oxidation, cancer, brain tumor, chemotherapy, saliva, taste and smell

4.1 Introduction

Taste and smell functions are critical aspects of human physiology. They provide us with the pleasures of experiencing flavors and aromas in foods and beverages and the smells of fragrances. They also protect us from the dangers of ingested or inhaled toxins. Thus, disorders of taste and smell functions can have important health implications as well as debilitating effects on our quality of life. Many factors have been associated with taste and smell loss and/or disorders, namely, normal aging, environmental exposure, nasal congestion and allergies, prescription medication, head trauma, neurological diseases such as Alzheimer's, and cancer and its treatment (Ackerman and Kasbekar 1997; Bernhardson et al. 2007; Boyce and Shone 2006; Bromley 2000; Deems et al. 1991; Doty 2009).

It is widely known that cancer patients experience loss or altered taste and smell functions due to the disease itself and/or the effects of chemotherapy and radiation. As early as the 1970s, unpleasant changes in taste and smell functions were reported in cancer patients (Dewys and Walters 1975); while taste alterations have been associated with the occurrence of anorexia (DeWys 1977) as well as negative short-term and long-term impact on the patients' quality of life (de Graeff et al. 2000; Hong et al. 2009).

Nearly two million cancer patients receiving chemotherapy and radiotherapy experience metallic flavor perception after consuming foods and beverages (Comeau et al. 2001). Oral pain and metallic taste phantom phenomenon have been found to be significantly higher in cancer survivors than in healthy individuals (Logan et al. 2008). The most

prevailing taste alteration experienced by cancer patients is the perception of a metallic and bitter taste or aftertaste (McGowan 2008). Taste qualities have also been described as “nauseating” or “unpleasant” by cancer patients of all ages, including pediatric patients (Di Fiore and Rigal 2009; McDaniel 1998; Skolin et al. 2006). These issues have broad impacts on the quality of life for these patients and often lead to other implications such as reduced oral intake and nutritional deficiency, weight loss, and depression (Epstein and Barasch 2010). While these problems are significant, there are only a limited number of studies exploring their causes and possible treatments.

The underlying causes of metallic flavor sensation in humans are not well understood. Recent findings have shown that a mechanism responsible for the generation of metallic flavor is lipid oxidation. On the human skin surface, lipid oxidation has been linked to the production of “metallic” odor compounds produced when a metallic object such as a key or a penny is held in the hand (Glindemann et al. 2006). Occurrence of metal-induced lipid oxidation has also been demonstrated in the oral cavity of healthy human subjects when drinking water spiked with copper and iron was consumed (Mirlohi et al. 2011; Ömur-Özbek 2008). Oxidative stress in a cellular environment can be caused by metal ions and result in the generation of highly reactive and unstable lipid hydroperoxides (Catala 2009). Within the oral cavity, lipid oxidation is associated with oxidation of polyunsaturated fatty acids in the oral mucosal cell membranes or the salivary fluid lipids (Larsson et al. 1996). The decomposition of the hydroperoxides leads to the formation of secondary by-products, such as aldehydes, and ketones including malondialdehyde (MDA) (Catala 2009). MDA and other by-products of lipid oxidation can be quantified colorimetrically after the controlled reaction with thiobarbituric acid (Yagi 1998). The measurement of thiobarbituric acid reactive substances (TBARS) has been a widely used method for screening and monitoring lipid peroxidation since the early 80’s (Guillen-Sans and Guzman-Chozas 1998). The TBARS method has been used to assess off-flavors in food products and oxidative stress in samples that include biological tissues and fluids associated with cellular toxicity and study of human diseases (Yagi 1998; Shpitzer et al. 2007; Sun et al. 2011).

It has been shown that metallic flavor can be reduced or prevented by use of antioxidants and/or chelating agents (Ömur-Özbek 2008), which act by preventing lipid oxidation reaction when administered orally. Taste disorders have often been associated with zinc deficiency, and zinc supplementation has been somewhat effectively used in such treatments (Fukasawa et al. 2008; Kettaneh et al. 2002; Stewart-Knox et al. 2005).

In cancer patients, metallic and bitter taste sensations have been associated with low levels of irradiation and chemotherapy using several tested chemotherapeutic drugs including cyclophosphamide, doxorubicin, 5-fluorouracil, methotrexate, paclitaxel and cisplatin (Comeau et al. 2001; Capra 2001). Seventy-seven percent of patients treated with cisplatin alone or in combination with other chemotherapeutic agents reported metallic taste perception (Ravasco 2005). Release of chemotherapy drugs into the salivary fluid and interaction with taste buds can cause impairments by direct contact with taste receptor cells. Additionally, damage to sensory nerves such as the chorda tympani can impact the olfactory system and consequently cause disturbances in taste and smell functions (Epstein and Barasch 2010).

Other possible causes of unpleasant taste alterations that cancer patients experience could be associated with poor oral hygiene and/or infections within the oral cavity (Hong et al. 2009; Mosel et al. 2011). Others have noted that taste alterations may in part be due to psychological causes when patients complain about taste (Comeau et al. 2001; Epstein et al. 2002).

As side effects of treatment, taste and smell abnormalities (TSA) are most commonly reported in head and neck cancer cases (Logan et al. 2008; Mirza et al. 2008), including cancers of the throat, nasal/oral cavities, and salivary glands (NCI, 2011). TSA symptoms are anticipated in other types of cancers where body regions associated with taste and smell functions are affected by the field of radiation treatment. This is the case for primary malignant brain tumors or gliomas. Currently, the most common treatment for such tumors is surgery, followed by concurrent radiation and chemotherapy—typically an orally administered drug called temozolomide (Temodar).

Temozolomide is then given on monthly cycles for 6-12 months as maintenance therapy (Stupp et al. 2005).

The Central Brain Tumor Registry of the U.S. (CBTRUS) has reported that approximately 24,000 new primary malignant brain tumors are diagnosed in the United States each year. While most prevalent among the elderly (greater than 50 years of age), brain tumors occur in all ages and are the second most frequent malignancy of childhood after leukemia (CBTRUS 2011). Currently, no prospective data is available on the incidence, severity or duration of the taste and smell abnormalities experienced by brain tumor patients undergoing concurrent radiation and chemotherapy. Anecdotally, a large number of these patients develop dramatic alterations in taste perception, food texture, quality and the sensation of a metallic taste within several weeks of beginning radiation therapy, with or without concurrent chemotherapy (Lesser 2011). These alterations lead to food aversion, weight loss and a profoundly decreased quality of life (Bernhardson et al. 2007; Hong et al. 2009). They may last for up to several months after the radiation has been completed and, on occasion, normal taste and odor sensation never returns (Bernhardson et al. 2007). The objectives of this study were 1) to quantify TSA abnormalities in a small cohort of newly diagnosed primary glioma patients treated with CMT; 2) to assess the etiologic role of salivary oxidative stress and constituents, as cause of the observed sensory changes; and 3) to compare the salivary constituents in the cancer patients with healthy subjects. It is hoped that the findings from this research will provide baseline data and lead to future trials utilizing an intervention to prevent or treat taste and smell disturbances in similar patient populations.

4.2 Materials and Methods

4.2.1 Human Subjects. This study was approved by the Institutional Review Boards (IRB) at Virginia Tech and the Wake-Forest School of Medicine. Twenty-two cancer patients (10 Females), age range 20 to 79 years (median age 60), were recruited from the brain tumor clinic at the Comprehensive Cancer Center of Wake Forest University (CCCWFU). Eligibility criteria included age greater than 18 years, a newly diagnosed primary malignant brain tumor, anticipated combined modality therapy, and an expected

survival of at least 6 months. Ineligible patients included those with an extreme dry mouth syndrome that prevented them from producing adequate amounts of saliva (about 2 mL in 15-20 minutes), known HIV positive, and patients with any of the following conditions: untreated gastroesophageal reflux disease; uncontrolled diabetes; active oral infections including thrush; or evidence of active mucositis. Additionally, 22 healthy subjects (14 Females), age range 21 to 82 years (median age 58), were recruited from the community, students, faculty and staff of Virginia Tech and Blacksburg, Virginia. Healthy subjects were required to have no chronic oral or general health problems, be non-smokers, and not pregnant. All subjects read and signed an informed consent form in accordance with the approved IRB protocols.

4.2.2 Cancer Treatment Plan. Cancer patients were subjected to a combined modality treatment (CMT), comprised of standard radiation therapy (RT) with concurrent temozolomide (TEM) given over 6 weeks followed by adjuvant TEM given for 5 days each month for an additional 6 months. Patients underwent an initial saliva analysis and chemosensory assessment prior to beginning CMT. Repeat analyses were performed following 3, 6, 10, 18, and 30 weeks of treatment. For healthy subjects, saliva analysis was performed at 0 (baseline), 3, 6, 10, 18, and 30 weeks (Figure 4.1)

4.2.3 Saliva Collection and Analysis. At baseline and each time point (Figure 4.1), saliva samples were collected from subjects (that had not consumed food or beverages and had not smoked for at least one hour prior to testing) as follows. First, subjects rinsed their mouth using purified water (Aquafina®). After a 1-minute rest, they sipped 2 mL of purified water (Aquafina®), as the control sample, then, swished it around their mouth for 20 seconds, and, without swallowing, expectorated saliva into a clean sample collection tube until approximately 4 mL of saliva (control) was collected. After a short rest period, subjects sipped 2 mL of ferrous sulfate solution (at 10 $\pm$ 1 mg/L ferrous), swished the solution around their mouth, and expectorated the second (ferrous) 4 mL saliva sample. Subjects were asked to put on nose clips before sipping the metal salt solution to help evaluate the retronasal component of the metallic flavor sensation. Subjects' oral pH was measured using a pH indicator strip (Cen-Med/Fisher M95883) after sampling the control solution and again after sampling the metal salt solution.

Saliva samples were frozen immediately and stored at -50 ºC for up to three months until analysis. Saliva analysis included the measurement of salivary oxidative stress response as TBARS, total protein, and total metals, nonmetals, and electrolytes.

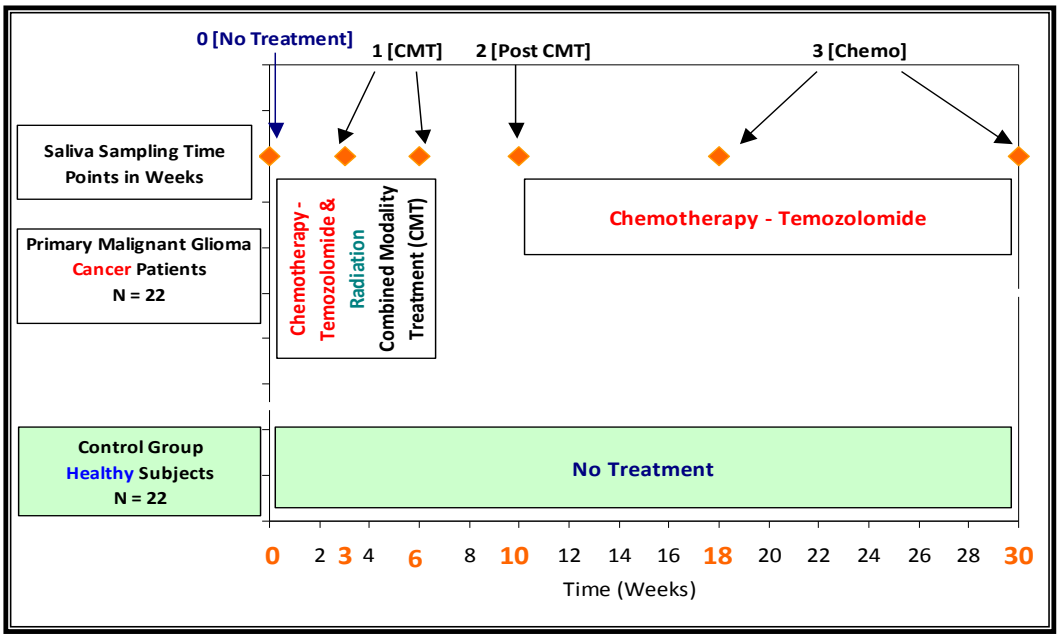

Figure 4.1: Saliva collection and cancer treatment regimen. Study subjects donated saliva at time 0, 3, 6, 10, 18, and 30-weeks. For cancer patients, time 0 represents the baseline (prior to the start of cancer treatment). Treatment designation for data plotting and analysis purposes include: 0 [No treatment or None], representing baseline or 0 time; 1 [CMT] representing 3- and 6-week times; 2[Post CMT], representing 10-week time; and 3 [Chemo], representing 18- and 30-week times. CMT = Combined Modality Treatment.

4.2.4 Salivary Oxidative Stress. TBARS method was used for the measurement of salivary oxidative stress response. The method was modified from Spanier's to work with liquid samples and to enhance readings at low concentrations (Spanier 1991; Wang 2009). For TBARS analysis, 1 mL of saliva sample and known concentrations of MDA standard were mixed with 2 mL of thiobarbituric acid (TBA) solution and digested for 60 minutes at 95℃ in a water bath. Then, the samples were immediately cooled in an ice bath, mixed with 2 mL of n-butanol/pyridine mixture (at 15:1 ratio), and centrifuged for 15 minutes at a speed of 3000 g. The absorbance of the supernatant was measured with a spectrophotometer at 532 nm for the samples and standard curve. The concentration of TBARS was obtained from the standard curve and absorbance values. The dilution effect was taken into consideration in the calculations.

4.2.5 Salivary Protein. Since the protein content of saliva can vary among individuals, to report the TBARS results more accurately, the total protein content of the saliva of the patients, as well as the healthy individuals, was measured and the results were reported as μM TBARS produced per gram of protein. Total protein content of saliva was analyzed using the Bradford assay (Copeland 1994). A standard curve was obtained by using bovine serum albumin (BSA) at 1mg/mL concentration. To perform the analysis, 1 mL of Bradford reagent was mixed with 8 to 20 μL of saliva. The samples were vortexed and 100 μL of the mixture was transferred to the well plates and read by spectrophotometer at 595 nm. Duplicates of each sample were analyzed and the total protein content of the saliva was determined from the BSA standard curve.

4.2.6 Salivary Metals, Nonmetals, and Electrolytes. Saliva samples were first thawed at room temperature. Then, they were diluted with deionized water at 1:10 ratio and digested with trace metals grade nitric acid at 90 ℃ for 45 minutes; hydrogen peroxide was added and the solution was heated up to 130 ℃ until the mixture became clear as described by the method from the U.S. Environmental Protection Agency (EPA method 3050B). After digestion, the samples were adjusted back to their original dilution and acidified with 2% trace metals grade nitric acid prior to being analyzed by emission spectroscopy using Inductively Coupled Plasma (ICP) technique (EPA method 3050B).

4.2.7 Chemosensory Assessment. There are no standard validated tools specifically designed to assess taste and smell in cancer patients. Thus, as an exploratory endpoint, self-perceived taste and smell functions were assessed using a questionnaire (Appendix B) which has been used to evaluate chemosensory functions in AIDS patients (Hutton et al. 2007). As part of the questionnaire, patients were asked to rate their individual taste and smell abnormalities as “insignificant,” “mild,” “moderate,” “severe,” or “incapacitating.” The tool yields a taste complaint score (0-10) based on the subjects’ responses to nine questions addressing changes to the sense of taste since the start of their cancer treatment. The questionnaire was completed prior to the start of treatment (baseline) and at 3, 6, 10, 18, and 30-week time points following the baseline; the cancer treatment regimen during this monitoring period was presented

earlier in Figure 4.1. To score the questionnaire, one point was added for each reported taste complaint and two points for a rating of “severe” or “incapacitating” on the severity of the taste abnormality. Similarly, a smell complaint score (0-6) was generated by adding one point for a positive response to each of five questions addressing self-perceived changes to the sense of smell. Two points were assigned to a severity rating of “severe” or “incapacitating” for the smell abnormality. The total chemosensory complaint score, CSCS (0-16), was calculated by adding the taste and smell complaint scores.

4.2.8 Data Analyses. Analysis of variance (ANOVA) with repeated measures, treating subjects as random effect and utilizing a univariate or adjusted univariate approach, was used; while, the multivariate repeated measure ANOVA was used where there were no missing data. For comparison of the means on the continuous variables, Student’s t-Test and/or Tukey HSD or Wilcoxon/Kruskal Wallis Rank sum test, were performed. For the salivary parameters, comparative analysis was performed on the mean responses between the healthy subjects and cancer patients, averaged within a person, across all times. For the cancer group, data analysis also compared the mean responses of salivary parameters at baseline (time 0), during CMT (3-week and 6- week time points; averaged within a person across those times), post- CMT (10-week time point), and during adjuvant monthly TEM (18- and 30-week time points; averaged within a person across those times). For the iron-induced oral oxidative stress response, delta OLO, as the arithmetic difference between the measured salivary TBARS before (control) and after (ferrous) oral rinse with ferrous spiked water was used for the data analysis. For chemosensory assessment, reported TSA responses as quantified by the CSC scores were plotted against time-points, 0 (baseline), CMT (3-week and 6-week), and post CMT (10-week). Using bivariate linear correlation analysis at each time point, the CSC scores were compared to the corresponding iron-induced OLO responses to identify any correlations between the observed taste and smell abnormalities and iron-induced oral oxidative stress response. Statistical software, JMP 9.0 (SAS, Cary, NC), was used for most data analyses; p-values less than 0.05 were considered statistically significant. Additionally, for the delta OLO and CSC data, repeated analysis of

variances were performed using a mixed modeling approach available through the SAS software, not JMP 9.0. The additional SAS analyses were conducted by the statistician at Wake-Forest cancer center.

4.3 Results and Discussion

4.3.1 Salivary Oxidative Stress Response. Salivary lipid oxidation (OLO) levels before and after oral rinse with ferrous-spiked water, as well as the pre/post change (denoted delta OLO) are summarized in Table 4.1 by group and time. Mixed models were used separately for the cancer patients and the healthy controls to assess the significance of the pre/post rinse differences and to assess the significance of the changes in delta OLO over time. Salivary lipid oxidation (OLO) levels typically increased after oral rinse with ferrous-spiked water in both cancer patients and healthy subjects. This increase in OLO response after ferrous rinse, comparing the mean OLO responses before and after ferrous rinse, was consistently significant in healthy subjects ($p < 0.004$ for all times) and for cancer patients ($p < 0.034$ for all times). To simplify and facilitate comparisons between OLO responses at different time points, subsequent analysis of the data was performed using delta OLO as the dependent variable. As noted earlier, delta OLO represents the iron-induced OLO response calculated by the arithmetic difference between the measured salivary TBARS before (control) and after (ferrous) oral rinse with ferrous spiked water. The change in delta OLO over time was not statistically significant for either the cancer patients [$F(5,16) = 2.00$, $p = 0.134$] or the healthy subjects [$F(5, 21) = 2.05$, $p = 0.113$]. As shown in Figure 4.2, the changes in delta OLO were highly variable for the cancer patients, ranging from 0.53 to 5.94 TBARS/g protein; the changes in delta OLO over time were much more consistent for the control patients with mean delta OLO ranging from 0.71 to 1.04 µM TBARS/g protein. Likewise, when mean delta OLO responses for the cancer patients were evaluated by treatment phase, the differences were nonsignificant [$F(3,16) = 2.12$, $p = 0.138$].

To better display the within-subject variability over time for both cancer patients and healthy subjects, additional plots of the delta OLO data are presented in Appendix C.

Table 4.1: Summary of oxidative stress response and chemosensory assessment scores.

Cancer Group			Mean ± Standard Deviation Salivary Oxidative Stress/Oral Lipid Oxidation (OLO) Response [1] (uM TBARS/g Protein)			Mean ± Standard Deviation	Mean ± Standard Deviation Chemosensory Complaint Score (CSCS) (#, 0 - 16)
Time (Weeks)	Cancer Treatment	N	Before Fe^{+2} Rinse (Control)	After Fe^{+2} Rinse (Ferrous)	Delta OLO [3]	Oral pH	
0 (Baseline)	None	17	0.84 ± 1.43 [0.11 - 1.58] [2]	1.90 ± 2.01 [0.87 - 2.94]	1.16 ± 1.60 [0.33 - 1.98]	6.54 ± 0.88 [6.01 - 7.07]	1.59 ± 2.37 (N = 17) [4] [0.37 - 2.81]
3	CMT	15	1.06 ± 1.22 [0.39 - 1.74]	1.83 ± 1.64 [0.92 - 2.74]	0.79 ± 1.14 [0.16 - 1.43]	6.30 ± 0.70 [5.91 - 6.69]	3.93 ± 4.14 (N = 14) [1.54 - 6.32]
6	CMT	15	0.67 ± 1.33 [-0.06 - 1.41]	0.89 ± 0.68 [0.51 - 1.27]	0.78 ± 1.08 [0.19 - 1.38]	6.25 ± 0.50 [5.93 - 6.57]	5.07 ± 4.13 (N = 15) [2.78 - 7.35]
10	Post CMT	15	0.69 ± 0.60 [0.36 - 1.02]	6.60 ± 9.83 [1.15 - 12.04]	5.94 ± 9.45 [0.71 - 11.18]	6.06 ± 0.53 [5.65 - 6.46]	3.57 ± 3.39 (N = 14) [1.62 - 5.53]
18	Chemo	11	0.90 ± 1.28 [0.04 - 1.76]	6.00 ± 12.28 [-2.25 - 14.24]	5.10 ± 11.88 [-2.87 - 13.09]	6.20 ± 0.27 [5.86 - 6.54]	4.44 ± 4.10 (N = 9) [1.30 - 7.59]
30	Chemo	12	0.47 ± 0.45 [0.19 - 0.75]	0.94 ± 0.61 [0.55 - 1.33]	0.53 ± 0.43 [0.26 - 0.81]	6.38 ± 0.44 [6.00 - 6.74]	2.30 ± 4.22 (N = 10) [-0.72 - 5.32]
Healthy Group							
0 (Baseline)	None	22	0.38 ± 0.40 [0.20 - 0.56]	1.26 ± 1.28 [0.69 - 1.83]	1.04 ± 1.21 [0.50 - 1.58]	6.60 ± 0.52 [6.36 - 6.83]	
3		22	0.27 ± 0.21 [0.18 - 0.37]	1.14 ± 1.25 [0.59 - 1.70]	0.89 ± 1.27 [0.32 - 1.45]	6.56 ± 0.56 [6.31 - 6.81]	
6		22	0.34 ± 0.36 [0.18 - 0.50]	1.12 ± 0.94 [0.70 - 1.54]	0.78 ± 0.85 [0.40 - 1.15]	6.63 ± 0.52 [6.40 - 6.86]	
10		22	0.40 ± 0.58 [0.14 - 0.66]	1.07 ± 0.66 [0.78 - 1.36]	0.85 ± 0.67 [0.56 - 1.15]	6.77 ± 0.56 [6.53 - 7.02]	
18		22	0.43 ± 0.46 [0.22 - 0.63]	1.28 ± 1.35 [0.68 - 1.88]	0.86 ± 1.14 [0.35 - 1.36]	6.82 ± 0.55 [6.58 - 7.05]	
30		22	0.27 ± 0.32 [0.13 - 0.41]	0.98 ± 0.93 [0.57 - 1.40]	0.71 ± 0.86 [0.33 - 1.40]	6.82 ± 0.51 [6.60 - 7.05]	

1. Salivary oxidative stress response as measured by oral lipid oxidation (OLO) by TBARS method.
2. Values in bracket represent the 95% confidence interval.
3. Delta OLO is the difference between the OLO response after and before ferrous-spiked water rinse.
4. Specified N is the number of cancer patients with reported chemosensory complaint scores (CSCS).

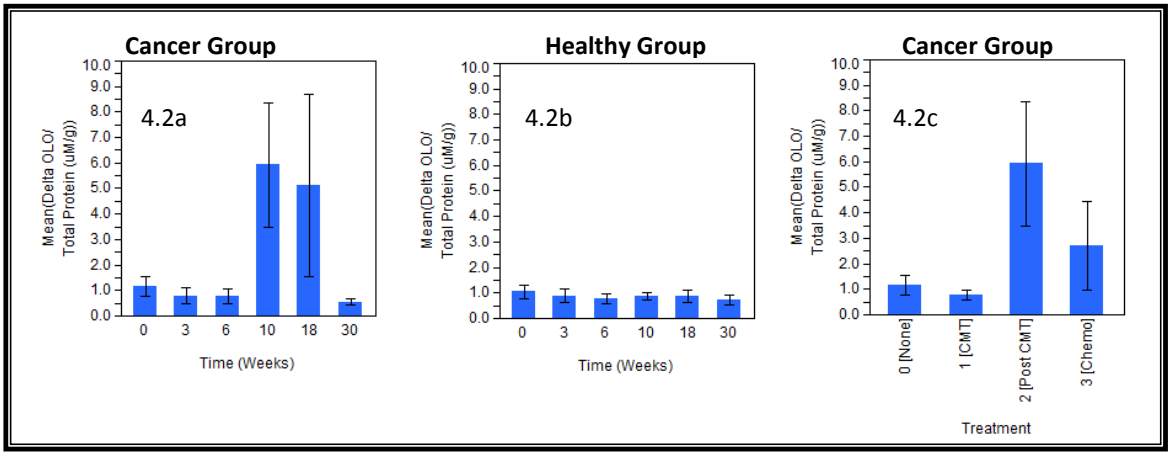

Figure 4.2: Salivary oxidative stress reponses in cancer patients and healthy subjects. Oxidative stress response was measured using the TBARS method (cancer patients: N = 17; healthy subjects: N = 22). The plotted data represent delta TBARS indicative of iron-induced oxidative stress response for cancer patients over time (4.2a) or over the treatment (4.2c), and delta TBARS for healthy subjects over time (4.2b). All responses over time and treatment were nonsignificant ($p > 0.05$). Error bars were constructed using $\pm$ 1 standard error from the mean.

4.3.2 Chemosensory Assessment. Self-reported taste and smell abnormalities (TSA) for individual cancer patients, as assessed by the CSC score scale of 0 to 16 (none to maximal impairment), ranged from 0 to 12. The mean CSC scores for cancer patients are summarized in Table 4.1 and plotted against time in weeks and treatment phase as shown in Figure 4.3. Repeated analysis of variance of the mean CSC scores indicated no significant differences with respect to time [$F(5, 16) = 3.06$, $p = 0.040$; Figure 4.3a]. The significant mean differences were between the baseline (time 0), and all the times, expect 30-week (range of p-values: 0.013 – 0.022). Likewise, when the mean CSC scores were compared by treatment phase, the repeated analysis of variance showed significant treatment effect [$F(3, 16) = 4.03$, $p = 0.026$]. Follow-up comparison of mean CSC scores by treatment indicated significant differences between the mean CSC scores at all CMT and post CMT phases when compared to the baseline (range of p-values: 0.036 – 0.006; Figure 4.3b).

To better display the within-subject variability over time for both cancer patients and healthy subjects, additional plots of the CSC scores are presented in Appendix C.

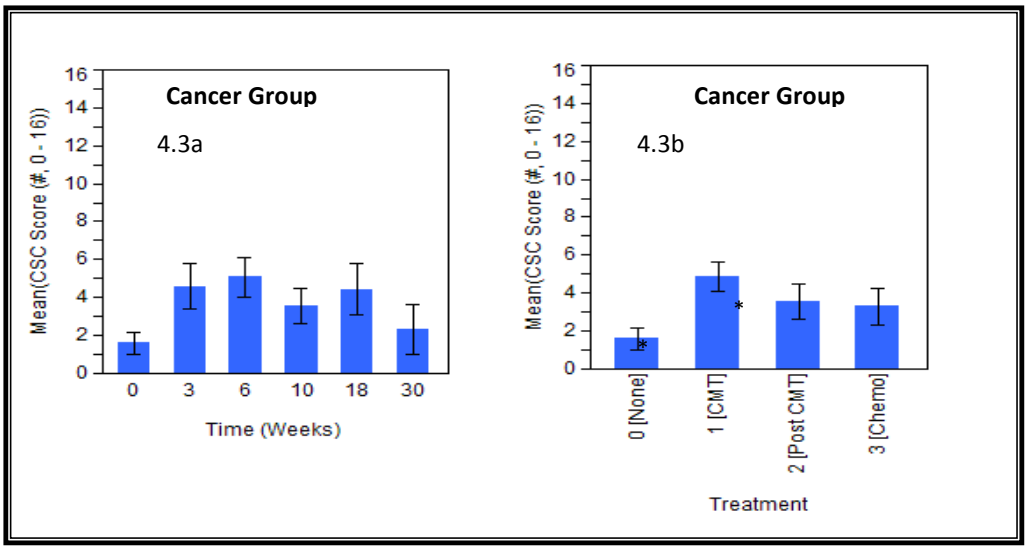

Figure 4.3: Chemosensory complaint (CSC) scores for cancer patients as measured by self-reported questionnaire. The plotted data (N = 17) represent mean CSC scores over time (4.3a) and course of the cancer treatment (4.3b). Error bars were constructed using ± 1 standard error from the mean.

4.3.3 Salivary Protein. The within-subject variations in the total salivary protein levels were not significant over time for healthy subjects (p = 0.49; Figure 4.4a; Table 4.2) or cancer patients (p = 0.70; Figure 4.4b; Table 4.2). Additionally, for cancer patients, the within-subject variations in the total protein levels were not significant when examined by the treatment phase (p = 0.90). In contrast, when examined by subject group (cancer patients and healthy subjects), the mean total salivary protein levels were significantly different (p < 0.0001), with the mean total protein level being higher in cancer patients than in healthy subjects (Figure 4.5). Within each subject group, the mean total salivary protein levels were not significantly different when comparing saliva samples before (Control) and after ferrous rinse with respective p-values from the paired t-test at p= 0.16 for the cancer group and p = 0.29 for the healthy subjects.

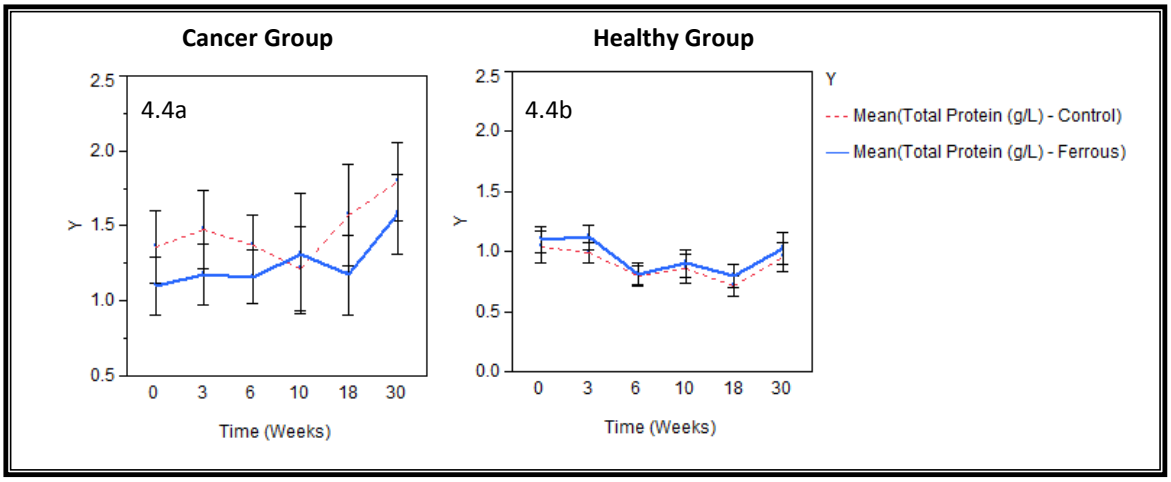

Figure 4.4: Variations in the mean total salivary protein levels in cancer patients and healthy subjects. Figure 4.4a shows the data for cancer patients (N = 17); Figure 4.4 shows the data for healthy subjects (N = 22). Error bars were constructed using ± 1 standard error from the mean. The dotted red lines represent saliva samples collected before ferrous rinse (Control); the blue lines represent samples collected after mouth rinse with ferrous (Ferrous).

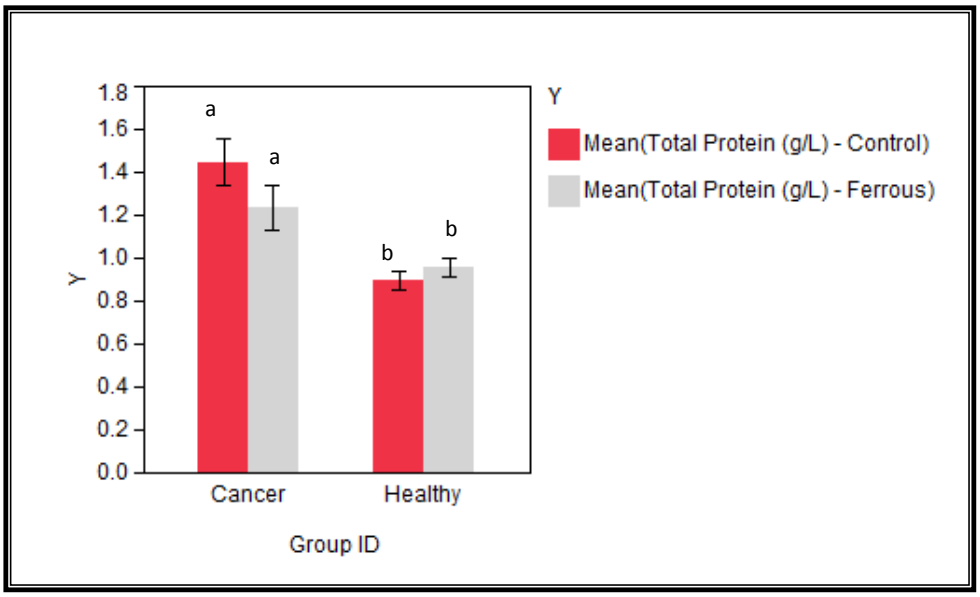

Figure 4.5: Comparison of the mean total salivary protein levels in cancer patients and healthy subjects. Bars with different letters indicate statistically significant ($p < 0.0001$ for Control and $p = 0.016$ for Ferrous) difference between the mean responses for the two groups. Error bars were constructed using ± 1 standard error from the mean. The red bars represent saliva samples collected before ferrous rinse (Control); the blue bars represent samples collected after mouth rinse with ferrous (Ferrous). The data set represents all oral pH measurements taken at time 0 (baseline) through 30 weeks for each group. Neither time nor treatment had significant effects on oral pH levels ($p > 0.05$).

Table 4.2: Total salivary protein levels in cancer patients and healthy subjects.

Cancer Group			Total Salivary Protein (g/L) Mean ± Standard Deviation, [95% CI]	
Time (Weeks)	Cancer Treatment	N	Before Fe^{+2} Rinse (Control)	After Fe^{+2} Rinse (Ferrous)
0 (Baseline)	None	17	1.36 ± 1.00 [0.85 - 1.88]	1.10 ± 0.80 [0.68 - 1.51]
3	CMT	15	1.48 ± 1.00 [0.92 - 2.03]	1.18 ± 0.78 [0.75 - 1.61]
6	CMT	15	1.37 ± 0.78 [0.94 - 1.80]	1.16 ± 0.68 [0.79 - 1.54]
10	Post CMT	15	1.22 ± 1.08 [0.62 - 1.82]	1.31 ± 1.55 [0.45 - 2.17]
18	Chemo	11	1.57 ± 1.13 [0.82 - 2.33]	1.17 ± 0.88 [0.58 - 1.77]
30	Chemo	12	1.80 ± 0.89 [1.22 - 2.36]	1.58 ± 0.92 [0.95 - 2.16]
Healthy Group				
0 (Baseline)	None	22	1.04 ± 0.60 [0.77 - 1.31]	1.10 ± 0.52 [0.87 - 1.33]
3		22	0.99 ± 0.40 [0.81 - 1.17]	1.12 ± 0.49 [0.90 - 1.34]
6		22	0.80 ± 0.41 [0.62 - 0.98]	0.81 ± 0.40 [0.64 - 0.99]
10		22	0.86 ± 0.57 [0.61 - 1.11]	0.90 ± 0.54 [0.67 - 1.14]
18		22	0.71 ± 0.41 [0.53 - 0.90]	0.80 ± 0.44 [0.60 - 0.99]
30		22	0.95 ± 0.57 [0.70 - 1.21]	1.03 ± 0.64 [0.74 - 1.31]

Values in bracket represent the 95% confidence interval

4.3.4 Measurements of Oral pH. In cancer patients, the oral pH levels showed no significant within-subject variation over time ($p = 0.69$) or from the treatment effect ($p = 0.36$). Likewise, for the healthy subjects, the within-subject variation in oral pH levels was not significant over time ($p = 0.37$). In contrast, when examined by subject group (cancer patients and healthy subjects), the oral pH levels were significantly different for the control sample (before ferrous rinse; $p < 0.0001$), but not for the ferrous sample (after ferrous rinse; $p = 0.18$), with the mean oral pH levels being lower in cancer patients than healthy subjects (Figure 4.6). Within each subject group, the mean oral

pH levels were significantly different when comparing saliva samples before (Control) and after ferrous rinse in cancer patients (p = 0.03), but not in healthy subjects (p = 0.93).

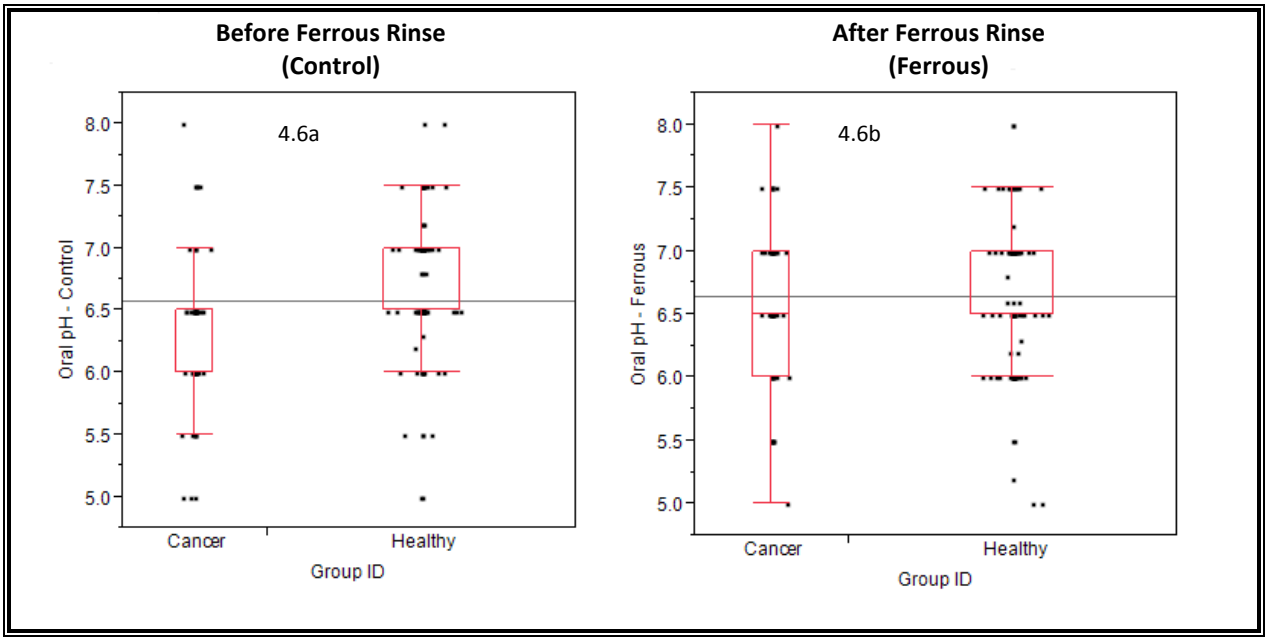

Figure 4.6: Comparison of Oral pH levels between cancer patients and healthy subjects. The mean oral pH levels were significanlty different in the Control sample (Fig 4.6a; $p < 0.0001$), but not in the Ferrous sample (Fig 4.6b; $p = 0.18$). The data set represents all oral pH measurements taken at time 0 (baseline) through 30 weeks for each group. Neither time nor treatment had significant effects on oral pH levels ($p > 0.05$).

4.3.5 Salivary Metals, Nonmetals, and Electrolytes. In cancer patients, the concentrations of salivary constituents show no statistically significant within-subject variations over time (p = 0.10 - 0.82) or over the treatment effect (p = 0.05 - 0.78). For the healthy subjects the variation in the salivary constituents was significant over time only for mean salivary zinc (p = 0.02) and iron levels (p < 0.0001). When examined over time, comparative means for salivary zinc levels for healthy subjects (HS) and cancer patients (CP) were significantly different only at time 0 (p < 0.0001), while for salivary iron levels, the means were significantly different at all time points (p < 0.0001). Regardless, comparative analysis of the means for all the parameters were performed only by subject group (i.e., cancer patients and healthy subjects). When compared by subject group, and with the exception of Ca and Cu, the mean salivary Na, Mg, Cl, K, S,

P, Zn, and Fe levels (both before and after ferrous rinse) were significantly different between cancer patients and healthy subjects (Table 4.3 and Figure 4.7 – 4.10).

Table 4.3: Salivary electrolytes, metals, and nonmetals in cancer patients and healthy subjects

Parameter (ppm)	Salivary Constituents Mean ± standard deviation, [95% CI]		Means Comparison[1]
	Cancer Group	Healthy Group	p-value (t-Test)
Sodium (23Na)[2]	210.80 ± 96.24 [189.91 - 231.68]	169.23 ± 66.51 [157.78 - 180.68]	0.0007
Magnesium (25Mg)	8.49 ± 4.63 [7.48 - 9.49]	5.49 ± 2.89 [4.99 - 5.98]	< 0.0001
Phosphorus (31P)	220.33 ± 117.81 [194.76 - 245.89]	166.69 ± 79.40 [153.02 - 180.36]	0.0003
Sulfur (34S)	342.46 ± 181.26 [303.12 - 381.79]	237.98 ± 119.62 [217.38 - 258.58]	< 0.0001
Chlorine (35Cl)	630.21 ± 292.16 [566.81 - 693.61]	553.20 ± 190.17 [520.45 - 585.94]	0.034
Potassium (39K)	1076.77 ± 478.44 [972.94 - 1180.59]	776.28 ± 264.12 [730.80 - 821.75]	< 0.0001
Calcium (43Ca)	73.96 ± 34.97 [66.37 - 81.55]	67.04 ± 26.56 [62.47 - 71.62]	0.123
Copper 65Cu	0.09 ± 0.08 [0.07 - 0.10]	0.06 ± 0.11 [0.04 - 0.08]	0.062
Zinc 66Zn	0.41 ± 0.25 [0.36 - 0.47]	0.54 ± 0.30 [0.49 - 0.60]	0.001
Iron (54Fe) (Before Fe^{+2} Rinse)	0.49 ± 0.28 [0.43 - 0.55]	1.28 ± 0.50 [1.20 - 1.37]	< 0.0001
Iron (54Fe) (After Fe^{+2} Rinse)	1.98 ± 0.79 [1.81 - 2.15]	3.68 ± 0.71 [3.56 - 3.80]	< 0.0001

1. For each group, means were averaged within subjects across all times.
2. 23Na indicates the element's molecular weight and symbol.

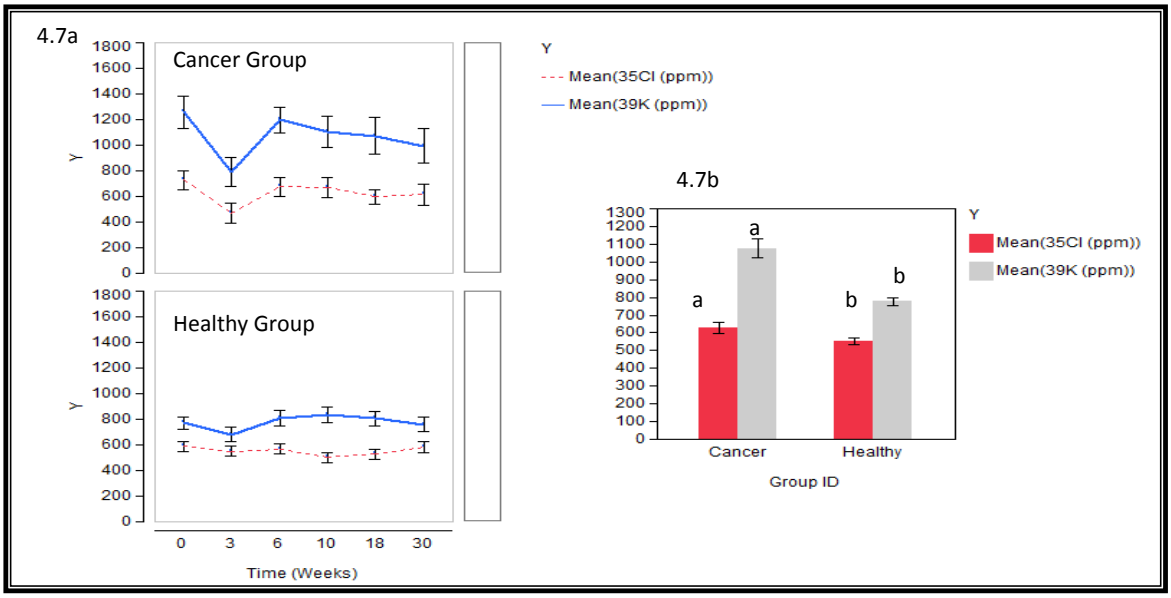

Figure 4.7: Comparison of the mean salivary potassium (K) and chloride (Cl) levels in cancer patients and healthy subjects. Figures display plots showing variations over time (Fig. 4.7a) and by subject group (Fig. 4.7b). In Figure 4.7b, bars with different letters indicate statistically significant ($p < 0.05$) difference between the mean responses for the two groups. Error bars were constructed using $\pm$ 1 standard error from the mean.

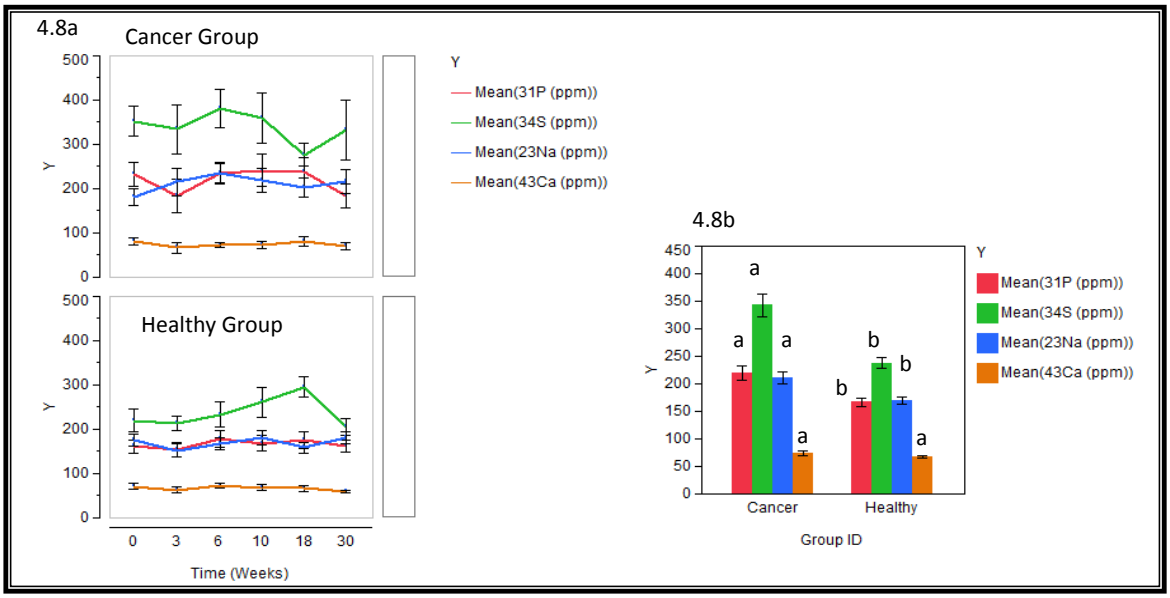

Figure 4.8: Comparison of the mean salivary phosphorus, sulfur, sodium, and calcium levels in cancer patients and healthy subjects. Figures display plots showing variations over time (Fig. 4.8a) and by subject group (Fig. 4.8b). In Figure 4.8b, bars with different letters indicate statistically significant ($p < 0.05$) differences between the mean responses for the two groups. Error bars were constructed using $\pm$ 1 standard error from the mean.

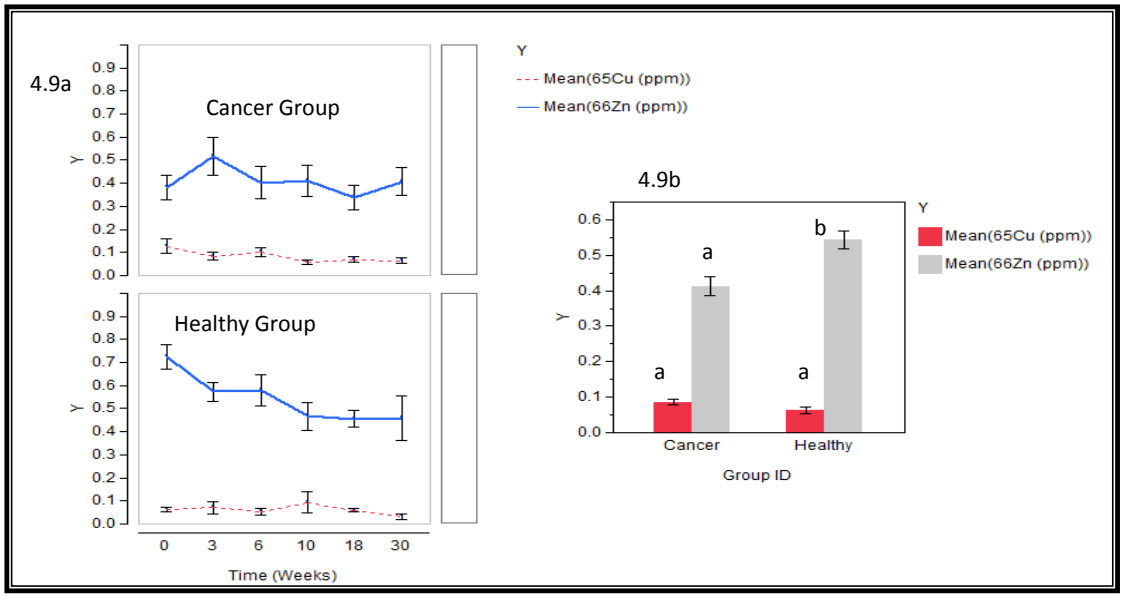

Figure 4.9: Comparison of the mean salivary copper and zinc levels in cancer patients and healthy subjects. Figures display plots showing variations over time (Fig. 4.9a) and by subject group (Fig. 4.9b). In Figure 4.9b, bars with different letters indicate statistically significant ($p < 0.05$) differences between the mean responses for the two groups. Error bars were constructed using $\pm$ 1 standard error from the mean.

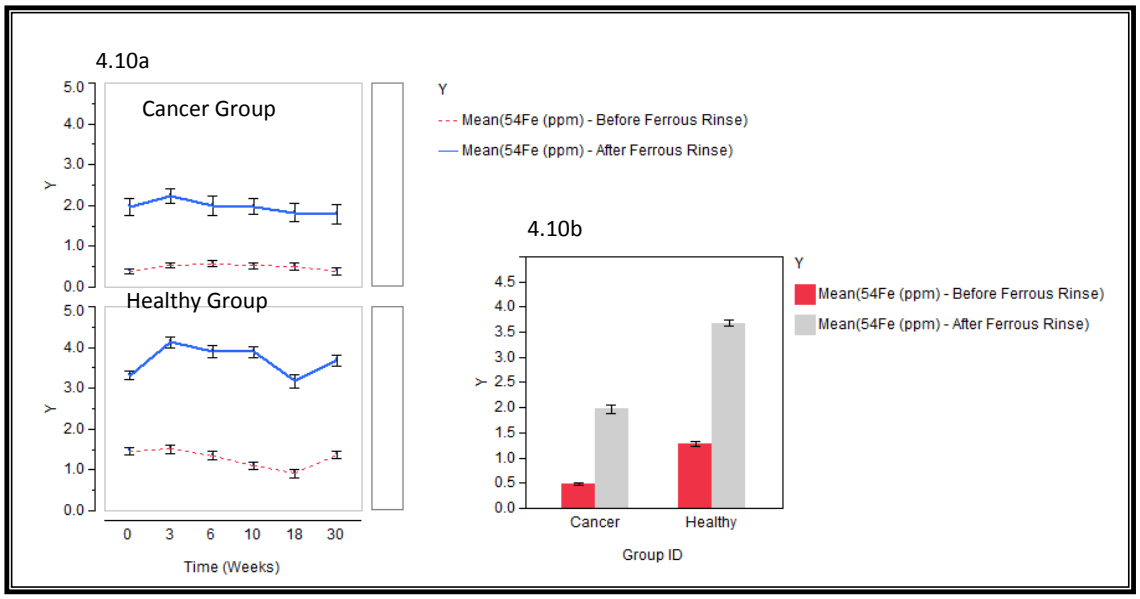

Figure 4.10: Comparison of the mean salivary iron levels in cancer patients and healthy subjects. Figures display plots showing variations over time (Fig. 4.10a) and by subject group (Fig. 4.10b). In Figure 4.10b, bars with different letters indicate statistically significant ($p < 0.05$) differences between the mean responses for the two groups. Error bars were constructed using $\pm$ 1 standard error from the mean. The dotted red lines/bars represent salivary iron levels before mouth rinse with ferrous containing water; the blue lines/bars represent samples collected after mouth rinse with ferrous (Ferrous).

4.3.6 Discussions on Findings. Salivary Oxidative Stress Response and Chemosensory Assessment. The overall mean oral lipid oxidation levels in cancer patients were significantly higher than that of healthy subjects. This was the case both prior to ($p < 0.0001$) and after ($p = 0.003$) iron-induced OLO response due to the oral rinse with ferrous spiked drinking water. Elevated oxidative stress response in biological fluids such as serum and saliva is a common occurrence in some disease conditions including cancer (Adibhatla and Hatcher 2010; Angeli et al. 2011). The increase in oxidative stress response is associated with free radical reactions that cause damage to macromolecular components, such as lipids, proteins, and DNA in cells and tissues (Catala 2009). Transition metals, such as iron, are known for their catalytic effects on free-radical oxidative reactions (Jomova and Valko 2011). Based on this understanding, the significantly higher mean OLO response in cancer patients, relative to that of healthy subjects is not surprising. However, an interesting finding is that the mean iron-induced OLO (i.e., delta OLO) response was dramatically increased in some patients at post CMT phase. Although the overall delta OLO response was considerably high, it was not statistically significant at the CMT phase when compared to the baseline due to the high variability in the oxidative stress responses. This observation is consistent with the chemosensory responses measured by the mean CSC scores, which increased significantly at the CMT phase ($p < 0.05$) and when compared to the baseline. These results suggest that in some cancer patients, the self-reported TSA, as measured by the mean CSC score is parallel to that of the iron-induced oxidative stress response as measured by delta OLO, although the significance of the oxidative stress response was noted at a later stage of the treatment than that of the self-reported TSA (i.e., the post CMT phase). A previous study on patients with breast cancer or gynecological malignancies showed that taste and smell functions, as assessed by sniff sticks and taste strips, declined significantly during chemotherapy, but normal functioning was returned three months after chemotherapy (Steinbach et al. 2009).

Salivary Proteins and Possible Association with Taste Perception and Oxidative Stress Response. While our research showed a significant difference in the level of proteins

present in cancer patients compared to healthy subjects, it did not focus on identification of specific proteins as potential biomarkers of TSA and/or oxidative stress in saliva. As an evolving research area, the composition of proteins in saliva is very complex. Research studies have identified more than 1400 salivary proteins (Scarano et al. 2010). Studies have shown that at the cellular level, some proteins, such as lactoferrin and transferrin, can act as antioxidants through chelation of metals that catalyze lipid oxidation (LO) reactions or capturing of free radicals that propagate LO reactions; whereas other proteins such as serum albumin can promote oxidation reactions (Elias et al 2008). Among the major salivary proteins, alpha amylase and mucin have shown varying binding capacities to oxidative metals such as copper in artificial saliva, thus indicating their potentials to indirectly limit or enhance lipid oxidation reactions in the oral cavity (Tang 2010) which could ultimately impact perception of metallic off-flavors associated with lipid oxidation reactions.

Variations in Oral pH and Potential Influence on Taste Perception. As presented earlier, our results indicate that the mean oral pH level in cancer patients was significantly lower ($p < 0.0001$) than that of healthy subjects. Since pH is an important factor in speciation of metals in aqueous environments, variations in pH could be an implicating factor in taste perception. A previous study on copper has shown that taste perception is associated with soluble species of copper and that the particulate form of the metal is poorly tasted (Cuppett et al. 2006). In the case of iron, within the typical pH range of saliva (5.5 – 8.0), iron is expected to remain in the dissolved ferrous form (Benjamin 2002). In the presence of salivary proteins, the role of pH can be further complicated. Studies with artificial saliva have shown that the metal binding capacities of major salivary proteins, mucin and alpha amylase, can decrease or increase based on the metal concentration as well as the salivary pH (Tang 2010). Additionally, depending on their isoelectric points in relation to the pH of saliva, different salivary proteins can be influenced to either inhibit or enhance lipid oxidation reactions (Elias et al 2008).

Salivary Metals, Nonmetals, and Electrolytes. As a noninvasive method of biological sample collection, analysis of salivary fluid for metals and electrolytes has been extensively studied in the literature for the purposes of exposure assessment to toxic

metals or diagnostics in clinical applications (Wang et al. 2008; Watanabe et al. 2005). It is recognized that there are inherently wide variations between and within subjects on salivary parameters due to variations in salivary flow, while possibly age and sex could be sources of variations as well (Tenovuo 1989). Our research showed that mean levels of salivary electrolytes, metals and nonmetals, specifically, Na, Cl, K, Mg, P, S, Zn were significantly higher in the cancer patients ($P < 0.05$) when compared to the healthy subjects, while the mean level of total iron was significantly lower in the cancer patients than healthy subjects ($p < 0.0001$). It has been reported in the literature that a high sodium and chloride concentration under the condition of low salivary flow rate is indicative of damage to the cells of the striated ducts of the salivary glands (Tenovuo 1989). Reduced salivary flow rate has also been shown to impact salivary pH, buffering capacity, and other constituents (Tenovuo 1989). Radiation therapy targeting tumors of the head and neck region often impact salivary gland functions, which can result in reduced salivary flow (Lal et al. 2010). In one study with oral cancer patients, it was shown that numerous salivary parameters, including Na, K, Ca, Mg, P, and total protein levels were considerably higher (in some cases statistically significant) in cancer patients when compared to a corresponding healthy group (Shpitzer et al. 2007). With regard to taste disorders, zinc deficiency has been widely associated with decline in taste acuity. In a clinical study with a group of head and neck cancer patients, (Ripamonti et al. 1998) it was demonstrated that oral administration of zinc sulfate alleviated taste abnormalities in cancer patients treated with external beam radiation therapy; their post treatment recovery of taste acuity was also improved. Similar findings have been reported in other studies (Watanabe et al. 2005) although a phase III randomized placebo-controlled, double blind trial of zinc supplementation did not prevent taste alterations in head and neck cancer patients undergoing radiotherapy.

One interesting finding from our study is the consistently lower level of salivary iron measured in cancer patients compared to that of healthy subjects ($p < 0.0001$). This observation remained even after the oral rinse with drinking water containing 10 ± 1 mg/L of ferrous sulfate. Recent studies in the literature have reported higher levels of some trace metals, including iron in serum samples from lung cancer patients

(Cobanoglu et al. 2010) and malignant glioma patients (Arslan et al. 2011) when compared to healthy subjects; however, serum levels of trace metals are often not correlated with that of saliva (Gil et al. 2011). Among side effects of chemotherapy, conditions of anemia and iron deficiency are common and often treated with iron supplementation or therapeutic agents that boost red blood cells production (Cunningham 2003; Steinmetz et al. 2011). In some types of malignancies, namely breast cancer, both conditions of iron deficiency and iron excess can serve as tumor growth factors, although through different mechanisms; one being hypoxia regulated gene expression and the other through induced oxidative stress (Jian et al. 2011).

4.4 Conclusions

Impact of cancer treatment on chemosensory functions can range from minimal to moderate impairment with maximum impairment developing during a 6-week CMT. Study of salivary constituents may provide clues to the causes of taste and smell disorders. Our study indicates that in addition to self-reported taste and smell assessment, a measure of iron-induced salivary oxidative stress response by oral lipid oxidation could be useful in assessing changes in taste and smell abnormalities during cancer treatment. However, the usefulness of salivary lipid oxidation as a biomarker for taste and smell assessment may be limited, as the results appear to be patient specific, rather than inclusive. Additionally, our results indicated that as a group, cancer patients had significantly higher total salivary protein level than healthy subjects. Since metallic sensation is a common taste complaint in cancer patients and proteins play an import role in the storage and transport mechanisms of flavor producing metals such as iron and copper, study of specific salivary proteins could provide insights into the causes of metallic taste discomfort associated with cancer therapies and other disease conditions. Among other salivary constituents, cancer patients as a group, had lower salivary zinc levels than healthy subjects; zinc deficiency could be an implicating factor in taste abnormalities as shown by other researchers.

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References

Ackerman BH, Kasbekar N. **1997**. Disturbances of taste and smell induced by drugs. Pharmacotherapy 17(3): 482-496.

Adibhatla RM, Hatcher JF. **2010**. Lipid Oxidation and Peroxidation in CNS Health and Disease: From Molecular Mechanisms to Therapeutic Opportunities. Antioxid Redox Signal 12(1): 125-169.

American Brain Tumor Association. **2011**. Moving Research into Treatment, Glioblastoma Multiforme, web site: www.abta.org. [Accessed September 28, 2011]

Angeli JPF, Garcia CCM, Sena F, Freitas FP, Miyamoto S, Medeiros MHG, et al. **2011**. Lipid hydroperoxide-induced and hemoglobin-enhanced oxidative damage to colon cancer cells. Free Radic Biol Med 51(2): 503-515.

Arslan M, Demir H, Arslan H, Gokalp AS, Demir C. **2011**. Trace Elements, Heavy Metals and Other Biochemical Parameters in Malignant Glioma Patients. Asian Pac J Cancer Prev 12(2): 447-451.

Benjamin MM. **2002**. Water Chemistry, 1st ed.: McGraw-Hill.

Bernhardson B, Tishelman C, Rutqvist L. **2007**. Self-reported taste and smell changes during cancer chemotherapy. Support Care Cancer DOI 10.1007/s00520-007-0319-7.

Boyce JM, Shone GR. **2006**. Effects of ageing on smell and taste. Postgraduate Medical Journal 82(966): 239-241.

Bromley SM. **2000**. Smell and taste disorders: A primary care approach. Am Fam Physician 61(2): 427-436.

Capra S, Ferguson M, Ried K. **2001** Cancer: impact of nutrition intervention outcome - nutrition issues for patients. Nutrition 17: 769-772.

Catala A. **2009**. Lipid peroxidation of membrane phospholipids generates hydroxy-alkenals and oxidized phospholipids active in physiological and/or pathological conditions. Chemistry and Physics of Lipids 157(1): 1-11.

Cobanoglu U, Demir H, Sayir F, Duran M, Mergan D. **2010**. Some mineral, trace element and heavy metal concentrations in lung cancer. Asian Pac J Cancer Prev 11(5): 1383-1388.

Comeau TB, Epstein JB, Migas C. **2001**. Taste and smell dysfunction in patients receiving chemotherapy: a review of current knowledge. Support Care Cancer 9(8): 575-580.

Copeland RA. **1994**. Methods for protein quantification.Chap.3.:Methods for Protein Analysis: A Practical Guide to Laboratory Protocols. In: Chapman and Hall (Ed), New York, NY, p 39-58.

Cunningham RS. **2003**. Anemia in the oncology patient - Cognitive function and cancer. Cancer Nurs 26(6): 38S-42S.

Cuppett JD, Duncan SE, Dietrich AM. **2006**. Evaluation of copper speciation and water quality factors that affect aqueous copper tasting response. Chemical Senses 31(7): 689-697.

de Graeff A, de Leeuw JRJ, Ros WJG, Hordijk GJ, Blijham GH, Winnubst JAM. **2000**. Long-term quality of life of patients with head and neck cancer. Laryngoscope 110(1): 98-106.

Deems DA, Doty RL, Settle G, Mooregillon V, Shaman P, Mester AF, et al. **1991**. Smell and taste disorders, a study of 750 patients from the University-of-Pennsylvania Smell and Taste Center. Arch Otolaryngol Head Neck Surg 117(5): 519-528.

Dewys WD, Walters K. **1975**. Abnormalities of taste sensation in cancer patients. Cancer 36(5): 1888-1896.

DeWys WD. **1977**. Anorexia in cancer patients. Cancer Res 37(7 Pt 2): 2354-2358.

Di Fiore F, Rigal O. 2009. Chemotherapy-induced oral food intake impairment. Oncologie 11(4): 204-210.

Doty RL. 2009. The Olfactory System and Its Disorders. Semin Neurol 29(1): 74-81.

Epstein JB, Barasch A. **2010**. Taste disorders in cancer patients: Pathogenesis, and approach to assessment and management. Oral Oncol 46(2): 77-81.

Epstein JB, Phillips N, Parry J, Epstein MS, Nevill T, Stevenson-Moore P. **2002**. Quality of life, taste, olfactory and oral function following high-dose chemotherapy and allogeneic hematopoietic cell transplantation. Bone Marrow Transplantation 30(11): 785-792.

Fukasawa T, Orii T, Tanaka M, Suzuki N, Kanzaki Y. **2008**. Relation between drug-induced taste disorder and chelating behavior with zinc ion; Statistical approach to the drug-induced taste disorder, Part II. Chem Pharm Bull 56(8): 1177-1180.

Gil F, Hernandez AF, Marquez C, Femia P, Olmedo P, Lopez-Guarnido O, et al. **2011**. Biomonitorization of cadmium, chromium, manganese, nickel and lead in whole blood, urine, axillary hair and saliva in an occupationally exposed population. Sci Total Environ 409(6): 1172-1180.

Glindemann D, Dietrich AM, Staerk HJ, Kuschk P. **2006**. The two smells of touched or pickled iron (skin) carbonyl-hydrocarbons and organophosphines. . Angewandte Chemie Intl Ed (European Journal "Applied Chemistry") 45: 7006-7009.

Guillen-Sans R, Guzman-Chozas M. **1998**. The thiobarbituric acid (TBA) reaction in foods: A review. Crit Rev Food Sci Nutr 38(4): 315-330.

Hong JH, Omur-Ozbeck P, Stanek B, Dietrich AM, Duncan S, Lee YW, et al. **2009**. Taste and Odor Abnormalities in Cancer Patients. Journal of Supportive Oncology 7.

Hutton JL, Baracos VE, Wismer WV. **2007**. Chemosensory dysfunction is a primary factor in the evolution of declining nutritional status and quality of life in patients with advanced cancer. Journal of Pain and Symptom Management 33(2): 156-165.

Jian JL, Yang Q, Dai JS, Eckard J, Axelrod D, Smith J, et al. **2011**. Effects of iron deficiency and iron overload on angiogenesis and oxidative stress-a potential dual role for iron in breast cancer. Free Radic Biol Med 50(7): 841-847.

Jomova K, Valko M. **2011**. Advances in metal-induced oxidative stress and human disease. Toxicology 283(2-3): 65-87.

Kettaneh A, Fain O, Stirnemann J, Thomas M. **2002**. Taste disorders. Rev Med Interne 23(7): 622-631.

Lal P, Bajpai R, Khurana R, Das KJM, Kumar P, Tiwari A, et al. **2010**. Changes in salivary flow rates in head and neck cancer after chemoradiotherapy. J Canc Res Ther 6(4): 458-462.

Larsson B, Olivecrona G, Ericson T. **1996**. Lipids in human saliva. Archives of Oral Biology 41(1): 105-110.

Lesser G, Case, D., Mirlohi S., M. Harmon, M. Chan, S. Duncan, A. Dietrich. Taste and smell abnormalities (TSA) in patients with newly diagnosed malignant gliomas (MG) treated with combined modality therapy (CMT). **2011**. Proceedings of the ASCO Annual Meeting, J Clin Oncol 29: 2011 (suppl; abstr e19646)

Logan HL, Bartoshuk LM, Fillingim RB, Tomar SL, Mendenhall WM. **2008**. Metallic taste phantom predicts oral pain among 5-year survivors of head and neck cancer. Pain 140(2): 323-331.

McDaniel RRV. **1998**. Development of a preparatory sensory information videotape for women receiving chemotherapy for breast cancer. Cancer Nurs 21: 143-148.

McGowan D. **2008**. Chemotherapy-induced oral dysfunction: a literature review. Br J Nurs 17(22): 1422-1426.

Mirlohi S, Dietrich AM, Duncan SE. **2011**. Age-Associated Variation in Sensory Perception of Iron in Drinking Water and the Potential for Overexposure in the Human Population. Environ Sci Technol 45(15): 6575-6583.

Mirza N, Machtay M, Devine PA, Troxel A, Abboud SK, Doty RL. **2008**. Gustatory Impairment in Patients Undergoing Head and Neck Irradiation. Laryngoscope 118(1): 24-31.

Mosel DD, Bauer RL, Lynch DP, Hwang ST. **2011**. Oral complications in the treatment of cancer patients. Oral Diseases 17(6): 550-559.

Ömur-Özbek P. **2008**. Macromolecular Reactions and Sensory Perceptions at the Air-Water-Human Interface. [PhD Dissertation]. Blacksburg, VA: Virginia Tech.

Ravasco P. **2005**. Aspects of taste and compliance in patients with cancer. European Journal of Oncology Nursing 9: 84-91.

Ripamonti C, Zecca E, Brunelli C, Fulfaro F, Villa S, Balzarini A, et al. **1998**. A randomized, controlled clinical trial to evaluate the effects of zinc sulfate on cancer

patients with taste alterations caused by head and neck irradiation. Cancer 82(10): 1938-1945.

Scarano E, Fiorita A, Picciotti PM, Passali GC, Calo L, Cabras T, et al. **2010**. Proteomics of saliva: personal experience. Acta Otorhinolaryngol Ital 30(3): 125-130.

Shpitzer T, Bahar G, Feinmesser R, Nagler RM. **2007**. A comprehensive salivary analysis for oral cancer diagnosis. J Cancer Res Clin Oncol 133(9): 613-617.

Skolin I, Wahlin YB, Broman DA, Hursti UKK, Larsson MV, Hernell O. **2006**. Altered food intake and taste perception in children with cancer after start of chemotherapy: perspectives of children, parents and nurses. Support Care Cancer 14(4): 369-378.

Spanier AM. **1991**. A rapid, direct chemical assay for the quantitative determination of thiobarbituric acid reactive substances in raw, cooked, and cooked/stored muscle foods. Journal of Muscle Foods 2(3): 165-175.

Steinbach S, Hummel T, Bohner C, Berktold S, Hundt W, Kriner M, et al. **2009**. Qualitative and Quantitative Assessment of Taste and Smell Changes in Patients Undergoing Chemotherapy for Breast Cancer or Gynecologic Malignancies. J Clin Oncol 27(11): 1899-1905.

Steinmetz HT, Tsamaloukas A, Schmitz S, Wiegand J, Rohrberg R, Eggert J, et al. **2011**. A new concept for the differential diagnosis and therapy of anaemia in cancer patients. Support Care Cancer 19(2): 261-269.

Stewart-Knox BJ, Simpson EEA, Parr H, Rae G, Polito A, Intorre F, et al. **2005**. Zinc status and taste acuity in older Europeans: the ZENITH study. Eur J Clin Nutr 59: S31-S36.

Stupp R, Mason W, van den Bent M, Weller M, Fisher B, Taphoorn M, et al. **2005**. Radiotherapy plus Concomitant and Adjuvant Temozolomide for Glioblastoma. The New England Journal of Medicine 352: 987-996.

Sun YE, Wang WD, Chen HW, Li C. **2011**. Autoxidation of Unsaturated Lipids in Food Emulsion. Crit Rev Food Sci Nutr 51(5): 453-466.

Tenovuo J. **1989**. Human Saliva: Clinical Chemistry and Micriobiology, Volume I. 205.

Wang DX, Du XQ, Zheng W. 2008. Alteration of saliva and serum concentrations of manganese, copper, zinc, cadmium and lead among career welders. Toxicol Lett 176(1): 40-47.

Wang H. **2009**. Determination of thiobarbituric acid reactive substances (TBARS) for water base liquid food with low oxidation. In: Unpublished: Virginia Tech.

Watanabe M, Asatsuma M, Ikui A, Ikeda M, Yamada Y, Nomura S, et al. **2005**. Measurements of several metallic elements and matrix metalloproteinases (MMPs) in saliva from patients with taste disorder. Chemical Senses 30(2): 121-125.

Yagi K. **1998**. Simple assay for the level of total lipid peroxides in serum or plasma. Meth Mol Biol 108: 101-106.

Chapter V

Water Consumption in Healthy Adults: Implications of Age, Flavor Preference, and Beverage Choices

Abstract

Consumption of water or water containing beverages is a necessity to sustain life for all organisms. In humans, the prescribed daily adequate intake (AI) levels for water ranges from approximately 0.7L in infants to 3.7L in adults. Infants and elderly are at a greater risk of dehydration. Inadequate intake of water as compared to calorie containing beverages has been linked to obesity in children and adults. This study examined the possible association between drinking water intake, consumers' age, water preference, taste/flavor sensitivity, and beverage choices in healthy adults. Thirty-three subjects aged 19 to 84 years old (22 Females) participated in the study. On three occasions, 26 of the 33 subjects completed a previously validated beverage questionnaire, which assessed their daily beverage intake during previous month. They also answered a general questionnaire, which assessed their drinking water preference. Because iron is often present in well water at high concentrations, the subjects' sensitivity to metallic flavor perception of ferrous iron in drinking water was assessed. Of the 33 subjects, 70% preferred tap water, 18% had no preference, and the remaining 12% had other preferences such as filtered or bottled water. Average daily drinking water intake ranged from 0.54 L to 1.4 L with tap water consumers having the lowest average daily drinking water intake. Plain water intake was considerably lower among the elderly (60 – 84 year) and the average total beverage intake for all age groups was below the AI. In the elderly group reduced intake of plain water coincided with reduced sensitivity to metallic flavor. Sugar-sweetened beverages (SSB) constituted 43% of the beverage caloric intake for age group 19 – 39 compared to 28% and 23% in age groups 40 – 59 and 60 –84, respectively; while, at 78%, percent caloric consumption from other beverages was highest among the elderly group. Increased consumption of plain water should be encouraged among all age groups to minimize caloric intake from SSB and other caloric beverages.

5.1 Introduction

The issue of water consumption among human populations is important in many aspects, in terms of both environmental sustainability as well as global health concerns. In addition to the global concerns for safety and sustainability, water consumption is relevant to public health as it has been promoted as healthy choice to replace calorie containing beverages (CDC 2012). Intrinsically, consumption is linked to water quality, safety, and flavor perception and/or preference. In the United States, the national drinking water standards established by the Environmental Protection Agency (USEPA) are intended to ensure safety and quality of drinking water for public consumption, as are the standards established by the World Health Organizations (USEPA 2009; WHO 2011). Some standards are health-based and regulated by the primary maximum contaminate levels (PMCLs) not to be exceeded in potable waters; others are aesthetic-based, secondary maximum contaminant levels (SMCLs), recommended to ensure acceptable appearance and palatability of drinking water (USEPA 2009). Public perception of drinking water safety, while difficult to measure, is known to influence consumers' behavior. In this regard, attributes such as health effects as well as taste and odor are often used as indicators of water purity and safety by consumers (Lee et al. 2009; Whelton et al. 2007). Among unpleasant flavor attributes, metallic taste is a common consumer complaint. Incidentally, metal contamination of drinking water by copper, which produces a distinct metallic flavor in water (Ömur-Özbek and Dietrich 2011), was identified as the source of 27 illness outbreaks in the US since 1971 (Craun et al. 2010). Iron is another commonly occurring metal responsible for imparting metallic flavor to drinking water (WHO 2011).

With regard to health benefits of water consumption, a number of studies have investigated the role of water consumption on energy intake and its ultimate effect as a weight loss/control measure (Daniels and Popkin 2010; Davy et al. 2008; Dennis et al. 2009; Dubnov-Raz et al. 2011). Additionally, numerous studies have linked obesity to increased intake of sugar-sweetened beverages (SBB) (Daniels and Popkin 2010; Davy et al. 2008; Dennis et al. 2009; Bleich et al. 2009). Epidemiological studies conducted by the US National Health and Nutrition Examination Survey (NHANES) have indicated

a positive correlation between total energy intake and consumption of beverages other than water (IOM 2004). Other studies have reported reduced caloric intake when water replaced other beverages (Kant et al. 2009; Popkin et al. 2005; Stookey et al. 2007). According to the NHANES, in the period of 2005-2006, American adults consumed 3.18 L of total water in a given day, with plain water and beverages representing 33% and 48% of the total beverage intake, respectively (Kant et al. 2009). The United States Institute of Medicine, Food and Nutrition Board recommends an Adequate Intake (AI) of daily total water in healthy adult males and females at 3.7 and 2.7 liters, respectively. In spite of this recommendation, the amount of water consumed in a day varies considerably among different age groups. For example, for the most recent period of 2005 – 2006, U.S. adults in age groups 19 - 39 and 40 – 59 consumed 1.1 liters of plain water per day, while those greater than age 60 consumed 0.8 liters. Likewise, the daily total beverage intake was less in the elderly (i.e., age > 60) to 2.1 L compared to 2.7 L and 2.9 L for the 19 – 39 and 40 – 59 age groups, respectively (Popkin 2010).

Intake of adequate fluid and/ water is vital for the maintenance of human body functions, such as cellular metabolism, regulation of body heat, and maintaining the osmolarity of various bodily fluids (Jequier and Constant 2010; Popkin et al. 2010). Dehydration has been associated with increased risk of developing kidney stones, urinary tract infections and bladder cancer, while mild day-to-day dehydration has been associated with fatigue and impaired cognitive performance (Popkin et al. 2010; Ritz and Berrut 2005). The elderly and infants are typically at greater risk of dehydration (IOM 2004; Jequier and Constant 2010). In the elderly, diminished sensation of thirst as well as behavioral reasons such as fear of incontinence and/or disabling conditions associated with aging can contribute to dehydration risk. Age-associated decline in taste functions as well as swallowing disorders have also been recognized to contribute to dehydration risks among the elderly (Asai 2004; Bratlund et al. 2010).

Since water consumption is so vital to bodily functions, and plain drinking water is being encouraged as a healthy beverage in place of calorie containing beverages, factors that influence human consumption of drinking water should be taken into consideration when examining beverage consumption behavior. These factors may include consumer

preference for a certain taste or flavor, such as tap, bottled, well, or mineral water as well as the availability of a safe and clean source of water. Choice of drinking water may also be driven by their perception of risks associated with a given water source (Doria 2010; Doria et al. 2009). For example, in public water systems, when water utilities experience problems with a certain contaminant of concern, consumers may resort to drinking alternative source, such as bottled and/or filtered water due to safety concerns. In fact, this was shown to be the case in a study on Canadian consumers (Dupont et al. 2010). In this study, we examined the beverage consumption pattern in adults who participated in a sensory study that assessed sensitivities to metallic flavor of iron in drinking water. Our objectives were to identify correlations between drinking water consumption, age, other beverage choices, water preference, and individual taste sensitivity as assessed by metallic flavor threshold for iron. Metallic flavor was studied in this case, since it is among the most common consumer complaints in drinking water (Benham et al. 2010).

5.2 Materials and Methods

5.2.1 Human Subjects. The study was approved by the Institutional Review Board (IRB) at Virginia Tech (IRB Project No. 06-395). Human subjects were recruited from the community, students, faculty and staff of Virginia Tech and Blacksburg, Virginia by means of paper and email flyers. Subjects were required to have no chronic oral or general health problems, be non-smokers, and not pregnant. All subjects read and signed an informed consent form in accordance with the approved IRB protocols. Thirty-three, (22 females) multinational subjects, ages 19 – 84 years, participated in the study. Each subject completed a brief demographics questionnaire which, provided information on their age, gender, smoking, and general health.

5.2.2 Beverage Intake Questionnaire. To estimate the mean daily intake of water, sugar-sweetened beverages, and total beverages (in volume and calories), a previously validated beverage questionnaire (BEVQ) was used (Hedrick et al. 2010). The questionnaire consisted of 19 beverage categories and one, open-ended section for "other" beverages (Figure 5.1). On three occasions, initial, 18-week, and 30-week, subjects completed the beverage questionnaire. BEVQ measurements were taken at

initial time point for all 33 subjects. For 26 of the 33 subjects, repeated BEVQ measurements were also taken at 18-week and 30-week time points as part of another study that assessed their sensitivities to metallic flavor of iron and monitored biochemical measures of iron-induced metallic flavor in their saliva.

Briefly, to score the BEVQ, frequency ("How often") was converted to the unit of times per day and then multiplied by the amount consumed ("How much each time") to provide average daily beverage consumption in fluid ounces. Total caloric intake of each beverage was determined by multiplying the number of fluid ounces per day by the calorie per fluid ounce for each beverage. To quantify SSB consumption, beverage categories containing added sugars were summed (sweetened juice beverages/drinks, regular soft drinks, sweet tea, sweetened coffee, energy drinks, mixed alcoholic drinks, and meal replacement beverages). For the purpose of identification, beverages not listed under the category of SSB will be referred to as "other" beverages. These include 100% fruit juices, milk, unsweetened or artificially sweetened tea and/or coffee, and alcoholic beverages.

5.2.3 Sensory Threshold Determination. The sensory protocol used for individual flavor threshold determinations has been described in our previous publication (Mirlohi et al. 2011). Briefly, for threshold determinations, an ascending concentration one-of-five forced choice test was used which had a 20% chance of guessing correctly (ASTM 1997). Samples were served at 22 - 24 ºC in taste-and odor free 3-oz Solo plastic cups (Solo Cup Company, Lake Forest IL) filled with 20 ml of sample and/or control water. Only one sensory session that tested a single ferrous concentration was conducted per day. A single test and concentration per day was necessary to avoid any effect of aftertaste, which is typical of metallic flavor. Subjects were instructed to avoid consuming food or beverages for at least one hour prior to each sensory testing session. Tests were conducted in a quiet room with no distracting odors or sounds. To familiarize subjects with metallic flavor, they were given deionized water with a high iron concentration before threshold testing began. At each session, subjects received 5 cups each labeled with a different 3-digit number. Four cups contained deionized water and one contained the ferrous solution. The cups were presented in a random order

such that the ferrous solution could be in any of the five positions. Subjects were instructed to taste the samples from left to right without going back, wait 1 minute in between samples, and to select the metallic tasting sample and mark it on their score sheet. For a given subject, testing was complete when the subject correctly identified three sequential ferrous concentrations or reached the last and highest concentration.

In preparing taste samples for metallic flavor threshold determination, a 100 mg/L iron stock was prepared daily using Iron (II) Sulfate (Sigma-Aldrich, PA, CAS # 13463-43-g) and deionized water which was taste and odor free. Ferrous solutions were prepared daily by diluting the stock solution with deionized water, which also served as the control. The concentrations tested were 0.005, 0.01, 0.025, 0.05, 0.1, 0.25, 0.5, 1, 2, 4, 5, 10, and 20 mg/L ferrous. Solutions were monitored to prevent the oxidation of ferrous iron t ferric iron. The concentrations were verified using inductively coupled plasma – mass spectroscopy (Thermo Electronic Corporation, X-Series ICP-MS, Waltham, MA), following Standard Method 3120B.

For each participant, the individuals flavor thresholds were plotted against their corresponding water intake levels in order to identify correlations between an individual's measure of taste sensitivity and their level of water intake.

5.2.4 Data Analyses. Statistical software, JMP 9.0 (SAS, Cary, NC), was used for all data analyses. One-way analysis of variance (ANOVA) and comparison of the means, using Student's t-Test and Tukey HSD or Wilcoxon/Kruskal Wallis Rank sum test, were performed on the continuous variables. An alpha level of 0.05 was for all statistical tests. Associations among variables (beverage intake variables and age; beverage intake and metallic flavor threshold; beverage intake and total energy intake) were assessed using linear correlation analyses (Spearman's R). Additionally, descriptive evaluation of data included examining the mean beverage intake levels among age groups 19 – 39, 40 – 59, and 60 – 84 to facilitate comparisons to the previously reported NHANES data.

Beverage Questionnaire

Instructions:
In the past month, please indicate your response for each beverage type by marking an "X" in the bubble for "how often" and "how much each time"
1) Indicate how often you drank the following beverages, for example, you drank 5 glasses of water per week, therefore mark 4-6 times per week
2) Indicate the approximate amount of beverage you drank each time, for example, you drank 1 cup of water 2 times per day, therefore mark 1 cup under "how much each time"

Subject ID _____

Date _____

	HOW OFTEN (MARK ONE)							HOW MUCH EACH TIME (MARK ONE)				
Type of Beverage	**Never or less than 1 time per week (go to next beverage)**	**1 time per week**	**2-3 times per week**	**4-6 times per week**	**1 time per day**	**2+ times per day**	**3+ times per day**	**Less than 6 fl oz (3/4 cup)**	**8 fl oz (1 cup)**	**12 fl oz (1 1/2 cups)**	**16 fl oz (2 cups)**	**More than 20 fl oz (2 1/2 cups)**
Water	O	O	O	O	O	O	O	O	O	O	O	O
100% Fruit Juice	O	O	O	O	O	O	O	O	O	O	O	O
Sweetened Juice Beverage/Drink (fruit ades, lemonade, punch, Sunny Delight)	O	O	O	O	O	O	O	O	O	O	O	O
100% Vegetable Juice (V8, etc.)	O	O	O	O	O	O	O	O	O	O	O	O
Whole Milk	O	O	O	O	O	O	O	O	O	O	O	O
Reduced Fat Milk (2%)	O	O	O	O	O	O	O	O	O	O	O	O
Low Fat/Fat Free Milk (Skim, 1%, Buttermilk, Soymilk)	O	O	O	O	O	O	O	O	O	O	O	O
Soft Drinks, Regular	O	O	O	O	O	O	O	O	O	O	O	O
Diet Soft Drinks/Artificially Sweetened Drinks (Crystal Light)	O	O	O	O	O	O	O	O	O	O	O	O
Sweetened Tea	O	O	O	O	O	O	O	O	O	O	O	O
Coffee, with cream and/or sugar (includes non-dairy creamer)	O	O	O	O	O	O	O	O	O	O	O	O
Tea or Coffee, black, with/ without artifical sweetener (no cream or sugar)	O	O	O	O	O	O	O	O	O	O	O	O
Non-alcoholic or Light Beer	O	O	O	O	O	O	O	O	O	O	O	O
Beer, Ales, Wine Coolers	O	O	O	O	O	O	O	O	O	O	O	O
Hard Liquor (shots, rum, tequila, etc.)	O	O	O	O	O	O	O	O	O	O	O	O
Mixed Alcoholic Drinks (daiquiris, margaritas, etc.)	O	O	O	O	O	O	O	O	O	O	O	O
Wine (red or white)	O	O	O	O	O	O	O	O	O	O	O	O
Meal Replacement Shakes/Protein Drinks (Slimfast, shakes, etc.)	O	O	O	O	O	O	O	O	O	O	O	O
Energy Drinks (Red Bull, Rockstar, Full Throttle, etc.)	O	O	O	O	O	O	O	O	O	O	O	O
Other (list):	O	O	O	O	O	O	O	O	O	O	O	O
Other (list):	O	O	O	O	O	O	O	O	O	O	O	O

Virginia Polytechnic Institute and State University, 2008

Figure 5.1: The beverage intake questionnaire (BEVQ). Questionnaire was developed by Hedrick et al (2010) and reproduced here with permission from Copyrights Clearance Center, Inc. The BEVQ was used to assess subjects' daily beverage intakes. BEVQ measurements were taken at initial time point for all 33 subjects. For 26 (18 females) of the 33 subjects, BEVQ measurements were also taken at 18-week and 30-week time points.

5.3 Results and Discussion

5.3.1 Relationship between Drinking Water Consumption and Age. Distribution of subjects by age and gender was as follows: ages 19 – 39: 6 subjects (4 females); ages 40 – 59: 11 subjects (8 females); ages 60 – 84: 16 subjects (10 females). The average daily drinking water intake levels among the subjects ranged from 5.7 fl oz. (0.2 L) to 48 fl oz (1.4 L). Repeated measure ANOVA indicated no significant time effect for within subject variability ($p = 0.34$); therefore, subsequent analysis of the means was performed using BEVQ data taken at initial time point for all 33 subjects. The mean daily drinking water intake was weakly correlated with age ($R^2 = 0.15$) and showed a significant declining trend by increasing age (slope = -0.19, $p = 0.026$). When the data were group by age categories: 19 – 39, 40 – 59, and 60 - 84, following the grouping used by the NHANES data, the mean daily water intake level was notably lower in the age group 60 – 84 when compared to the other two age groups; however, the differences in the means were marginally non-significant [$F(2, 32) = 3.13$, $p = 0.058$; Figure 5.2a]. When grouped by gender, the mean daily water intake levels were not significantly different between the genders (t ratio= 0.092, $p = 0.93$; Figure 5.2b).

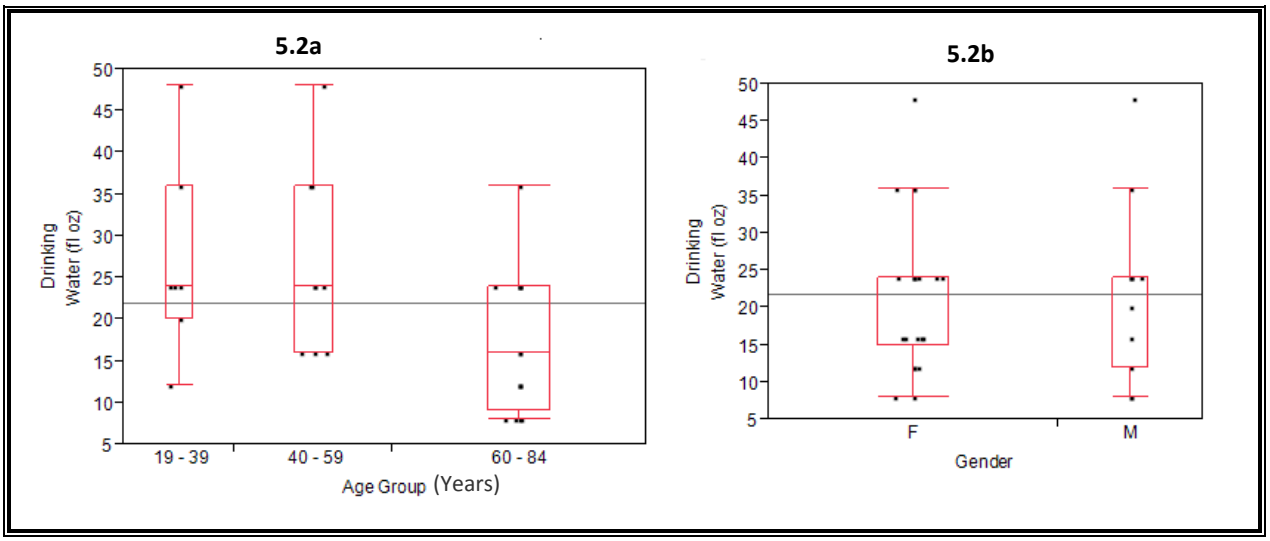

Figure 5.2: Comparison of the average daily drinking water intake in healthy adults. Data presented as grouped by age (5.2a) and gender (5.2b). Plotted data (N = 33) represent responses on the BEVQ from 33 subjects (22 females). The average daily water intake among the three age groups: 19 – 39 (N = 6), 40 – 59 (N = 11) and 60 – 84 (N = 16) were marginally non-significant (p = 0.058).

5.3.2 Variations in Sugar-Sweetened and Total Beverage Intakes. 1) Sugar-sweetened beverages (SBB): Repeated measure ANOVA indicated no significant effect for within subject variability for repeated BEVQ measurements taken over time (p = 0.51); therefore, subsequent analyses were performed on BEVQ data taken at the initial time for all 33 subjects. The average daily intake of SSB varied widely among subjects, ranging from 0 fl oz. to 38 fl oz (1.1 L); while, the average daily total beverage intake varied from 18 fl oz. (0.5 L) to 98 fl oz (2.9 L). There was no correlation neither between the intake of SSB and age (R^2 = 0.03, p = 0.18). When grouped by age categories: 19 – 39, 40 – 59, and 60 – 84, the mean SSB intake levels were not significantly different among the three age groups [F (2, 32) = 1.67, p = 0.21; Figure 5.3a). Likewise, when the data were grouped by gender, the mean SSB intake levels were not significantly different between the genders (t ratio = 1.06, p = 0.30; Figure 5.3b).

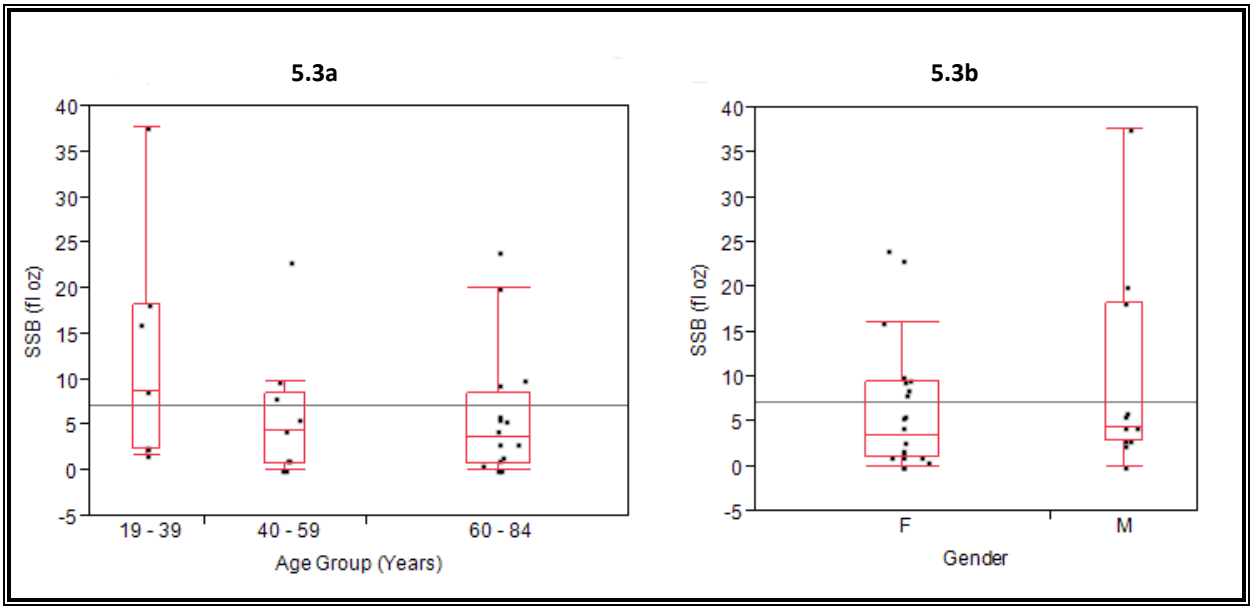

Figure 5.3: Comparison of the average daily sugar-sweetened beverage (SSB) intake in healthy adults. Data presented as grouped by age (5.3a) and gender (5.3b). Plotted data (N = 33) represent responses on the BEVQ from 33 subjects (22 females). The differences in average daily SSB intake among the three age groups: 19 – 39 (N = 6), 40 – 59 (N = 11) and 60 – 84 (N = 16) were non-significant (p = 0.21).

2) Total beverage intake: the self-reported BEVQ measurements for the total beverage intake were not significantly varied over time as assessed by repeated ANOVA (p = 0.65). Subsequent analysis performed on the initial BEVQ data indicated no significant difference between the mean daily total beverage intake levels among the three age

groups [F (2, 32) = 1.78, p = 0.19; Figure 5.4a]. Likewise, when the data were grouped by gender, the mean daily total beverage intake levels were not significantly different between the genders (t ratio = 0.29 p = 0.78; Figure 5.4a]. There was no correlation between the mean total daily beverage intake and age (R^2 = 0.09, p = 0.09).

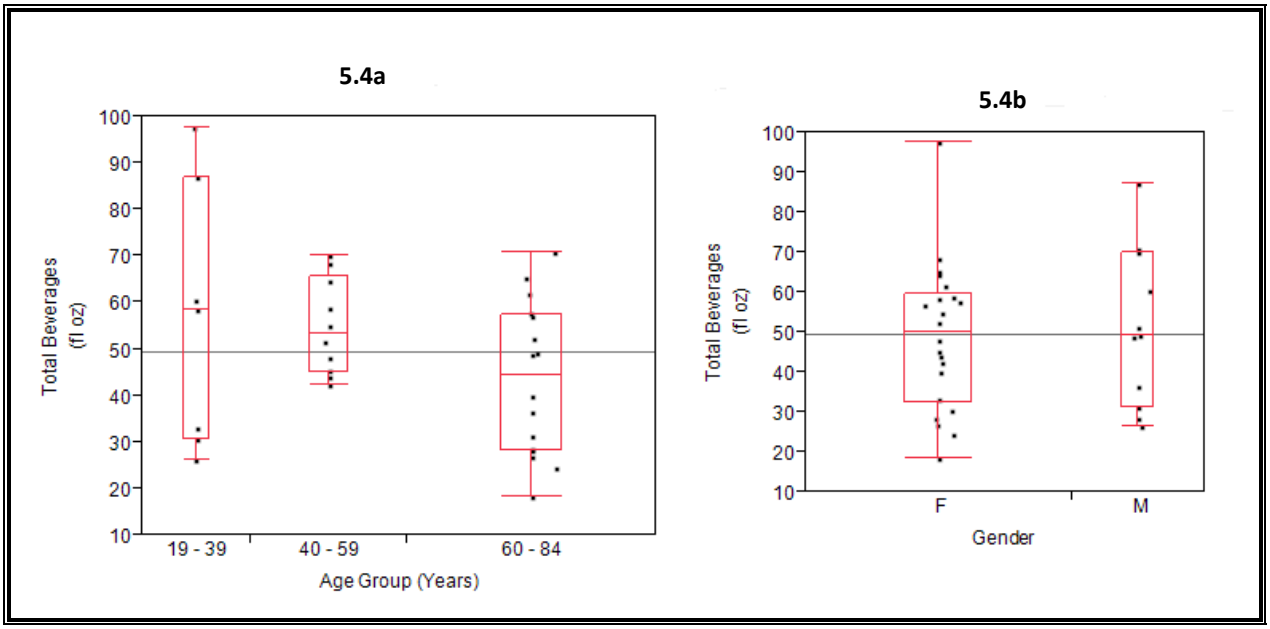

Figure 5.4: Comparison of the average daily total beverage intake in healthy adults. Data presented as grouped by age (5.4a) and gender (5.4b). Plotted data (N = 33) represent responses on the BEVQ from 33 subjects (22 females). The differences in average daily SSB intake among the three age groups: 19 – 39 (N = 6), 40 – 59 (N = 11) and 60 – 84 (N = 16) were non-significant (p = 0.19).

5.3.3. Impact of Drinking Water and Other Beverages Choices on Total Water and Calorie Intake. Subjects varied in their drinking water preferences (Table 5.1 and Figure 5.5). The majority of subjects preferred tap water over the other choices, namely, bottled water, no preference (i.e., any), or filtered tap water. Only one of the 33 subjects indicated a preference for bottled water. When the mean daily drinking water intake levels were compared to water preferences, the highest level of water consumption (48 fl oz. or 1.4 L) corresponded to the one bottled water user, while the lowest mean water intake level (18.4 fl oz. or 0.54 L) corresponded to tap water users (Figure 5.6a). Excluding the one bottled water wateruser, comparison of the mean intake levels for each water preference indicated a statistical significance [F(2, 31) = 4.33; p = 0.023]. Post hoc comparisons using the Wilcoxon's Rank Sum test indicated

that the mean intake level for tap water was significantly different from the filtered water users (p = 0.026); although not statistically significant, the mean intake levels for tap and no preference or "Any" were notably different (p = 0.058). When categorized by age groups, among all three age groups, tap water was most commonly preferred followed by filtered water; while among the age groups 40 – 59 and 60 – 84, no preference or "Any" was the second most common response (Table 5.2 and Figure 5.6).

Table 5.1: Variations in Subjects Drinking Water Preference and Water Intake.

Drinking Water Preference	**Number of Subjects (N)**	Mean ± Standard Deviation (fl oz.), Mean Daily Water Intake	95% CI
Tap	23	18.4 ± 8.8 or [0.54 ± 0.26 L]	14.6 – 22.3
Bottled	1	48	--
Any	6	26.0 ± 9.0 or [0.77 ± 0.27 L]	16.5 – 35.5
Other	3	32.0 ± 6.9 or [0.95 ± 0.20 L]	14.8 – 49.2

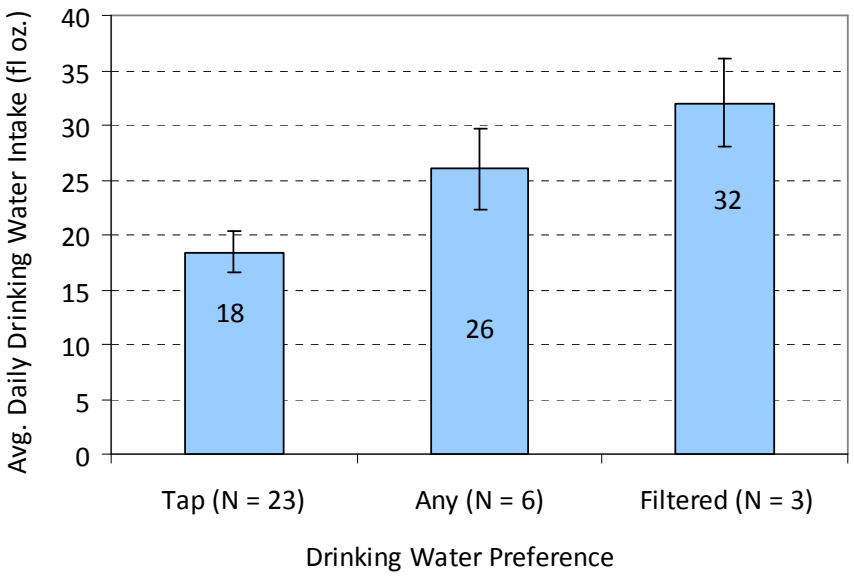

Figure 5.5: Relationship between water preference and average daily water intake levels in healthy adults. Plotted data (N = 33) represent responses on the BEVQ from 33 subjects (22 females). Error bars represent 1 standard error from the mean. Of the 33 subjects, only one preferred bottled water; this subject had the highest drinking water intake at 48 fl oz.

Table 5.2: Variations in Subjects' Drinking Water Preference by Age Group

Age Group	Number of Subjects (N)	Drinking Water Preference			
		Tap	Bottled	Any	Filtered
19 – 39 (4 females)	6	5	0	0	1
40 – 59 (8 females)	11	7	1	2	1
60 – 84 (10 females)	16	11	0	4	1

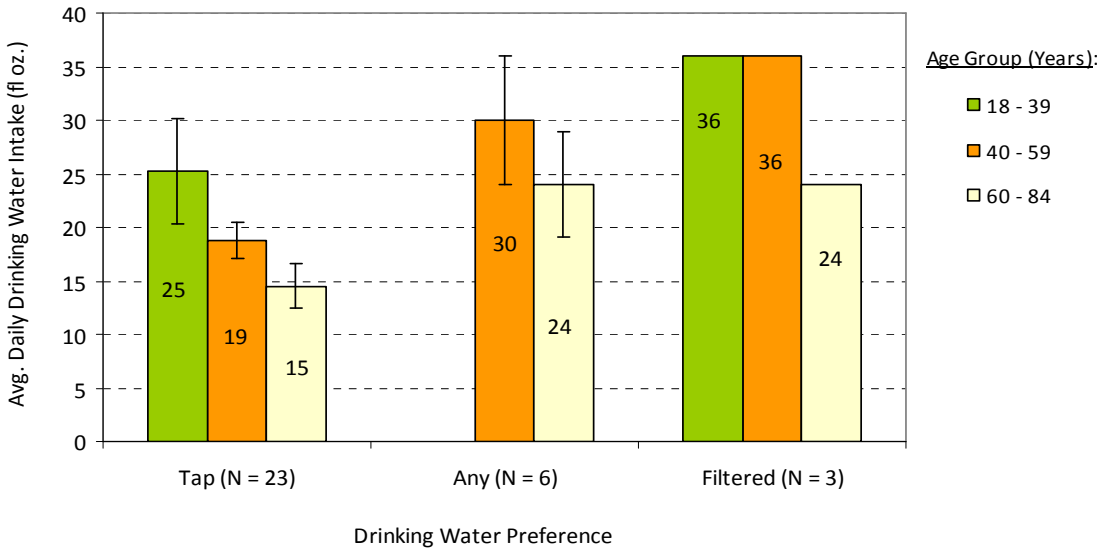

Figure 5.6: Relationship between water preference and average daily water intake levels in healthy adults, as grouped by age. Plotted data (N = 33) represent responses on the BEVQ from 33 subjects (22 females). Of the 33 subjects, only one preferred bottled water; this subject had the highest drinking water intake at 48 fl oz.

With regard to calorie intake, sugar-sweetened beverage (SSB) and "other" beverage choices, such as milk, 100% fruit juice, and alcoholic drinks contributed to the bulk of the total daily calorie intake. As categorized by age groups, the 19 – 39 year olds represented the highest mean consumption of plain water and sugar sweetened beverages in terms of volume; while the age group, 49 – 59 consumed the highest volume of "other" beverage choices. Among the age group 60 – 84, plain water and SSB represented the lowest mean level of consumption in terms of volume (Figure 5.7a). In terms of calorie intake, the highest mean calorie intake from SSB was observed within the age group 19 – 39, while the highest mean calories intake from "other" beverages was observed within the age group 60 – 84 (Figure 5.7b).

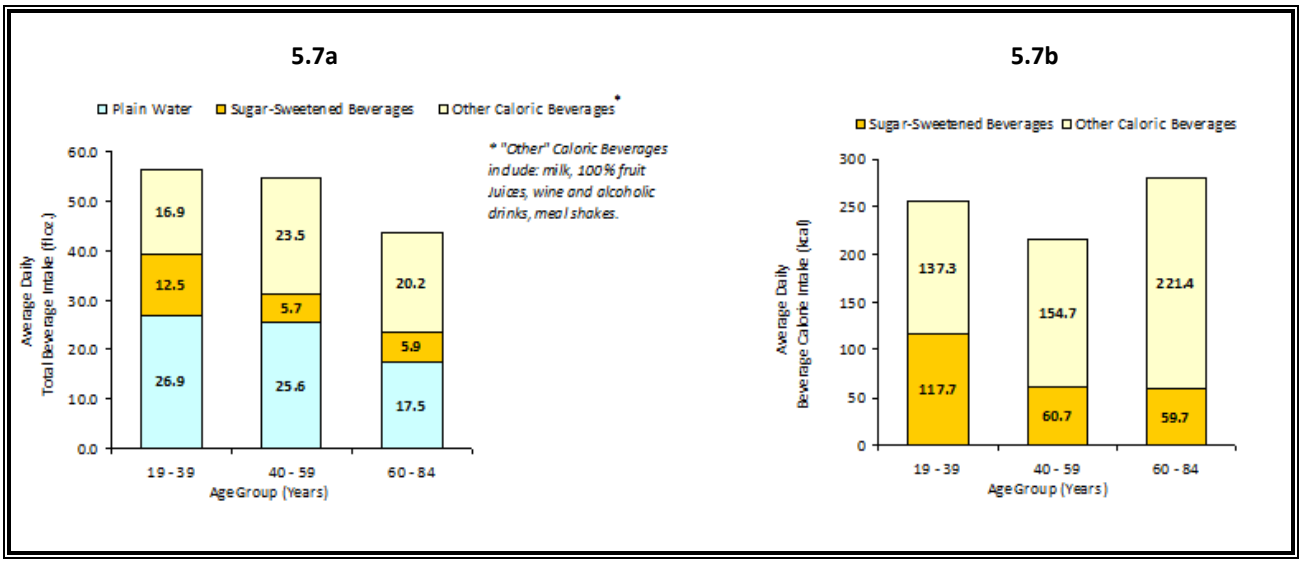

Figure 5.7: Contributions of beverage types to overall average daily beverage intake in healthy adults. Beverage intakes are presented in terms of volume by fluid ounce (Fig. 5.7a) and calorie intake (Fig. 5.7b) within different age groups. Plotted data (N = 33) represent responses on the BEVQ from 33 subjects (22 females).

In terms of calories, the linear correlations between daily intake of beverages and the total daily calorie intake was notably higher for the SSB (R^2 = 0.20, p = 0.009) than "other" beverages, which showed no correlation (R^2 = 0.07, p = 0.13; Figure 5.8). The finding indicates that in this group of subjects, contribution to daily caloric intake from beverages is higher for SBB than "other" caloric beverages. With regard to all beverages, the correlation between total beverage intake volume and caloric intake remained significant, but relatively small (R^2 = 0.31, p = 0.0007; Figure 5.8).

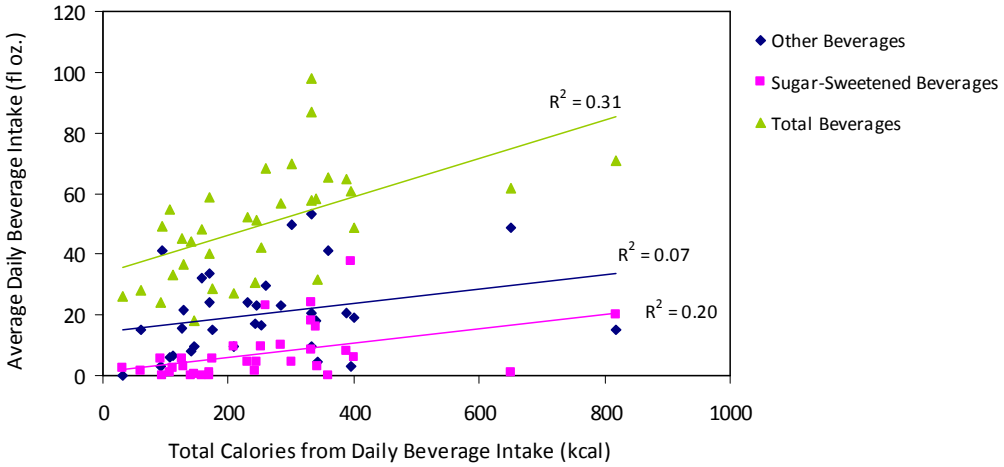

Figure 5.8: Correlations between average daily beverage intake and total caloric intake from beverages in healthy adults. Plotted data (N = 33) represent responses on the BEVQ from 33 subjects (22 females).

5.3.4 Relationship between Water Consumption and Sensitivity to Metallic Flavor. The sensitivity levels for ferrous iron flavor varied greatly among subjects and ranged from 0.003 - 14.14 mg/L Ferrous. These values are both under and exceed the USEPA guideline of 0.3 mg/L total iron for aesthetic standard. Subjects who were >50 years of age had higher threshold values and were less sensitive to the flavor of iron. There was no correlation between individuals' estimate of metallic flavor threshold and their drinking water intake levels (R^2 = 0.05, p = 0.25); however, there was a weak correlation and a significant declining trend in total beverage intake and individual taste/flavor sensitivity as measured by metallic flavor threshold (R^2 = 0.35, p = 0.0014; Figure 5.9).

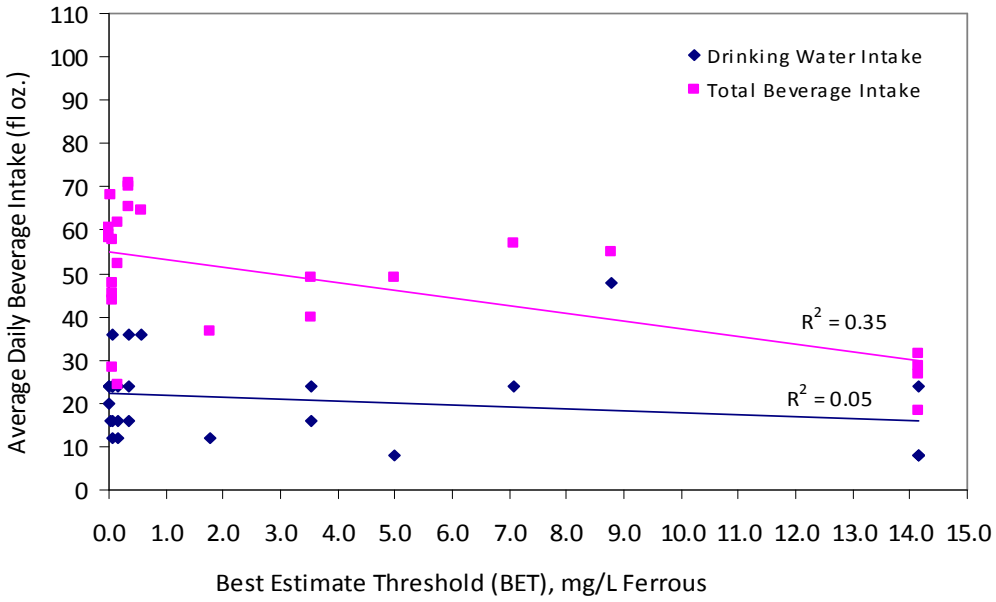

Figure 5.9: Correlations between drinking water intake, total beverage intake, and individual sensitivity to metallic flavor, as measured by flavor recognition threshold for iron in drinking water. Plotted data represent 26 subjects (18 females). Sensory-associated decrease in consumption was significant for total beverage intake (R^2 = 0.35, p = 0.001).

When the average flavor threshold data and corresponding drinking water intake levels were plotted by age group, the low water intake level in the elderly age group of 60 – 84 coincided with a lower sensitivity to metallic flavor as indicated by a considerably higher mean metallic flavor threshold level in this group (Figure 5.10). Regardless, the mean flavor threshold levels of the three age groups were not significantly different [F (2, 25) = 1.93; p = 0.17].

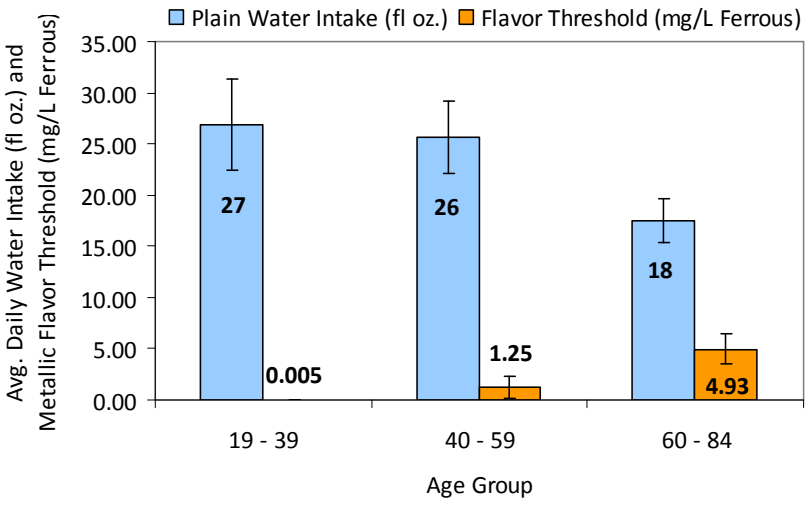

Figure 5.10: Comparison of average daily water intake and metallic flavor threshold levels by age group among healthy adults. Flavor threshold data were available for 26 subjects (22 females). Plotted data (N = 33) represent responses on the BEVQ from 33 subjects (22 females). BEVQ measurements were taken at initial time point for all 32 subjects. Error bars represent 1 standard error from mean.

5.3.5 Discussions on Findings. This study provides additional data in support of previous findings indicating a declining trend in water intake among elderly population greater than 60 years of age. The water needs of individuals can vary widely based on age, gender, body size, and environmental conditions (IOM 2004; Popkin 2010). The adequate intake level (AI) for water from all sources, namely, drinking water, beverages, and foods, is based on estimated median intakes among healthy people in the U.S. national survey data. In terms of plain water intake, our study showed that the decline in elderly group of 60 – 84 was highly significant while the total water intake from all beverage sources was only marginally significant when compared to the younger group of 19 – 39 year olds. Additionally, the average daily total water intake levels for all three adult age groups, ranging from 1.9 to 2.5 liters were below the AI levels as established by the Institute of Medicine (IOM) (Figure 5.11); however, our conclusions are limited by the fact that the contribution of water from food sources was not determined by our study. The national survey data for adults estimates that water intake from food sources constitute about 20% of the total daily water intake in a typical American diet. Based on this estimate, 20% water contribution from food sources would respectively equal to 0.54 L and 0.74 L of the total AI level for adult females and males. If these

amounts were added to the average total intake levels from our study, the average total daily beverage intake would still remain below the AI levels for both genders.

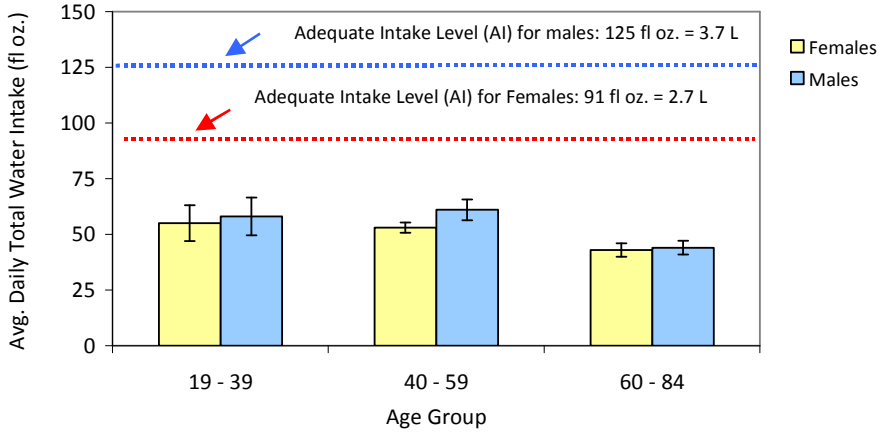

Figure 5.11: Average daily total water intake by age group among healthy adults in comparison to the adequate intake (AI) levels. The AI is based on the U.S. national Survey data and as established by the Institute of Medicine. Plotted data (N = 33) represent responses on the BEVQ from 33 subjects (22 females). Data represent BEVQ measurements were taken at initial time point for all 32 subjects. Error bars represent 1 standard error from mean.

According to the dietary intake panel of the institute of medicine, for a healthy person, daily consumption below the AI may not be indicative of additional risk for dehydration and related chronic diseases because a normal hydration status is associated with a wide range of intakes (IOM 2004). The composition of body weight is an important factor in variations between individual water needs since the majority of body water is contained in fat-free body mass (IOM 2004; Jequier and Constant 2010). Additionally, water needs in individuals may vary based on activity level, diet, and environmental conditions. In older adults, a 3% loss of body weight is considered significant and indicative of a risk for dehydration. Since aging has been associated with loss of body weight, the risk of dehydration is considered to be higher in the elderly than younger adults. Likewise, infants are at a greater risk of dehydration due to their smaller total body weight. However, the risk of dehydration in the elderly is primarily associated with those in assisted living facilities and or hospitals; otherwise, in healthy older adults, maintenance of body water balance is comparable to that of younger adults (Bossingham et al. 2005).

As indicated by our study, the significant decline in the intake of plain water among elderly adults in the age group 60 – 84, coincided with reduced taste sensitivity, in this case to the flavor of metals in drinking water. More significantly, the decline in total beverage intake correlated with reduced sensitivity to metallic flavor (Figures 5.9 and 5.10). The significance of the observed relationship between total water intake and metallic flavor is important, in terms of our current understanding of mechanisms of metallic flavor perception. Our previous research has shown that diminished sensitivity to metallic flavor in elderly adults is associated with impaired smell functions (Mirlohi et al. 2011). Since both taste and smell functions are essential components of flavor perception (Meilgaard and Civille 2007), impairment of sensory function can ultimately impact overall sensitivity to flavor perception from foods and beverages.

Additionally, in our study, total beverage intake among elderly adults was dominated by a higher intake of "other" beverages, such as 100% fruit juice, unsweetened and/or artificially sweetened coffee or tea, milk, and alcoholic beverages (typically wine) compared to sugar-sweetened beverages (SSB) and plain drinking water (Figure 5.7). One possible reason for this is that reduced taste sensitivity may be a factor in consumption of more flavorful beverages instead of plain water. Although previous studies have associated aging with decline in taste sensitivity (De Graaf and Zandstra 1999; Mojet et al. 2001), whether this reduced sensitivity influences the preference for specific beverages is a subject of debate (Kennedy et al. 2010; Mojet et al. 2005). Other noted factors associated with reduced fluid intake and dehydration risk in older adults are swallowing disorders, decreased olfactory sensation and decreased taste (Asai 2004). Oral problems associated with aging, such as the use of dentures and missing teeth can also discourage or inhibit fluid intake (Wotton et al. 2008).

With regard to SSB intake, it is encouraging to note that it represented the lowest intake of total beverage consumption in all three age groups as assessed by our study (Figure 5.7). However, it should be noted that our subject group consisted of mainly university-educated adults, either students, and/or current or retired staff and faculty members. Additionally, no data on body mass indices were obtained on our subjects, although no participant was observationally identified as obese or overweight. Previous researchers

have shown that socioeconomic factors have association with higher intake of SSB in low-income households (Rehm et al. 2008). On the other hand, our study showed that in terms of caloric intake, at 49%, the contribution to the average total daily intake of calories from SSB was highest among younger adults (age group: 19 – 39). While at 75% and 76%, older adults in respective age groups of 40 – 59 and 60 – 84, consumed their highest beverage calories from non-SSB beverages. These findings are consisted with national trends as reported by other researchers (Bleich et al. 2009). Regardless, it is important to note that consumption of plain water in place of caloric beverages is widely promoted as a healthy beverage alternative (Popkin et al. 2005), as well as a simple measure to minimize weight gain and/or even contribute to weight loss (Davy et al. 2008; Dennis et al. 2009; Patel and Hampton 2011).

Finally, with drinking water being a vital resource, a necessity to maintain health, and a widely promoted beverage for weight control in children as well as adults, understanding of the factors that implicate consumers' tendencies to drink water is important. Our findings indicate that factors such as water preference and flavor perception may influence the level of consumption. Interestingly, among our subjects, tap water was the most preferred water choice, yet the least consumed. Previous research has identified multiple factors such as flavor, risk perception, chemical perception, prior experience, and trust in water utilities as contributors to consumer's perception of water quality which ultimately influence their consumption and choice of water (Doria 2010; Doria et al. 2009; Imgrund et al. 2011; Jones et al. 2006). In our study, lower drinking water intake in older adults aged 60 – 84 years coincided with lower sensitivity to metallic flavor. Although reduced taste sensitivity may not necessarily be associated with reduced consumption level as indicated by a recent study which reported tap water consumers showed similar taste sensitivity to chlorine but differed in their acceptability of chlorine flavor (Puget et al. 2010).

5.4 Conclusions

The relationship between water consumption, water requirement, and osmotic balance in humans is rather complex. While consumption of adequate water intake is essential for maintenance of health, the level of intake is widely varied depending on life stage,

activity level, and environmental conditions. There is a wide range of prescribed recommendations for adequate or minimum daily intake levels for water, as lows as 1.1 liters to as high as 3.7 liters; whether consumption levels below the prescribed recommendations is associated with dehydration and risk of diseases remains to be subject for debate. Regardless of the level of uncertainty that may be associated with our knowledge on the precision of the human body's regulatory mechanism for water balance under varying hydration statuses and life stages, the benefits of drinking water consumption as a preferred beverage for weight management and maintenance of health can not be underestimated. In this regard, understanding variables that influence individual consumption is important both in terms of public health as well as consumer acceptability. While relatively limited in scope, the results of our study indicate that in addition to consumer acceptability and choices, physiological factors such as age-associated variations in individual and/or population taste sensitivities should be taken into consideration when examining beverage intake patterns and dehydration risks among susceptible populations, such as the elderly.

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References

Asai JL. **2004**. Nutrition and the geriatric rehabilitation patient - Challenges and solutions. Top Geriatr Rehabil 20(1): 34-45.

Benham B, Ling EJ, Scott JP, Lutz J, Thompson J, Thompson J. **2010**. Evaluation of household water quality in Fluvanna and Louisa counties, Virginia. October 2010, Virginia household water Quality program. Virginia Cooperative Extension.

Bleich SN, Wang YC, Wang Y, Gortmaker SL. **2009**. Increasing consumption of sugar-sweetened beverages among US adults: 1988-1994 to 1999-2004. Am J Clin Nutr 89(1): 372-381.

Bossingham MJ, Carnell NS, Campbell WW. **2005**. Water balance, hydration status, and fat-free mass hydration in younger and older adults. Am J Clin Nutr 81(6): 1342-1350.

Bratlund CV, O'Donoghue CR, Rocchiccioli JT. **2010**. Dehydration and Dysphagia: Challenges in the Older Adult. J Med Speech-Lang Pathol 18(3): 1-10.

CDC. **2012**. Center for Disease Control and Prevention: Water and Nutrition. Website: http://www.cdc.gov/healthywater/drinking/nutrition/index.html. (Accessed on 2/20/12)

Craun GF, Brunkard JM, Yoder JS, Roberts VA, Carpenter J, Wade T, et al. **2010**. Causes of Outbreaks Associated with Drinking Water in the United States from 1971 to 2006. Clin Microbiol Rev 23(3): 507-+.

Daniels MC, Popkin BM. **2010**. Impact of water intake on energy intake and weight status: a systematic review. Nutr Rev 68(9): 505-521.

Davy BM, Dennis EA, Dengo AL, Wilson KL, Davy KP. **2008**. Water consumption reduces energy intake at a breakfast meal in obese older adults. J Am Diet Assoc 108(7): 1236-1239.

De Graaf C, Zandstra EH. **1999**. Sweetness intensity and pleasantness in children, adolescents, and adults. Physiology & Behavior 67(4): 513-520.

Dennis EA, Flack KD, Davy BM. **2009**. Beverage consumption and adult weight management: A review. Eat Behav 10(4): 237-246.

Doria MD, Pidgeon N, Hunter PR. **2009**. Perceptions of drinking water quality and risk and its effect on behaviour: A cross-national study. Sci Total Environ 407(21): 5455-5464.

Doria MD. **2010**. Factors influencing public perception of drinking water quality. Water Policy 12(1): 1-19.

Dubnov-Raz G, Constantini NW, Yariv H, Nice S, Shapira N. **2011**. Influence of water drinking on resting energy expenditure in overweight children. Int J Obes 35(10): 1295-1300.

Dupont D, Adamowicz WL, Krupnick A. **2010**. Differences in water consumption choices in Canada: the role of socio-demographics, experiences, and perceptions of health risks. J Water Health 8(4): 671-686.

Hedrick VE, Comber DL, Estabrooks PA, Savla J, Davy BM. **2010**. The Beverage Intake Questionnaire: Determining Initial Validity and Reliability. J Am Diet Assoc 110(8): 1227-1232.

Imgrund K, Kreutzwiser R, de Loe R. **2011**. Influences on the water testing behaviors of private well owners. J Water Health 9(2): 241-252.

IOM. **2004**. Dietary reference intakes for water, potassium, sodium, chloride, and sulfate / Panel on Dietary Reference Intakes for Electrolytes and Water, Standing Committee on the Scientific Evaluation of Dietary Reference Intakes, Food and Nutrition Board. Institute of Medicine (U.S.), Panel on Dietary Reference Intakes for Electrolytes and Water. Washington D.C.

Jequier E, Constant F. **2010**. Water as an essential nutrient: the physiological basis of hydration. Eur J Clin Nutr 64(2): 115-123.

Jones AQ, Dewey CE, Dore K, Majowicz SE, McEwen SA, David WT, et al. **2006**. Public perceptions of drinking water: a postal survey of residents with private water supplies. BMC Public Health 6.

Kant AK, Graubard BI, Atchison EA. **2009**. Intakes of plain water, moisture in foods and beverages, and total water in the adult US population-nutritional, meal pattern, and body weight correlates: National Health and Nutrition Examination Surveys 1999-2006. Am J Clin Nutr 90(3): 655-663.

Kennedy O, Law C, Methven L, Mottram D, Gosney M. **2010**. Investigating age-related changes in taste and affects on sensory perceptions of oral nutritional supplements. Age Ageing 39(6): 733-738.

Lee J, Kleczyk E, Bosch D, Tanellari E, Dwyer S, Dietrich A. **2009**. Case Study: Preference Trade-Offs toward Home Plumbing Attributes and Materials. Journal of Water Resources Planning and Management-Asce 135(4): 237-243.

Meilgaard M, Carr T, Civille G. **2007**. Sensory Evaluation Techniques.

Mirlohi S, Dietrich AM, Duncan SE. **2011**. Age-associated variation in sensory perception of iron in drinking water and the potential for overexposure in the human population. Environ Sci Technol 45(15): 6575-6583.

Mojet J, Christ-Hazelhof E, Heidema J. **2001**. Taste perception with age: generic or specific losses in threshold sensitivity to the five basic tastes? Chemical Senses 26(7): 845-860.

Mojet J, Christ-Hazelhof E, Heidema J. **2005**. Taste perception with age: pleasantness and its relationships with threshold sensitivity and supra-threshold intensity of five taste qualities. Food Qual Prefer 16(5): 413-423.

Ömur-Özbek P, Dietrich AM. **2011**. Retronasal perception and flavour thresholds of iron and copper in drinking water. J Water Health 9(1): 1-9.

Patel AI, Hampton KE. **2011**. Encouraging Consumption of Water in School and Child Care Settings: Access, Challenges, and Strategies for Improvement. Am J Public Health 101(8): 1370-1379.

Popkin BM, Barclay DV, Nielsen SJ. **2005**. Water and food consumption patterns of US adults from 1999 to 2001. Obes Res 13(12): 2146-2152.

Popkin BM, D'Anci KE, Rosenberg IH. **2010**. Water, hydration, and health. Nutr Rev 68(8): 439-458.

Popkin BM. **2010**. Patterns of beverage use across the lifecycle. Physiology & Behavior 100(1): 4-9.

Puget S, Beno N, Chabanet C, Guichard E, Thomas-Danguin T. **2010**. Tap water consumers differ from non-consumers in chlorine flavor acceptability but not sensitivity. Water Res 44(3): 956-964.

Rehm CD, Matte TD, Van Wye G, Young C, Frieden TR. **2008**. Demographic and behavioral factors associated with daily sugar-sweetened soda consumption in New York City adults. J Urban Health 85(3): 375-385.

Ritz P, Berrut G. **2005**. The importance of good hydration for day-to-day health. Nutr Rev 63(6): S6-S13.

Stookey JD, Constant F, Gardner CD, Popkin BM. **2007**. Replacing sweetened caloric beverages with drinking water is associated with lower energy intake. Obesity 15(12): 3013-3022.

USEPA. **2009**. National Drinking Water Standards and Health Advisories Tables. EPA-822-R09-011. Washington D.C.

Whelton AJ, Dietrich AM, Burlingame GA, Schechs M, Duncan SE. **2007**. Minerals in drinking water: impacts on taste and importance to consumer health. Water Sci Technol 55(5): 283-291.

WHO. **2011**. Guidelines for Drinking Water Quality, 4th Edition, World Health Organization Geneva, Switzerland.

Wotton K, Crannitch K, Munt R. **2008**. Prevalence, risk factors and strategies to prevent dehydration in older adults. Contemp Nurse 31(1): 44-56.

Chapter VI

Role of Salivary Fluid in Metallic Flavor Production Associated with Iron and Copper Induced Lipid Oxidation

Abstract

Iron and copper are recognized for their unpleasant metallic flavors, as well as their toxicities due to their abilities to induce oxidative stress in living cells through lipid oxidation (LO) processes. Human perception of metallic flavor is associated with retronasal detection of aromas from LO by-products formed and released through interaction of metals with salivary constituents. Using salivary LO (SLO) as an indicator of metallic flavor intensity, this study compared levels of iron and copper induced SLO in artificial saliva and investigated the individual and interactive influences of fatty acids, proteins, and nitrite in the production and/or inhibition of metallic flavor. A series of SLO experiments were conducted on five different mixtures of artificial saliva (AS) that contained different combinations of both inorganic and organic constituents. AS samples were separately spiked with Fe(II) and Cu(II) at concentration of 180 µM. AS mixtures supplemented with linoleic acid (LA), were also treated with varying concentrations of Fe(II) and Cu(II); test samples were subjected to 37°C heat for 15 minutes; SLO was measured in all samples using the TBARS method and reported as micromoles of TBARS/L of saliva, as well as normalized by LA or protein content. The results indicated that in the absence of the two salivary proteins, alpha-amylase and mucin, SLO levels in AS mixtures containing only inorganic salts and/or LA was significantly lower ($p < 0.0001$), while addition of nitrite in the presence of LA and proteins did not influence the SLO levels. The addition of Fe(II) and Cu(II) to AS supplemented with LA resulted in incremental increase in SLO with increasing metal concentration; Cu(II) induced SLO levels were 3-10 times less than that of Fe(II). The results indicate that SLO profiles for Fe(II) and Cu(II) are characteristic of their metallic flavor attributes; salivary proteins can be a source of positive interference in measures of SLO by the TBARS method.

Key Words: Copper, Iron, Metallic Flavor, Lipid Oxidation, Protein, Saliva, TBARS

6.1 Introduction

Transition elements, iron and copper, are recognized as two major flavor producing metals in drinking water (USEPA 2009; World Health Organization (WHO) 2003). They are associated with many taste complaints among drinking water consumers as indicated in a survey of North American utilities (Suffet et al. 1996). In human sensory studies, perceived flavors of iron and copper have been characterized as metallic, salty, bitter and astringent (Epke and Lawless 2007; Hong et al. 2010; Ömur-Özbek and Dietrich 2011; Zacarias et al. 2001). Ferrous iron typicaly imparts the strongest metallic flavor, followed by cupric and cuprous salts, while bitterness and astringency are typically associated with the taste of copper (Ömur-Özbek 2008).

In addition to their distinctive, unpleasant flavor attributes, iron and copper are essential elements for maintaining bodily functions in all organisms; iron for its oxygen carrying capcity and copper as an enzyme cofactor (Cvetkovic et al. 2010). While elemental iron and copper are vital nutrients, their toxicity potentials is widely recognized due to their ability to cause oxidative stress in living cells by damaging memberane lipids and DNA (Jomova et al. 2010). Oxidative stress is defined as an imbalance between production of free radicals and reactive oxygen species, and their destruction by the protective actions of antioxidants and enzymes within the body (Durackova 2010). As associated with inducing oxidative stress, excessive iron and copper accumulation in the body have been linked to many serious diseases, such as alzheimers, parkinsons, and cancer (Jomova et al. 2010; Letelier et al. 2010). Oxidation by-products of memebrane lipids and/or proteins are often used as biomarkers of disease and deterioration of food matrices (Adibhatla and Hatcher 2010; Alexandrova et al. 2007; Sun et al. 2011). Two widely used biomarkers of lipid oxidation are malondialdehyde (MDA) and hydroxynonenal (HNE). The method of thiobarbituric reactive substances (TBARS) is widely utilized as an indirect indicator of lipid oxidation by-products (including MDA) in biological tissues and fluids, as well as food products (Guillen-Sans and Guzman-Chozas 1998; Khalili and Biloklytska 2008; Livnat et al. 2010).

Intriguingly, metal-induced lipid oxidation in the oral cavity, as measured by TBARS in human saliva after oral exposure to iron and copper, has been linked to the mechanism by which humans are able to detect the metallic flavor of iron and to some extent copper due to the release of volatile and odorous by-products of lipid oxidation in the oral cavity (Epke and Lawless 2007; Ömur-Özbek and Dietrich 2011; Ömur-Özbek 2008). The metallic flavor is thought to be perceived through retronasal detection of aromas associated with odorous aldehydes, such as hexanal, heptanal, 1-octen-3-one produced due to iron-induced LO reactions (Dietrich 2009; Glindemann et al. 2006; Tamura et al. 2009), since nose closure results in diminished or loss of this metallic flavor perception (Ömur-Özbek and Dietrich 2011). Within the oral cavity, lipid oxidation is caused by free radicals attacking lipid membranes (Ömur-Özbek 2008) and salivary lipids that are produced through the salivary glands (Larsson et al. 1996). Metals act as catalysts in the free radical processes that breakdown polyunsaturated fats (Repetto et al. 2010), while salivary nitrite has been shown to inhibit lipid oxidation in meat products under acidic conditions (Gorelik et al. 2007). Salivary proteins also play an important role in interaction of saliva with metals and thus may influence flavor perception (Hong et al. 2010). A recent study has shown that interaction of salivary protein, alpha- amylase, with copper impacts its solubility and thus contributes to the sensation of astringency associated with the flavor of copper in drinking water (Hong et al. 2009a). Another major salivary protein, mucin, has also been shown to have a high affinity for copper (Tang 2010). In biological samples, binding of lipid oxidation by-products, such as MDA and HNE, to proteins has been identified as an indirect cause of protein oxidation which also contributes to oxidative stress (Shacter 2000). In food systems, some proteins demonstrate antioxidant activities through their metal-binding properties which indirectly inhibit iron-catalyzed lipid oxidation reactions (Elias et al. 2008).

In addition to their unpleasant sensations, nutritional values, and toxicity potentials, metallic flavors such as those associated with iron and copper have often been experienced as phantom taste phenomenon by cancer patients undergoing chemotherapy and radiation (Comeau et al. 2001; Hong et al. 2009b). Unpleasant metallic sensations in the mouth have also been associated with side-effects of some

drugs as well as symptoms of some diseases (Doty and Bromley 2004; Nagler and Hershkovich 2005). While the causes of metallic taste disorders are not clear and subject of current research, study of salivary fluid and its constituents has been used in diagnostic applications (Bahar et al. 2007; Tenovuo 1989).

Using salivary lipid oxidation (SLO) as an indirect measure of metallic flavor intensity, the central aim of this study was to compare the levels of iron and copper induced SLO in artificial human saliva and to verify whether the presence of proteins in saliva influences the TBARS measurements intended to quantify lipid, not protein oxidation. Additional study objectives were to investigate the individual and interactive influences of fatty acids, proteins, and nitrite in the production and/or inhibition of metallic flavor. The findings will provide additional insights in understanding the implicating factors associated with metallic flavors of iron and copper upon interaction with salivary fluid as well as considerations in interpreting TBARS measurements in complex matrices, such as saliva where both lipids and protein can become oxidized upon exposure to redox active metals.

6.2 Materials and Methods

6.2.1 Artificial Saliva Mixture. Artificial saliva was prepared according to the recipes utilized by Tang (2010) and Hong et al (2006). This contained a mixture of inorganic components, consisting of NaCl (0.1256 g), KCl (0.9639 g), KSCN (0.189 g), KH_2PO_4 (0.655 g), Na_2SO_4 (0.337 g), NH_4Cl (0.178 g), $CaCl_2$ (0.172 g) and $NaHCO_3$ (0.631 g), dissolved in 1000 ml Nanopure water to make an artificial saliva stock solution. Both 0.216 g of mucin (Sigma-Aldrich, St. Louis, MO; CAS No. 84082-64-4) and 20,000 units, 0.541 g, of α-amylase (Sigma-Aldrich, St. Louis, MO; CAS No. 9001-19-8) were mixed in 100 mL of the inorganic saliva mixture to make the protein-spiked saliva solution. To make the lipid-amended saliva solution, 30 mg of linoleic acid (ACROS, New Jersey, USA, CAS No. 60-33-3) was added to 100 mL of the inorganic saliva mixture. Linoleic acid was used, as it is one of the major fatty acids in oral membrane lipids as well as a major constituent of total salivary lipids (Larsson et al, 1996). Nitrite-amended saliva solutions contained 250 mM of NO_2^- using sodium nitrite salt (Sigma-Aldrich, St. Louis, MO; CAS No. 237213). Reported concentrations of nitrite in human saliva range from

60 – 1600 mM (Gorelik et al. 2007), while total lipids and protein concentrations range from 2.4 to 80 mg/L and 0.6 to 4.0 g/L, respectively (Larsson et al. 1996; Tenovuo 1989; Actisa et al. 2005; Aydin 2007; Slomiany et al. 1983).

6.2.2 Metal Salts Stock Solutions. A 1.0 g/L iron and copper stock solutions were prepared using ACS grade iron (II) sulfate heptahydrate (Sigma-Aldrich, PA, CAS # 13463-43-g) and copper (II) sulfate pentahydrate (Sigma-Aldrich, PA, CAS # 13463-43-g) in Nanopure® water. Metal solutions were prepared immediately prior to use in testing to minimize air oxidation. The stock solutions were added in microliter amounts to achieve the targeted dosage of iron and/or copper in each of the artificial saliva test solutions described in the next section.

6.2.3 Salivary Lipid Oxidation Experiments with Artificial Saliva. Salivary lipid oxidation (SLO) experiments were conducted on artificial saliva samples that contained or excluded different salivary constituents in order to examine the extent of SLO under each condition. Test samples consisted of: 1) artificial saliva (AS) solution that contained only the inorganic constituents noted above, 2) AS solution amended with linoleic acid, (AS + LA), 3) AS solution supplemented with alpha-amylase and mucin (AS + Protein), 4) AS solution supplemented with both proteins and lipid (AS + LA + Protein), and 5) AS solution spiked with proteins, lipid, and nitrite (AS + LA + Protein + Nitrite). Test samples (1 through 5) were separately spiked with ferrous iron and cupric copper at concentration of 180 mM. Additionally, the test sample, AS + LA, was treated with varying concentrations of ferrous and cupric salts in order to examine the effect of metal concentration on the level of SLO induced by each metal. The concentration series for both ferrous and cupric consisted of 0, 4.5, 9, 18, 45, 90, 180, and 360 mM. The pH level was measured in each test sample using an ion selective pH electrode (Fisher Accumet). Upon addition of the metals, all test samples were placed in a 37 ℃ water bath for 15 minutes to simulate the temperature in the oral environment. The 15-minute time was based on a typical salivary flow rate of 5 mL per fifteen minute (Benaryeh et al. 1986). At the end of the incubation period, samples were immediately cooled and analyzed for lipid oxidation using the method of TBARS (Spanier 1991). The TBARS method was modified to work with liquid samples and to enhance readings

at low concentrations (Wang 2009). For all experiments, four replicate analyses were performed. Table 6.1 provides a summary of the artificial saliva test samples identifications and treatment conditions, along with designated abbreviations to be referred to in subsequent data analysis.

Table 6.1: Artificial Saliva test sample descriptions and abbreviations

Abbreviation	Artificial Saliva Sample Description	Organic Supplements (g/L)		Metal Concentration (μM): Fe(II) or Cu(II)
		Linoleic Acid, LA	Proteins (Alpha amylase + Mucin)	
AS1	AS	0	0	0, 180
AS2 - P	AS + Protein	0	3.78	0, 180
AS3 - LA	AS + LA	0.03	0	0, 4.5, 9, 18, 45, 90, 180, 360
AS4 - LAP	AS + LA + Protein	0.03	3.78	0, 180
AS5 - LAPN	AS + Protein + LA + Nitrite	0.03	3.78	0, 180

6.2.4 Data Analyses. Statistical software, JMP 9.0 (SAS, Cary, NC), was used for all data analyses. One-way analysis of variance (ANOVA) and comparison of the means, using Tukey HSD or Wilcoxon/Kruskal Wallis Rank sum test, were performed on the mean salivary lipid oxidation in the test samples. The salivary lipid oxidation (SLO) was reported as delta SLO, as the arithmetic difference between the measured salivary TBARS in the AS samples without and with iron or copper. SLO levels as TBARS (in micromoles) were normalized by the amount (in grams) of the added linoleic acid (LA) or protein. For statistical comparisons, three sets of analyses where performed. In one set, SLO data were normalized by the concentration of the added LA in the corresponding artificial saliva samples, which included AS3 - LA, AS4 - LAP, and AS5 – LAPN. In another set of analyses, SLO data were normalized by the amount of the added protein in the corresponding artificial saliva samples, which included AS2 - P, AS4 - LAP, and AS5 - LAPN. Lastly, a set of analyses were performed on the SLO data “as is” (i.e., not normalized by LA or protein); this included all five combinations of the AS samples. All statistical analyses were performed at alpha level of 0.05 and results were presented as means ± standard error (SEM).

6.3 Results and Discussion

6.3.1 Variations in Metal-induced Lipid Oxidation in Artificial Saliva. Addition of ferrous iron and/or cupric copper induced some salivary lipid oxidation (SLO) in all artificial saliva solutions, with the exception of the artificial saliva (AS) solution that contained no organic components (AS1); this solution showed very little or no notable amount of LO (Figure 6.1). Tables 6.2 and 6.3 provide summaries of the mean SLO data. Table 6.2 includes mean SLO data, reported as micromoles/L of saliva, without being normalized by the added linoleic acid (LA) and/or protein contents in the AS samples. Table 6.3 provides a summary of the data as normalized by LA and/or protein contents in the AS samples. The results of the data analyses on each set of data is presented as follows:

Analysis of the Non-Normalized Data (Table 6.2; Figure 6.1), the highest level of SLO was measured in the artificial saliva samples supplemented with LA and protein (AS4 - LAP) and protein (AS2 – P), followed by the LA-protein-nitrite amended samples (AS5 - LAPN), and lastly the artificial saliva supplemented with LA only (AS3). In samples treated with Fe(II) and Cu(II), there were significant differences between mean SLO levels in the LA-normalized artificial saliva samples [ANOVA for Cu(II): $F(4,19) = 5.39$; $p = 0.0007$) and for Fe(II): $F(4,19) = 16.04$; $p < 0.0001$]. Follow-up analysis on the means using Wilcoxon test showed significant differences between AS1 and all other saliva samples ($p = 0.03$), as well as AS3- LA and AS4 – LAP, and AS3 – LA and AS4 – LAPN sample means ($p = 0.03$).

Analysis of the Linoleic Acid (LA)-Normalized Data (Table 6.3; Figure 6.2). The highest level of SLO was measured in the artificial saliva samples supplemented with LA and protein (AS4 - LAP), followed by the LA-protein-nitrite amended samples (AS5 - LAPN), and lastly the artificial saliva supplemented with LA only (AS3). In samples treated with Fe(II), there were significant differences between mean SLO levels in the LA-normalized artificial saliva samples [$F(2,11) = 32.42$; $p < 0.0001$)]. Follow-up analysis on the means using Tukey-Kramer HSD test showed significant differences between AS2 – LA and AS2 – LAP sample means ($p < 0.0001$) and AS2 – LA and AS2 – LAPN sample means

(p = 0.0005). In contrast, in the Cu(II) treated samples, there were no significant differences between the mean SLO levels [F (2,11) = 1.93; p = 0.20)].

Table 6.2: Summary of salivary lipid oxidation (SLO) data, not normalized.

Artificial Saliva Sample	N	Metal-induced SLO (micromoles/L)	SEM (1)	95% Confidence Interval
Lipid Oxidation Experiments with Fe(II)				
AS1	4	0.06	0.01	[0.03 - 0.09]
AS2 - P	4	0.65	0.19	[0.04 - 1.23]
AS3 - LA	4	0.40	0.01	[0.36 - 0.44]
AS4 - LAP	4	1.00	0.08	[0.74 - 1.26]
AS5 - LAPN	4	0.88	0.05	[0.71 - 1.04]
Lipid Oxidation Experiments with Cu(II)				
AS1	4	0.05	0.01	[0.04 - 0.06]
AS2 - P	4	0.34	0.10	[0.19 - 0.50]
AS3 - LA	4	0.17	0.06	[0.06 - 0.27]
AS4 - LAP	4	0.34	0.19	[0.04 - 0.64]
AS5 - LAPN	4	0.24	0.09	[0.10 - 0.38]

(1) SEM: standard error of the mean.

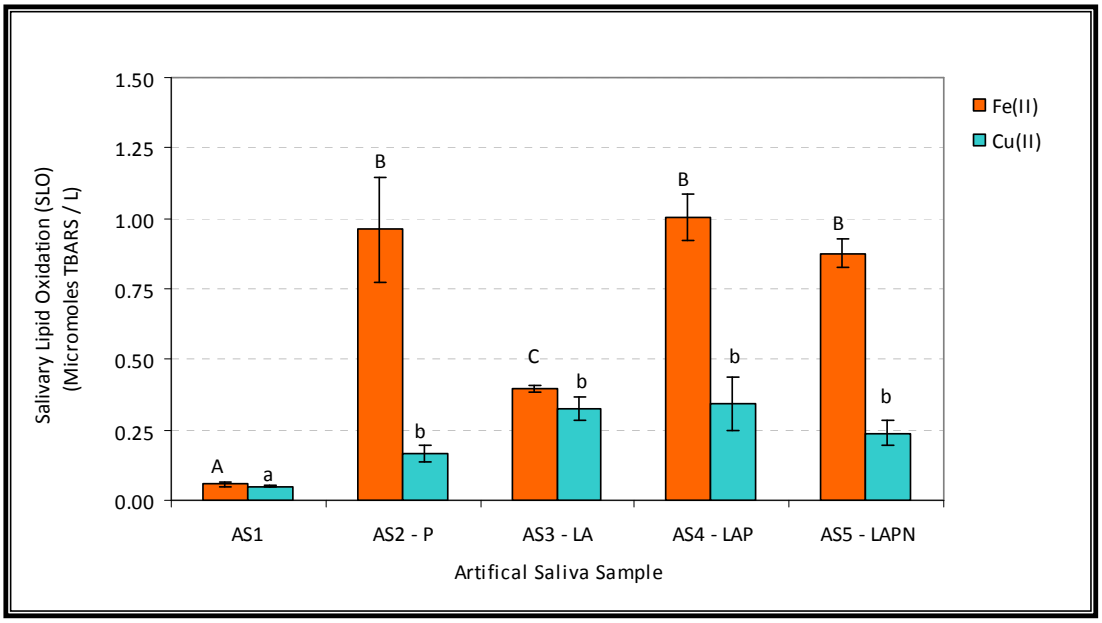

Figure 6.1: Mean Salivary lipid oxidation (SLO), measured in artificial saliva samples using thiobarbituric acid reactive substances (TBARS) methods; data not normalized. Error bars were constructed using 1 standard error from the mean of 4 replicate analyses. Within the same group [i.e., Fe(II) bars in capital letters and Cu(II) bars in small letters], bars with different letters indicate statistical significance ($p < 0.05$) between the compared pairs.

Table 6.3: Summary of salivary lipid oxidation (SLO) data, normalized by the protein and/or linoleic acid content in the artificial saliva mixtures.

Artificial Saliva Sample	N	Mean SLO (µmoles TBARS / g Linoleic Acid, LA)	SEM [1]	95% Confidence Interval	Mean SLO (µmoles TBARS / g Protein	SEM	95% Confidence Interval
Lipid Oxidation Experiments with Fe(II)							
AS2 - P	4	-- [2]	--	--	0.17	0.05	[0.01 - 0.32]
AS3 - LA	4	13.22	0.40	[11.94 - 14.50]	-- [3]	--	--
AS4 - LAP	4	33.40	2.73	[24.73 - 42.08]	0.26	0.02	[0.20 - 0.33]
AS5 - LAPN	4	29.23	1.70	[23.80 - 34.65]	0.23	0.01	[0.19 - 0.27]
Lipid Oxidation Experiments with Cu(II)							
AS2 - P	4	-- [2]	--	--	0.09	0.02	[0.05 - 0.13]
AS3 - LA	4	5.52	1.06	[2.16 - 8.89]	-- [3]	--	--
AS4 - LAP	4	11.34	3.16	[1.27 - 21.40]	0.09	0.01	[0.01 - 0.17]
AS5 - LAPN	4	7.97	1.47	[3.29 - 12.65]	0.06	0.01	[0.03 - 0.10]

(1) SEM: standard error of the mean.
(2) No linoleic acid was added to this artificial saliva sample (AS2 – P).
(3) No proteins were added to this artificial saliva sample (AS3 – LA).

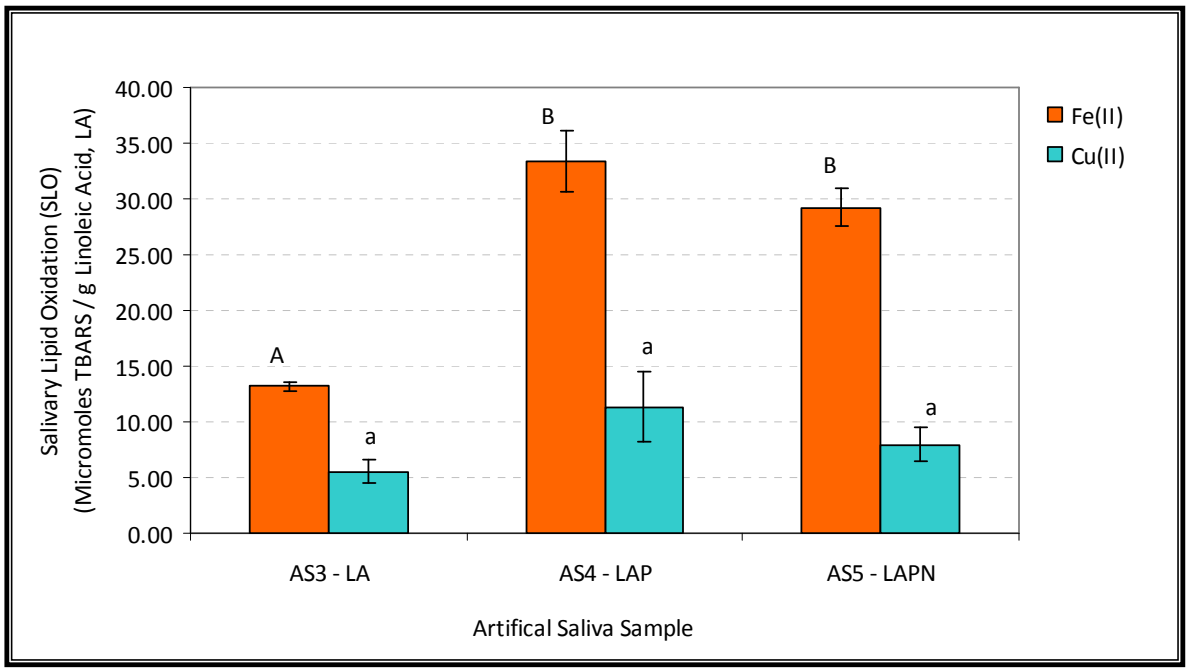

Figure 6.2: Mean Salivary lipid oxidation (SLO), measured in artificial saliva samples using thiobarbituric acid reactive substances (TBARS) methods; data linoleic acid (LA) normalized. Error bars were constructed using 1 standard error from the mean of 4 replicate analyses. Within the same group [i.e., Fe(II) bars in capital letters and Cu(II) bars in small letters], bars with different letters indicate statistical significance ($p < 0.05$) between the compared pairs.

Analysis of the Protein-Normalized Data (Table 6.3; Figure 6.3). In the protein-normalized samples, the highest level of SLO was measured in the artificial saliva samples supplemented with LA and protein (AS4 - LAP); however, in both Fe(II) and Cu(II) treated samples, there were no significant differences between mean SLO levels [for Fe(II), ANOVA: F (2,11) = 2.34; p = 0.15; for Cu(II), ANOVA: F (2,11) = 0.80; p = 0.48)].

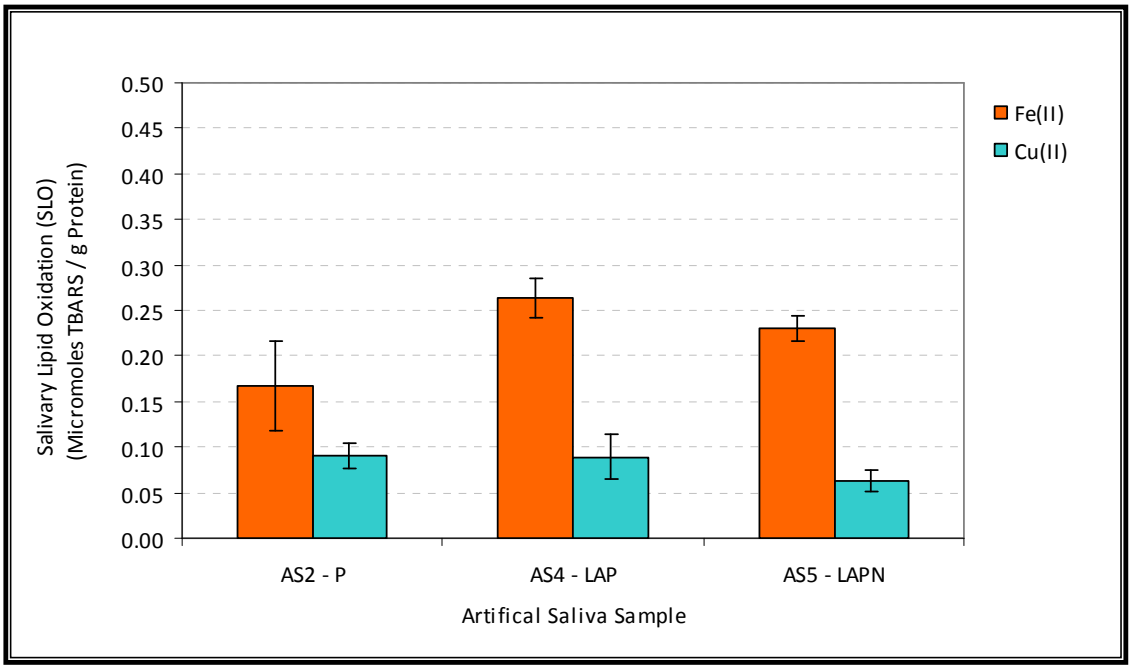

Figure 6.3: Mean Salivary lipid oxidation (SLO), measured in artificial saliva samples using thiobarbituric acid reactive substances (TBARS) methods; data protein normalized. Error bars were constructed using 1 standard error from the mean of 4 replicate analyses. Within each group [i.e. Fe(II) and Cu(II)], there were no significance differences between the mean SLO levels ($p < 0.05$).

6.3.2 Effect of Metal Concentration on Inducing Salivary Lipid Oxidation. The addition Fe(II) and Cu(II) to the artificial saliva sample supplemented with linoleic acid (LA) resulted in incremental increase in SLO with increasing metal concentration (Figure 6.4) as measured by TBARS, and the data normalized by the amount (in grams) of LA added to the sample . At a concentration range of 0 to 360 µM, Fe(II) induced SLO beginning at a concentration of 9 µM (corresponding to 0.5 mg/L Fe) and continued to increase incrementally up to the maximum tested concentration of 360 µM (corresponding to 20 mg/L Fe). Unlike Fe(II), treatment of a the LA-supplemented

artificial saliva sample with Cu(II) did not induce SLO until the cupric concentration reached 90 µM and it continued to rise, until appearing to level off at about 360 µM. In all the artificial saliva solutions, the pH level was measured at 6.8 ± 1 units and the metals appeared to remain dissolved in solution.

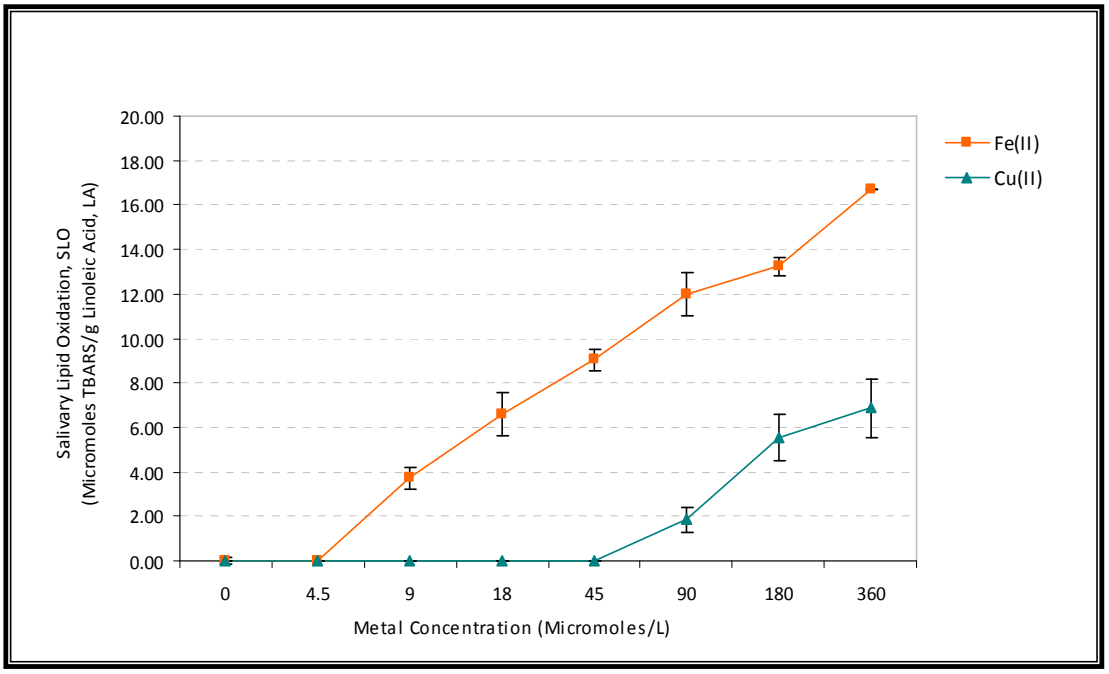

Figure 6.4: Variations in the mean salivary lipid oxidation (SLO) in artificial saliva versus metal concentration as measured using the thiobarbituric acid reactive substances (TBARS) method. Data is linoleic acid (LA) normalized. Error bars were constructed using 1 standard error from the mean of 4 replicate analyses.

6.3.3 Discussions on Findings. Assessing the influence of salivary constituents on the measure of metal-induced lipid oxidation by TBARS. The results of our study indicate that the presence of proteins, namely mucin and alpha-amylase, strongly influences the measure of lipid oxidation (LO) in artificial saliva by the TBARS method. While the presence of nitrite does not appear to exert inhibitory effects on salivary LO. When present in saliva, the inhibitory effect of nitrite on LO has been attributed to its conversion to nitric oxide (NO), which can ultimately alter LO pathways through binding with reduced metals such as ferrous iron (Gorelik et al. 2007). With regard to salivary proteins, both mucin and alpha amylase have been recognized for their varying potentials to bind with copper in artificial saliva (Hong et al. 2009a; Tang 2010) as well as in-vitro using actual human saliva (Hong and Kim 2011). The binding of copper and

iron to salivary protein occurs when these metals are in their free ionic forms, namely ferrous and cupric; this binding in turn can influence their flavor attributes through changing the speciation of metals in saliva (Hong et al. 2009a; Hong and Kim 2011). Similar binding interactions are expected for iron, although a recent study demonstrated that flavor intensity for copper was highly correlated to the concentration of soluble copper in saliva, but this was not the case for iron (Hong and Kim 2011). Recent experiments with artificial saliva have shown alpha-amylase to have varying binding capacities for free copper, with strongest binding occurring at relatively low concentrations of copper (< 2.5 mg/L); for example, at pH 6.5 and 2.5 mg/L total copper, the free copper, Cu(II), concentration in artificial saliva was less than 0.1 mg/L compared to about 0.6 mg/L at 5 mg/L total copper (Tang 2010). This study also showed that at higher copper concentration (10 mg/L), the binding capacity of alpha amylase decreased relative to inorganic constituents in saliva. In the same study, similar experiments with the salivary protein, mucin, demonstrated a considerably higher binding capacity to copper. In relating these findings to our present study, one can infer that in the presence of these proteins, Cu(II) becomes less available to induce lipid oxidation, hence, the measure of SLO in Cu(II) treated artificial saliva (AS) samples was notably lower than that of Fe(II) treated samples. For the same reasoning, our results suggest that at the given AS solution pH of 6.8, Fe(II) has a lower binding capacity for the two salivary proteins when compared to copper, therefore, it resulted in a higher iron-induced LO in the AS solution.

With regard to the use of TBARS as a measure of LO in saliva, our results highlight an important finding, that the presence of proteins, alpha-amylase and mucin, in saliva can be detected by the method of TBARS, thus significantly influencing the LO measurements. In fact, as noted earlier, the SLO data that were not normalized by the amount of protein in the artificial saliva samples, showed a significantly higher level of LO in the protein containing samples treated with Fe(II) as compared to the lipid-amended samples. However, in the case of Cu(II), the presence or absence of the proteins in the artificial saliva did not significantly influence the measure of LO by TBARS. Again, these results support the previous inference that the Cu(II) due to its

higher binding capacity to alpha-amylase and mucin than Fe(II), had less of an influence on inducing LO in the protein amended saliva samples.

The specificity of TBARS method to primarily detect LO by-products has been long questioned (Janero 1990). As one of several secondary end-products of LO, malondialdehyde (MDA) is formed by decomposition of certain primary and secondary lipid oxidation products (Fernandez et al. 1997). MDA is measured by the TBARS method through its reaction with thiobarbitric acid (TBA) under low pH and heated conditions that results in the formation of a pink product (MDA-TBA) that is colorimetrically measured at a wavelength of 532 nm (Spanier 1991; Fernandez et al. 1997). Previous studies have shown that there are many classes of non-lipid biological molecules, such as proteins, nucleic acids, bile pigments, and carbohydrates that have been supposed to be sources of MDA production in biochemical processes (Janero 1990; Fernandez et al. 1997). For this reason, in cases where quantification of MDA or other targeted LO by-products are of interest, the use of more sensitive methods such as high performance liquid chromatography has been recommended (Janero 1990; Jardine et al. 2002). While recognizing such implicating factors, our research finding on the contribution of proteins to the measure of LO by the TBARS method is reasonable. Additionally, with regard to saliva, other considerations, such as the use of a normalizing factor, namely protein and/or lipid content, may be warranted in providing additional insights in interpretation of results. Since the TBARS method is intended to measure LO, normalizing the TBARS levels by protein content in saliva will help to account for the protein interference. In fact, other researchers have recommended the use of a normalization factor to reduce the high variability associated with salivary TBARS measurements on human subjects, which is often used as a biomarker of oxidative stress in the study of oral diseases (Behuliak et al. 2009).

Comparing the Fe(II) and Cu(II) induced salivary lipid oxidation by metal concentration as related to metallic flavor attributes. The results of our experiments evaluating the influence of Fe(II) and Cu(II) concentration on inducing lipid oxidation (LO) in artificial saliva amended with linoleic acid, clearly indicate that iron has a notably higher LO inducing capacity than copper. This is expected since Fe(II) iron is a stronger reducing

agent than Cu(II) copper (Jensen 2003). Additionally, the differences between the LO profiles of Fe(II) and Cu(II) may be reasonable, given the recently reported findings (Tang 2010). Using the MINEQL+ chemical equilibrium modeling software, Tang modeled chemical speciation of Fe(II) and Cu(II) in artificial saliva containing only inorganic constituents. According to the model, at a given pH of 7.0 and metal concentration of 10 mg/L, the contribution of free iron, as Fe(II), to the total soluble iron in solution is 2.5 times greater than Cu(II), while at a lower pH of 5.5, the relative contributions of Fe(II) and Cu(II) are nearly equal (ratio = 1.09). Since our experiment was only conducted at a pH of 6.8, it is not possible to state whether at a lower pH of 5.5, the LO profiles for the two metals may have been more similar as it could be predicted based on the speciation model.

In range with previously reported measures of free fatty acids in human saliva (Larsson et al 1996), the concentration of linoleic acid (LA) in artificial saliva samples for our experiments was 30 mg/L, while the maximum metal concentration tested was about 20 mg/L. As noted earlier, in the concentration range of 0 to 360 µM (equivalent to 20 mg/L Fe(II)), metal-induced LO continued to rise with increasing metal concentration. Conducting similar experiments with artificial saliva at concentrations equal or higher than the amount of fatty acid in the sample, would provide additional insights on the LO profiles for the two metals, specifically, identifying the concentration at which LO will cease to rise any further, given a fixed reaction time. Regardless, it is clearly noted that in the presence of LA, Cu(II)-induced LO is considerably lower than the Fe(II)-induced LO. This difference in pattern of iron and copper induced LO has been observed in previous studies (Repetto et al. 2010). As an example, a toxicological study by Repetto and colleagues investigated the role of transition metals, Fe(II), Cu(II), Co(II), and Ni(II) on inducing lipid oxidation in the presence of liposomes and showed that Fe(II) induced the highest level of LO, followed by Cu(II), while cobalt and nickel had the lowest LO (Repetto et al. 2010).

In relation to metallic flavor attributes for iron and copper, our results suggest that by virtue of its higher level of metal-induced LO, upon oral intake, Fe(II) would produce a higher metallic flavor than Cu(II). Reported population thresholds for metallic flavor of

ferrous iron range from 0.03 to 0.5 mg/L among individuals with varying sensitivities and ages (Omur-Ozbek and Dietrich 2011; Mirlohi et al. 2011), while for cupric copper reported population thresholds range from 0.4 to 2.5 mg/L (Zacarias et al. 2001; Cuppett et al. 2006). Recognizing the findings from previous studies that indicate metallic flavor sensations from foods and beverages are associated with the detection of odorous by-products of LO (Epke and Lawless 2007; Ömur-Özbek 2008; Tamura et al. 2009), metallic flavor detection threshold for Fe(II) and Cu(II) are consistent with their respective LO profiles as demonstrated by our study. For Fe(II), LO became measurable by the TBARS method at a concentration of 0.5 mg/L (9 µM), while for Cu(II), LO was measurable at a concentration of 5.7 mg/L (90 µM), approximately 10 times greater than that of Fe(II).

6.4 Conclusion

In addition to being used as an indicator of metallic flavor production in saliva for sensory studies, the TBARS method has been used as biomarker of oxidative stress in various disease conditions. Our research findings provide additional insights in understanding the implicating factors associated with metallic flavors of iron and copper upon interaction with salivary fluid as well as considerations in interpreting TBARS measurements in complex matrices, such as saliva where both lipids and protein can become oxidized upon exposure to redox active metals. In this regard, normalization of TBARS measurements based on protein and/or lipid content is warranted.

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References

Actisa B, Perovicb N, Defago D, Beccaceceb C, Eynarda A. **2005**. Fatty acid profile of human saliva: a possible indicator of dietary fat intake. Arch Oral Biol 50: 1-6.

Adibhatla RM, Hatcher JF. **2010**. Lipid Oxidation and Peroxidation in CNS Health and Disease: From Molecular Mechanisms to Therapeutic Opportunities. Antioxid Redox Signal 12(1): 125-169.

Alexandrova A, Kebis A, Misl'anova C, Kukan M. **2007**. Copper impairs biliary epithelial cells and induces protein oxidation and oxidative DNA damage in the isolated perfused rat liver. Exp Toxicol Pathol 58(4): 255-261.

Aydin S. **2007**. A comparison of ghrelin, glucose, alpha-amylase and protein levels in saliva from diabetics. J Biochem Mol Biol 40(1): 29-35.

Bahar G, Feinmesser R, Shpitzer T, Popovtzer A, Nagler RM. **2007**. Salivary analysis in oral cancer patients - DNA and protein oxidation, reactive nitrogen species, and antioxidant profile. Cancer 109(1): 54-59.

Behuliak M, Palffy R, Gardlik R, Hodosy J, Halcak L, Celec P. **2009**. Variability of thiobarbituric acid reacting substances in saliva. Disease Markers 26(2): 49-53.

Benaryeh H, Shalev A, Szargel R, Laor A, Laufer D, Gutman D. **1986**. The salivary flow-rate and composition of whole and parotid resting and stimulated saliva in young and old healthy subjects. Biochemical Medicine and Metabolic Biology 36(2): 260-265.

Comeau TB, Epstein JB, Migas C**. 2001**. Taste and smell dysfunction in patients receiving chemotherapy: a review of current knowledge. Support Care Cancer 9(8): 575-580.

Cuppett JD, Duncan SE, Dietrich AM. **2006**. Evaluation of copper speciation and water quality factors that affect aqueous copper tasting response. Chemical Senses 31(7): 689-697.

Cvetkovic A, Menon AL, Thorgersen MP, Scott JW, Poole FL, Jenney FE, et al. **2010**. Microbial metalloproteomes are largely uncharacterized. Nature 466(7307): 779-U718.

Dietrich AM. **2009**. The sense of smell: contributions of orthonasal and retronasal perception applied to metallic flavor of drinking water. J Water Supply Res Technol-Aqua 58(8): 562-570.

Doty RL, Bromley SM. **2004**. Effects of drugs on olfaction and taste. Otolaryngol Clin N Am 37(6): 1229-+.

Durackova Z. **2010**. Some Current Insights into Oxidative Stress. Physiol Res 59(4): 459-469.

Elias RJ, Kellerby SS, Decker EA. **2008**. Antioxidant activity of proteins and peptides. Crit Rev Food Sci Nutr 48(5): 430-441.

Epke EM, Lawless HT. **2007**. Retronasal smell and detection thresholds of iron and copper salts. Physiol Behav 92(3): 487-491.

Fernandez J, PerezAlvarez JA, FernandezLopez JA. **1997**. Thiobarbituric acid test for monitoring lipid oxidation in meat. Food Chem 59(3): 345-353.

Glindemann D, Dietrich AM, Staerk HJ, Kuschk P. **2006**. The two smells of touched or pickled iron (skin) carbonyl-hydrocarbons and organophosphines. Angewandte Chemie Intl Ed (European Journal "Applied Chemistry") 45: 7006-7009.

Gorelik S, Kohen R, Ligumsky M, Kanner J. **2007**. Saliva plays a dual role in oxidation process in stomach medium. Archives of Biochemistry and Biophysics 458: 236-243.

Guillen-Sans R, Guzman-Chozas M. 1998. The thiobarbituric acid (TBA) reaction in foods: A review. Crit Rev Food Sci Nutr 38(4): 315-330.

Hong JH, Duncan SE, Dietrich AM, O'Keefe SF, Eigel WN, Mallikarjunan K. **2009a**. Interaction of Copper and Human Salivary Proteins. J Agric Food Chem 57(15): 6967-6975.

Hong JH, Duncan SE, Dietrich AM, O'Keefe SF. **2010**. Evaluation of the operationally defined soluble, insoluble, and complexing copper consumed through drinking water in human saliva. Eur Food Res Technol 231(6): 977-984.

Hong JH, Kim KO. **2011**. Operationally defined solubilization of copper and iron in human saliva and implications for metallic flavor perception. Eur Food Res Technol 233(6): 973-983.

Hong JH, Omur-Ozbeck P, Stanek B, Dietrich AM, Duncan S, Lee YW, et al. **2009b** . Taste and Odor Abnormalities in Cancer Patients. Journal of Supportive Oncology 7.

Janero DR. **1990**. Malondialdehyde and Thiobarbituric acid reactivity as diagnostic indexes of lipid-peroxidation and peroxidative tissue injury. Free Radic Biol Med 9(6): 515-540.

Jardine D, Antolovich M, Prenzler PD, Robards K. **2002**. Liquid chromatography-mass spectrometry (LC-MS) investigation of the thiobarbituric acid reactive substances (TBARS) reaction. J Agric Food Chem 50: 1720-1724.

Jensen N. **2003**. A Problem-Solving Approach to Aquatic Chemistry: John Wiley & Sons, Inc.

Jomova K, Vondrakova D, Lawson M, Valko M. **2010**. Metals, oxidative stress and neurodegenerative disorders. Mol Cell Biochem 345(1-2): 91-104.

Khalili J, Biloklytska H. **2008**. Salivary malondialdehyde levels in clinically healthy and periodontal diseased individuals. Oral Diseases 14(8): 754.

Larsson B, Olivecrona G, Ericson T. **1996**. Lipids in human saliva. Arch Oral Biol 41(1): 105-110.

Letelier ME, Sanchez-Jofre S, Peredo-Silva L, Cortes-Troncoso J, Aracena-Parks P. **2010**. Mechanisms underlying iron and copper ions toxicity in biological systems: Pro-oxidant activity and protein-binding effects. Chem-Biol Interact 188(1): 220-227.

Livnat G, Bentur L, Kuzmisnsky E, Nagler RM. **2010**. Salivary profile and oxidative stress in children and adolescents with cystic fibrosis. J Oral Pathol Med 39(1): 16-21.

Mirlohi S, Dietrich AM, Duncan SE. **2011**. Age-Associated Variation in Sensory Perception of Iron in Drinking Water and the Potential for Overexposure in the Human Population. Environ Sci Technol 45(15): 6575-6583.

Nagler RM, Hershkovich O. **2005**. Relationships between age, drugs, oral sensorial complaints and salivary profile. Arch Oral Biol 50(1): 7-16.

Ömur-Özbek P, Dietrich AM. **2011**. Retronasal perception and flavour thresholds of iron and copper in drinking water. J Water Health 9(1): 1-9.

Ömur-Özbek P. **2008**. Macromolecular Reactions and Sensory Perceptions at the Air-Water-Human Interface. [PhD Dissertation]. Blacksburg, VA: Virginia Tech.

Repetto MG, Ferrarotti NF, Boveris A. **2010**. The involvement of transition metal ions on iron-dependent lipid peroxidation. Arch Toxicol 84(4): 255-262.

Shacter E. **2000**. Quantification and significance of protein oxidation in biological samples. Drug Metab Rev 32(3-4): 307-326.

Chapter VII

In-vitro Evaluation of Iron-induced Salivary Lipid Oxidation Associated with Exposure to Zero-valent Iron Nanoparticles (nZVI)

Abstract

Zerovalent iron nanotechnologies are widely used for groundwater remediation and increasingly considered for advance oxidation treatment in drinking water applications. Iron nanoparticles have been detected in drinking water systems and considered for food fortification; therefore, human exposure potential through ingestion can be a concern. This study aimed to assess whether ingestion of iron nanoparticles from drinking water could be detected through flavor perception using in-vitro salivary lipid oxidation as an indicator for metallic flavor perception. Ten female subjects ages 29 to 59 years donated saliva samples for use in the in-vitro experiments. Test samples consisted of 1:1 mixture of saliva and bottled drinking water (Control) and three treatment solutions, spiked with ferrous sulfate, stabilized nZVI, and an aggregated/microsized suspension of zerovalent iron metal powder, mZVI. Upon mixing, samples were subjected to 15-minute incubation at 37ºC to resemble oral conditions. Salivary lipid oxidation (SLO) was measured in all samples as micromoles of TBARS/mg Fe. Exposure to iron in all three forms induced significant amount of SLO in all treatment samples as compared to the control ($p < 0.0001$). The mean SLO levels were the highest in the ferrous, followed by nZVI, and mZVI treatments; the differences in the mean SLO levels were significant ($p < 0.05$). The findings indicate that oral exposure to ZVI nanoparticles may induce sensory properties different from that of ferrous salt, likely predictive of a diminished detection of metallic flavor by humans.

Key Words: Nanoscale iron, zerovalent iron, salivary lipid oxidation, oxidative stress, ferrous iron.

7.1 Introduction

Metallic iron in its zerovalent form has been widely used in the treatment of contaminated groundwater since the early 1990s when it was first discovered that chlorinated hydrocarbons such as trichlorethene (TCE) could be dehalogenated in the presence of iron metal (Gillham and Ohannesin 1994; Matheson and Tratnyek 1994). Since then, the use of metallic iron as applied in the form of permeable reactive barriers (PRBs) has been widely reported in groundwater remediation sites (USEPA 2008). More recently, scientific explorations in the field of nanoscience have opened many more intriguing possibilities with reported uses of zerovalent iron nanoparticles (nZVI) for the removal of toxic contaminants such as nitrate, perchlorates, arsenic, hexavalent chromium, uranium, and antibiotics from drinking water, and the use of magnetic iron oxides for targeted drug delivery and food fortification (Cao et al. 2005; Fang et al. 2011; Ghauch et al. 2009; Hilty et al. 2010; Tanboonchuy et al. 2011).

Consequently, with widespread usage and increasing applications, concerns for environmental exposure and toxicity impacts arise. For nZVI, the most likley potential for human exposure is through accidental dermal contact during manufacturing and slurry application, or ingestion of drinking water from contaminated wells and surface waters (Keenan et al. 2009). Inhalation can also be an important route of exposure through aerosals, iron oxide/carbon balck particles, and atmospheric dust (Keenan et al. 2009; Guo et al. 2009; Shi et al. 2011).

Few studies have investigated toxicity impacts of nZVI in the aquatic environment due to the ubiquitous nature of iron in the form of oxides as well as the limited mobility of iron nanoparticles once oxidized to form ferrous, ferric, and iron oxides. Although based on these characteristics, exposure potential to humans and higher organisms is believed to be unlikely, toxicity concerns are increasing as more engineered nanoparticles are being developed to enhance the mobility of nZVI through the use of particle stabilizing agents, such as surfactants, polymers, and polyelectrolytes. Additionally, the highly reductive property of nZVI that makes it so appealing in terms of removal of toxic contaminants, has been recognized as a mean by which surface and/or ground water contaminants can be effectively captured and transported through drinking

water systems. For example, nanoscale iron oxides have been found bounded to copper in surface waters many kilometers downstream from mining sites (Hochella et al. 2005). In a drinking water system, a sample of 20 nm particles was found to contain lead and iron, the iron was tentatively identified as its oxide form, hematite (Wigginton et al. 2007; Zhao et al. 2011).

While elemental iron is a vital nutrient for the maintenance of body functions in all organisms, its toxicity potential is widely recognized due to its ability to cause oxidative stress in living cells by damaging membrane lipids and DNA. Oxidative stress is defined as an imbalance between production of free radicals and reactive oxygen species, and their destruction by the protective actions of antioxidants and enzymes within the body (Durackova 2010). As associated with inducing oxidative stress, excessive iron accumulation in the body has been linked to many serious diseases, such as atherosclerosis, Alzheimer's, Parkinson's, and cancer. Several types of metal nanoparticles, namely, titanium, silver, iron oxides, copper, manganese oxide, and aluminum, are known to induce inflammation in brain tissues and alter key protein functions that can ultimately lead to development of neurodegenerative diseases (Bondy 2011).

The ability of iron to induce oxidative stress in biological tissues and fluids has been quantified by measuring lipid oxidation products using the method of thiobarbituric reactive substances (TBARS). Intriguingly, iron-induced salivary lipid oxidation in the oral cavity, as measured by TBARS, has been linked to the mechanism by which humans are able to detect the flavor of iron from the release of volatile and odorous by-products of lipid oxidation in the oral cavity (Ömur-Özbek 2008). Using salivary lipid oxidation (SLO) as an indirect measure of metallic flavor intensity, the aim of this study was to compare iron-induced SLO between soluble ferrous iron and nanoparticles of zerovalent iron in micro and nano scale suspensions.

7.2 Materials and Methods

7.2.1 Human Subjects. The study was approved by the Institutional Review Board (IRB) at Virginia Tech (IRB Project No. 06-395). Human subjects were recruited from the community, students, faculty and staff of Virginia Tech and Blacksburg, Virginia by means of paper and email flyers. Subjects were required to have no chronic oral or general health problems, be non-smokers, and not pregnant. All subjects read and signed an informed consent form in accordance with the approved IRB protocols. Ten female subjects, ages 29 – 59 years, donated saliva for use in this study.

7.2.2 Saliva Collection. Subjects were asked to refrain from eating, drinking, and smoking for at least one hour prior to collecting their saliva sample. Three daily saliva collection sessions were conducted within the same week and time frame of 10 am to 12 noon. For each daily session, subjects were first asked to thoroughly rinse their mouth with Aquafina® bottled water and wait 1 minute to stabilize the conditions in their mouth. Then, subjects' oral pH was measured using a pH indicator strip (Cen-Med/Fisher M95883) by placing the pH strip on their tongue until it became moist with their saliva; then, using the color scale, the pH was read and recorded after 1 minute and while the strip was still moist. Subjects were asked to expectorate approximately 4 mL of saliva into 50 mL propylene tubes during each session for a total volume of 12 mL collected over the three daily sessions. Between daily collections, saliva samples were kept stored in a freezer; at the end of final collection, saliva samples were frozen immediately and stored at -50 ºC for up to one month until analysis.

7.2.3 Preparation of Iron Solutions. Three different stock solutions of iron were prepared immediately before use in the experiments. The stock solutions included iron (II) sulfate (Sigma-Aldrich, PA, CAS # 13463-43-g) and two aqueous dispersions of manufactured nZVI products, NANOFER 25S and NANOFER STAR (kindly provided by Nanoiron Ltd., Rajhrad, Czech Republic, EU). As described by the manufacturer, NANOFER 25S is a stabilized water dispersion of nano zerovalent iron particles consisting of Fe, Fe_3O_4, C, and organic stabilizer. The NANOFER STAR is an air-stable nZVI powder, consisting of Fe(0) surface stabilized nanoparticles coated by a thin organic surface layer to protect against air oxidation and allow for ease of transport and

long-term storage. The iron solutions were prepared by mixing the iron salt or the nanoiron product with bottled drinking water (Aquafina®) to provide for 10 mg/L of total iron concentration in the resulting solution. For the NANOFER 25S, the suspension as provided by the manufacturer, contained approximately 15% Fe(0) and it was diluted accordingly to the targeted concentration of 10 mg/L total Fe. The NANOFER STAR solution was prepared by mixing 1 part (10 g) of the powder with 4 parts (40 mL) of the bottled drinking water to provide for approximately 15% Fe(0) suspension; then, the mixture was diluted to the targeted concentration of 10 mg/L total Fe. Since pH level is important in iron chemistry as well as stability of nanoparticles in suspension, pH was measured in the stock solutions as the prepared samples using pH indicator strips (Cen-Med/Fisher M95883). The total iron concentration of each stock solution was quantified using inductively coupled plasma – mass spectroscopy (Thermo Electronic Corporation, X-Series ICP-MS, Waltham, MA), following Standard Method 3120B (American Public Health Association (APHA); the American Water Works Association (AWWA); the Water Environment Federation (WEF) 2005).

7.2.4 Zerovalent Iron Nanoparticles (nZVI) Characteristics. Characteristics of the iron nanoparticles (NPs) were provided by the manufacturer: the average particle size was < 50 nm; the specific surface area was > 25 m^2/g with spherical morphology; the dispersion density was 1210 kg/cm. Additionally, freshly prepared solutions of the iron nanoparticles were analyzed by dynamic light scattering (Zetasizer™ Nano Series; Beckman Coulter Inc., CA, USA) to verify the size distribution and zeta potentials for each of the prepared nZVI suspensions. For the size and zeta potential determination, the suspensions had to be diluted 100 times (i.e., approximately 0.15% Fe(0) solutions).

7.2.5 In-vitro Experiments. In-vitro experiments were performed using individual saliva samples from each of the ten subjects, as well as a single pooled saliva sample from multiple subjects. In-vitro test samples were prepared by mixing equal volumes of saliva and iron stock or control solutions. The control sample consisted of equal volumes of saliva and bottled drinking water (Aquafina®). While, the treatment groups consisted of equal volumes of saliva and iron stock solutions. Corresponding to each iron stock solution, there were three treatment groups identified as Fe(II), nZVI, and

mZVI. For each experimental run, a minimum of three replicates per control and treatment group was prepared. After preparing the mixture of saliva and test samples in 50 mL propylene test tubes, they were placed in a 37ºC water bath for 15 minutes to resemble the oral cavity conditions. After the 15-minute incubation time, test samples were immediately analyzed for salivary lipid oxidation using the thiobarbituric acid reactive substances (TBARS) method as a measure of iron-induced oxidative stress within the oral cavity. The TBARS method (Spanier 1991) was modified to work with liquid samples and to enhance readings at low concentrations (Wang 2009).

7.2.6 Data Analyses. Statistical software, JMP 9.0 (SAS, Cary, NC), was used for all data analyses. One-way analysis of variance (ANOVA) and comparison of the means, using Tukey HSD or Wilcoxon/Kruskal Wallis Rank sum test, were performed on the mean oxidative stress responses as measured by salivary lipid oxidation in the control and test samples. The salivary lipid oxidation (SLO) was also reported as delta SLO, as the arithmetic difference between the measured salivary TBARS in the control and the iron containing test samples. To normalize the measured iron-induced SLO levels, the delta SLO values in micromoles of TBARS were divided by the concentration of total iron in milligrams. All statistical analyses were performed at alpha level of 0.05 and results were presented as means ± standard error (SEM).

7.3 Results and Discussion

7.3.1 Particle Size and Zeta Potential Characteristics of the Nanoparticles. The particle size distribution for the stabilized suspension of the nZVI, NANOFER 25S ranged from 18 to 110 nm with an average size of 52 nm; the zeta potential was measured at - 60 mV (Figure 7.1a). The prepared suspension using the stabilized powder form, NANAOFER STAR behaved as micro rather than nanoparticles, with the average size estimated at 635 nm and the zeta potential measured at - 19 mV. Plots of size and zeta potential could not be obtained due to difficulty of obtaining a stable suspension; however, an electron microscopic view of the air-stabilized particles as provided by the manufacturer is shown in Figure 7.1b. Based on this characterization, when the NANOFER 25S product was used in the in-vitro experiments, test samples

were identified as nZVI, whereas, designation of mZVI was used when NANOFER STAR product was used, thus characterizing the particles as greater than 100 nm.

7.3.2 Measure of pH in Saliva and Test Samples. The pH levels in saliva ranged from 6.0 to 7.0 (mean = 6.4; SD = 0.33). In the three iron stock solutions, the pH was 5.0; while in the control and all treatment samples, the measured pH was 7.0.

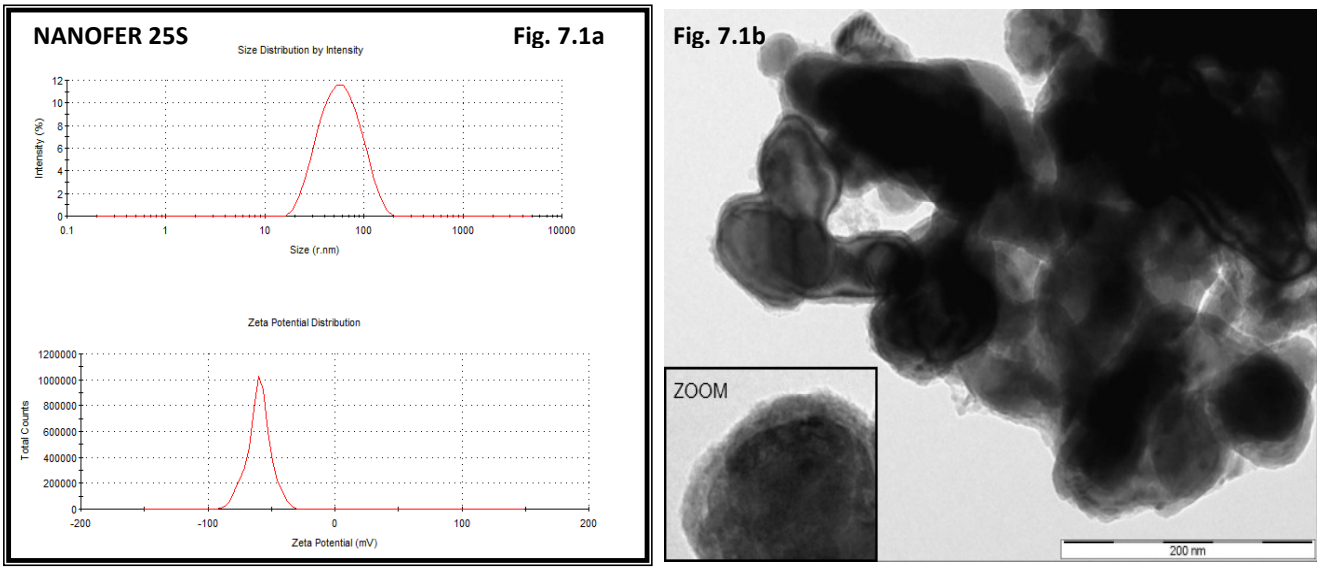

Figure 7.1: Particle size distribution and zeta potential for the stabilized suspension of zerovalent iron nanoparticles (nZVI). The nZVI products included NANOFER 25S (Figure 7.1a) and NANOFER STAR (Figure 7.1b: http://www.nanoiron.cz/en/nanofer-star). Both products were kindly provided by Nanoiron Ltd., Rajhrad, Czech Republic, EU.

7.3.3 Iron-induced Oxidative Stress Response. In the in-vitro experiments performed using individual saliva as well as the pooled saliva samples, addition of iron to human saliva induced oxidative stress response. This was indicated by significantly higher level of salivary lipid oxidation (SLO) in the test samples containing iron when compared to the control samples (Table 7.1); this observation was consistent in the experiments with individual saliva samples [$F(3,45) = 15.60$; $p < 0.0001$); Figure 7.2] as well as the pooled saliva sample [$F(3,11) = 164.32$; $p < 0.0001$); Figure 7.2].

In experiments conducted using individual saliva samples from the 10 subjects, the analysis of variance on the iron-induced delta SLO levels (i.e., SLO response in

treatment group minus SLO response in control group) indicated a significant difference between at least one pair of the mean responses [F (2, 35) = 3.72, p = 0.035]. Follow-up comparison of the mean delta SLO responses, using Wilcoxon's test, indicated a significance difference only between the Fe(II) and nZVI treatments (p = 0.038) and Fe(II) and mZVI (p = 0.044), but no significant differences between nZVI and mZVI [(p = 0.47); Table 7.1; Figure 7.3].

Table 7.1: Summary of oxidative stress response as measured by in-vitro salivary lipid oxidation (SLO)

Treatment	N	Mean SLO (µM TBARS)	SEM (1)	95% Confidence Interval	Delta SLO (2) (µM TBARS/mg Fe)	SEM	95% Confidence Interval
In-vitro Experiments with Individual Saliva Samples							
Control	10	0.229	0.031	[0.159 – 0.299]	-	-	-
Fe(II)	10	2.264	0.303	[1.578 – 2.950]	0.421	0.062	[0.282 – 0.561]
nZVI	13	1.494	0.208	[1.040 – 1.948]	0.235	0.037	[0.155 – 0.315]
mZVI	13	1.051	0.172	[0.676 – 1.425]	0.245	0.057	[0.120 – 0.369]
In-vitro Experiments with Pooled Saliva Samples							
Control	3	0.117	0.023	[0.017 – 0.216]			
Fe(II)	3	1.020	0.035	[0.868 – 1.171]	0.095	0.002	[0.087 – 0.103]
nZVI	3	0.777	0.042	[0.597 – 0.956]	0.062	0.006	[0.038 – 0.086]
mZVI	3	0.389	0.020	[0.303 – 0.475]	0.043	0.005	[0.022 – 0.064]

(4) SEM: standard error of the mean.

(5) Delta SLO: salivary lipid oxidation (SLO) in the treatment sample minus the SLO in the control sample; SLO levels were normalized based on the measured amount of total iron in the sample.

In experiments conducted with the pooled saliva sample, the analysis of variance on the iron-induced delta SLO levels indicated a significant difference between at least one pair of the mean responses [F (2, 8) = 34.75, p = 0.0005]. Follow-up comparison of the mean delta SLO responses, using Tukey HSD test, indicated a significance difference between the following treatment pairs: Fe(II) and nZVI (p = 0.0045), Fe(II) and mZVI (p = 0.0004), and marginally non-significant difference between nZVI and mZVI (p = 0.058); (Table 7.1; Figure 7.3).

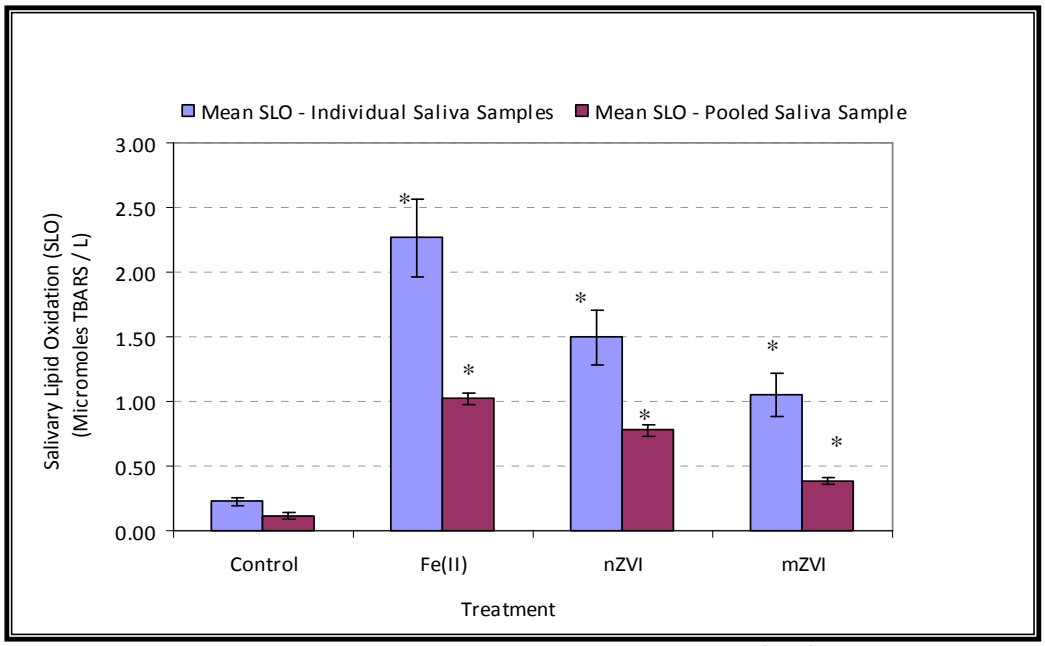

Figure 7.2: Oxidative stress response as measured by salivary lipid oxidation (SLO) using thiobarbituric acid reactive substances (TBARS) substances. Blue bars represent mean SLO responses in in-vitro experiments using individual saliva samples from 10 subjects. Purple bars represent the mean SLO response in in-vitro experiments using a single pooled saliva sample for 3 duplicate experiments. Error bars constructed using 1 standard error from the mean. Asterisks indicate statistical significance ($p < 0.05$) in treatment group when compared to the control.

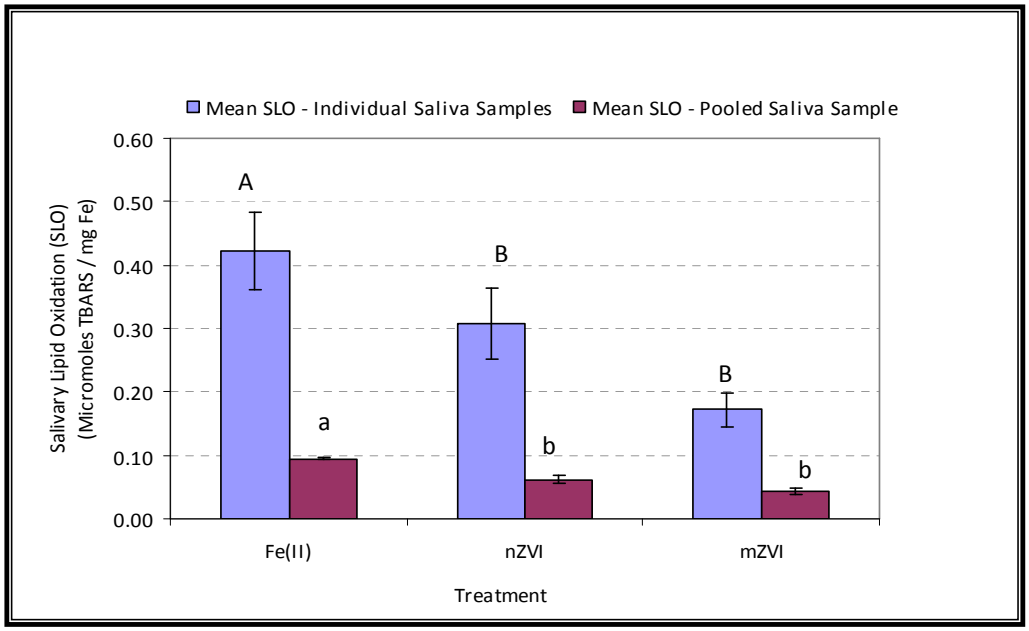

Figure 7.3: Iron-induced oxidative stress response as measured by delta salivary lipid oxidation (SLO) using thiobarbituric acid reactive substances (TBARS) substances. Delta SLO levels were normalized by mg of total Fe. Blue bars represent mean SLO responses in in-vitro experiments using individual saliva samples from 10 subjects. Purple bars represent the mean SLO response in in-vitro experiments using a single pooled saliva sample for 3 duplicate experiments. Error bars constructed using 1 standard error from the mean. For each set of experiments, mis-matched letters indicate statistical significance ($p < 0.05$) between the means responses among the treatment groups.

7.3.4 Discussions on Findings. Assessing the impact of nanoparticle size and/or aggregation on iron-induced salivary oxidative stress response. The results of our study indicate that ferrous iron salt, being the most soluble as compared to the two nanoiron products, induced the highest level of oxidative stress response in human salivary fluid as measured by lipid oxidation. After ferrous iron, the stabilized suspension of nZVI product, NAOFER 25S, produced the next highest level of iron-induced oxidative stress, followed by the stabilized powder, NANOFER STAR, showing the least oxidative stress response. Based on these observations, one can infer that in the case of zerovalent iron, potential for iron-induced salivary oxidative stress is higher when particle size becomes smaller. Whereas, when the particles get larger by size characteristics and/or aggregation effect, the oxidative stress declines. Prior research studies exploring the impact of particle size on nanometals toxicity have produced varying results. With regard to iron nanoparticles, toxicity, which is often associated with oxidative stress response in biological system, is induced by the production of reactive oxygen species (ROS) through Fenton reactions. In one study, nanometer or micrometer particle size toxicity varied by the type of metal; for example, cupper oxide in its nano form induced higher oxidative stress in human lung cells when compared to micro size particles. While in the case of iron, iron oxide (Fe_2O_3) induced lower toxicity with no considerable difference between the particle sizes in terms of induced oxidative stress (Karlsson et al. 2009). Other studies have shown that pH, particle surface chemistry, and presence of ligands are important factors in comparing toxicity levels of nZVI particles and ferrous iron (Keenan et al. 2009; Kim et al. 2011; Phenrat et al. 2009).

Exploring the role of pH and zeta potential on iron nanoparticles behavior in salivary fluid. In aqueous systems, the role of pH in influencing zeta potential is critical as it indirectly influences particle charge and stability through changing the zeta potential in aqueous suspensions (Zetasizer Nano Series Technical Notes). Nano sized particles can become attached to one another and effectively behave as micro sized particles by physical aggregation (Phenrat et al. 2008; Tratnyek and Johnson 2006). Solution pH, as well as zeta potential, influence zerovalent nanoparticles' tendencies to agglomerate (USEPA 2008). Additionally, other factors such as ionic strength and particle

concentration influence the agglomeration of particles (Phenrat et al. 2008). With regard to zeta potential, particles begin to agglomerate at zero zeta potential, thus becoming less mobile and reactive; while particles with large positive or negative zeta potentials are considered to be stable, thus resistant to agglomeration (Zhang and Elliott 2006). The particle stability range is defined at zeta potentials greater than +30 milivolts (mV) and less than -30 mV (Zhang and Elliott 2006). Previous research has indicated that the zeta potential for uncoated nZVI particles to be -30 ± 3 mV, making them virtually immobile (Saleh et al 2008); while stabilized nZVI become increasingly mobile with higher zeta potentials, as high as -50 ± 1.2 mV (Saleh et al. 2008). In groundwater applications, nZVI particles have been shown to have a 0 zeta potential at a pH of approximately 8.1; as the pH increases, zeta potential becomes negative, approaching –30 mV at pH 8.4, and likewise, gaining positive zeta potential below pH 8.1 (Zhang and Elliott 2006).

In human saliva, the pH remains relatively neutral, with levels ranging from 5.5 to 7.5 (Mirlohi et al. 2011). The zeta potential of human saliva has been estimated to be at 0 at pH of 3 and becoming negative with increasing pH levels, with a maximum zeta potential of –15 mV at pH 8.0 (Rykke et al. 1996). At the neutral pH of 7.0, a zeta potential of approximately –10 mV has been measured in human saliva (Rykke et al 1996). Based on these saliva characteristics and with consideration of zeta potentials for nZVI particles, one can infer that with higher negative charge, stabilized nZVI particles may be less likely to agglomerate when combined with salivary fluid at pH 7.0, and thus more reactive. On the other hand, already agglomerated nZVI particles, having a smaller negative zeta potential may be more likely to reach a 0 zeta potential, once mixed with saliva. Additionally, interaction of surfaced charged nZVI nanoparticles with the numerous salivary proteins present in human saliva can further impact their agglomeration properties (Rykke et al 1996); this factor could in turn influence the particles availability for inducing lipid oxidation.

Predicting flavor perception of iron nanoparticles based on salivary lipid oxidation phenomenon. Iron as well as copper induced lipid oxidation in the oral cavity has been associated with the perception of metallic flavor by human subjects (Mirlohi et al. 2011;

Ömur-Özbek and Dietrich 2011). With regard to oral lipid oxidation, flavor perception has been associated with retronasal detection of odors produced from volatile by-products of lipid oxidation in the oral cavity, namely odorous aldehydes and ketones (Ömur-Özbek 2008; Tamura et al. 2009). Prior research has demonstrated that the oral intake of iron-spiked drinking water has resulted in significant increase in in-vivo salivary lipid oxidation as measured by TBARS concentration in saliva before and after the oral intake or iron (Ömur-Özbek and Dietrich 2010; Mirlohi et al 2011). These findings are consistent with our current in-vitro study on iron-induced salivary lipid oxidation (SLO). With regard to flavor perception, the significantly reduced iron-induced salivary lipid oxidation by nZVI and mZVI particles observed in our study, may be predictive of sensory properties of ZVI nanoparticles different to that of ferrous salt. Possibly, reduced SLO may correspond to less metallic flavor detection, although smell functions are important in retronasal detection of metallic flavor as nose closure has been associated with significant decline or loss of metallic flavor perception by humans (Ömur-Özbek and Dietrich 2011; Lawless et al. 2004; Lim 2005). With regard to sensory properties, a recent study did in fact indicate that nanoscale iron particles, being less soluble than ferrous sulfate, were highly bioavailable when fed to rats in iron fortified food matrices; the nanosized particles also imparted considerably less color and taste change in food (Hilty et al. 2010). Although higher bioavailability would translate to benefits for iron-deficient populations (Zimmermann and Hilty 2011) and potential risks to vulnerable populations with hereditary iron overload disease (Brewer 2010).

Limitations of the current study and insights for future research. Our study aimed to gain some insights on predicting the ability of humans to detect iron nanoparticles through oral exposure, as human senses are often the first line of defense in detecting contaminants. While our research is limited by small scale, it provides many insights for future studies:

1. Our results indicate a high variability among human subjects on in-vitro salivary lipid oxidation response, with coefficient of variations ranging from 45% to 60% when using individual saliva samples. While with the pooled saliva sample, the coefficient of variation in the SLO response ranged from 3% to 16%, thus

enhancing the ability to detect significant differences between the mean SLO responses among the treatment groups. Therefore, in future research studies, in-vitro experiments should be conducted with pooled saliva.

2. Exploring the roles of salivary pH and zeta potential on how nZVI particles may be behave upon oral intake is intriguing. Further studies exploring the change in zeta potentials of salivary and nZVI fluid mixtures at varying pH levels would provide insights on agglomeration tendencies of nZVI particles and their subsequent impact on salivary lipid oxidation.

3. Lastly, with human salivary fluid enriched with numerous proteins, including those with metal binding capacities, fatty acids and lipids, electrolytes, and antimicrobial agents, human saliva can be used as an innovative and simple mean by which interactions of nZVI and other metal nanoparticles in biological fluids can be studied.

7.4 Conclusions

With increasing and innovative use of nanoparticles in variety of applications, concerns for potentially toxic exposure to humans as well as other organisms are well founded, as discussed earlier in this manuscript. While our research focused on iron nanoparticles, not typically a concern for toxic exposure to humans, similar in-vitro studies with toxic metals such as copper would be beneficial in assessing the potential for human sensory detection of copper nanoparticles through oral exposure. Like iron, copper is a flavor producing metal; however, due to its toxicity, it is regulated in drinking water based on aesthetics, as well as health-based standards (USEPA 2009). Additionally, both copper and iron nanoparticles have been found in drinking water systems due to release of corrosion products from pipe surfaces (Wigginton et al. 2007; Vargas et al. 2010).

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References

American Public Health Association (APHA); the American Water Works Association (AWWA); the Water Environment Federation (WEF), ed. **2005**. Standard Methods for Examination of Water and Wastewater. Washington DC.

Bondy SC. **2011**. Nanoparticles and Colloids as Contributing Factors in Neurodegenerative Disease. Int J Environ Res Public Health 8(6): 2200-2211.

Brewer GJ. **2010**. Risks of copper and iron toxicity during aging in humans. Chem Res Toxicol 23(2): 319-326.

Cao JS, Elliott D, Zhang WX. **2005**. Perchlorate reduction by nanoscale iron particles. J Nanopart Res 7(4-5): 499-506.

Durackova Z. **2010**. Some Current Insights into Oxidative Stress. Physiol Res 59(4): 459-469.

Fang ZQ, Qiu XQ, Huang RX, Qiu XH, Li MY. **2011**. Removal of chromium in electroplating wastewater by nanoscale zero-valent metal with synergistic effect of reduction and immobilization. Desalination 280(1-3): 224-231.

Ghauch A, Tuqan A, Assia HA. **2009**. Antibiotic removal from water: Elimination of amoxicillin and ampicillin by microscale and nanoscale iron particles. Environ Pollut 157(5): 1626-1635.

Gillham RW, Ohannesin SF. **1994**. Enhanced degradation of halogenated aliphatics by zero-valent iron. Ground Water 32(6): 958-967.

Guo B, Zebda R, Drake SJ, Sayes CM. **2009**. Synergistic effect of co-exposure to carbon black and Fe(2)O(3) nanoparticles on oxidative stress in cultured lung epithelial cells. Part Fibre Toxicol 6.

Hilty FM, Arnold M, Hilbe M, Teleki A, Knijnenburg JTN, Ehrensperger F, et al. **2010**. Iron from nanocompounds containing iron and zinc is highly bioavailable in rats without tissue accumulation. Nat Nanotechnol 5(5): 374-380.

Hochella MF, Kasama T, Putnis A, Putnis CV, Moore JN. **2005**. Environmentally important, poorly crystalline Fe/Mn hydrous oxides: Ferrihydrite and a possibly new vernadite-like mineral from the Clark Fork River Superfund Complex. American Mineralogist 90: 718-724.

Karlsson H, Gustafsson J, Cronholm P, Möller L. **2009**. Size-dependent toxicity of metal oxide particles-A comparison between nano- and micrometer size. Toxicol Lett(188): 112-118.

Keenan CR, Goth-Goldstein R, Lucas D, Sedlak DL. **2009**. Oxidative Stress Induced by Zero-Valent Iron Nanoparticles and Fe(II) in Human Bronchial Epithelial Cells. Environ Sci Technol 43(12): 4555-4560.

Kim JY, Lee C, Love DC, Sedlak DL, Yoon J, Nelson KL. **2011**. Inactivation of MS2 Coliphage by Ferrous Ion and Zero-Valent Iron Nanoparticles. Environ Sci Technol 45(16): 6978-6984.

Lawless HT, Shlake S, Smythe J, Lim J, Yang H, Chapman K, et al. **2004**. Metallic taste and retronasal smell. Chemical Senses 29(1): 25-33.

Lim J. 2005. Oral sensations from iron and copper sulfate. Physiology and Behavior(85): 5.

Matheson L, Tratnyek P. **1994**. Reductive Dehalogenation of Chlorinated Methanes by Iron Metal. Environ Sci Technol(28): 2045-2053.

Mirlohi S, Dietrich AM, Duncan SE. **2011**. Age-Associated Variation in Sensory Perception of Iron in Drinking Water and the Potential for Overexposure in the Human Population. Environ Sci Technol 45(15): 6575-6583.

Ömur-Özbek P, Dietrich AM. **2011**. Retronasal perception and flavour thresholds of iron and copper in drinking water. J Water Health 9(1): 1-9.

Ömur-Özbek P. **2008**. Macromolecular Reactions and Sensory Perceptions at the Air-Water-Human Interface. [PhD Dissertation]. Blacksburg, VA: Virginia Tech.

Phenrat T, Long TC, Lowry GV, Veronesi B. **2009**. Partial Oxidation ("Aging") and Surface Modification Decrease the Toxicity of Nanosized Zerovalent Iron. Environ Sci Technol 43(1): 195-200.

Phenrat T, Saleh N, Sirk K, Kim HJ, Tilton RD, Lowry GV. **2008**. Stabilization of aqueous nanoscale zerovalent iron dispersions by anionic polyelectrolytes:

adsorbed anionic polyelectrolyte layer properties and their effect on aggregation and sedimentation. J Nanopart Res 10(5): 795-814.

Rykke M, Young A, Smistad G, Rolla G, Karlsen J. **1996**. Zeta potentials of human salivary micelle-like particles. Colloid Surf B-Biointerfaces 6(1): 51-56.

Saleh N, Kim HJ, Phenrat T, Matyjaszewski K, Tilton RD, Lowry GV. **2008**. Ionic strength and composition affect the mobility of surface-modified Fe-0 nanoparticles in water-saturated sand columns. Environ Sci Technol 42(9): 3349-3355.

Shi ZB, Woodhouse MT, Carslaw KS, Krom MD, Mann GW, Baker AR, et al. **2011**. Minor effect of physical size sorting on iron solubility of transported mineral dust. Atmos Chem Phys 11(16): 8459-8469.

Spanier AM. **1991**. A rapid, direct chemical assay for the quantitative determination of thiobarbituric acid reactive substances in raw, cooked, and cooked/stored muscle foods. Journal of Muscle Foods 2(3): 165-175.

Tamura T, Taniguchi T, Suzuki Y, Okubo T, Takata R, Konno T. **2009**. Iron is an essential cause of fishy aftertaste formation in wine and seafood pairing. Journal of Agricultural and Food Chemistry 57: 8850-8856.

Tanboonchuy V, Hsu JC, Grisdanurak N, Liao CH. **2011**. Impact of selected solution factors on arsenate and arsenite removal by nanoiron particles. Environ Sci Pollut Res 18(6): 857-864.

Tratnyek PG, Johnson RL. **2006**. Nanotechnologies for environmental cleanup. Nano Today 1(2): 44-48.

USEPA. **2008**. Nanotechnology for Site Remediation Fact Sheet; United States Environmental Protection Agency, Solid Waste and Emergency Response; EPA 542-F-08-009.

USEPA. **2009**. National Drinking Water Standards and Health Advisories Tables. EPA-822-R09-011. Washington D.C.

Vargas IT, Pavissich JP, Olivares TE, Jeria GA, Cienfuegos RA, Pasten PA, et al. **2010**. Increase of the concentration of dissolved copper in drinking water systems due to flow-induced nanoparticle release from surface corrosion by-products. Corrosion Sci 52(10): 3492-3503.

Wang H. **2009**. Determination of thiobarbituric acid reactive substances (TBARS) for water base liquid food with low oxidation. In: Unpublished: Virginia Tech.

Wigginton NS, Haus KL, Hochella MF. **2007**. Aquatic environmental nanoparticles. J Environ Monit 9(12): 1306-1316.

Zetasizer Nanoseries Technical Notes. **2012**. Zeta Potential: An Introduction in 30 Minutes. Website: http://www.malvern.com/search?openagent=1&lang=unifiedeng&section=unified&count=10&query=Zeta+Potential%3A+An+Introduction+in+30+Minutes [Accessed: March 2, 2011)

Zhang WX, Elliott DW. **2006**. Applications of Iron Nanoparticles for Groundwater Remediation. Remediation 16(2): 7-21.

Zhao DL, Chen CL, Sheng GD, Wang XK. **2011**. Effect of environmental conditions on the retention behaviour of Pb(II) by hematite. J Chem Technol Biotechnol 86(8): 1099-1106.

Zimmermann MB, Hilty FM. **2011**. Nanocompounds of iron and zinc: their potential in nutrition. Nanoscale 3(6): 2390-2398.

CHAPTER VIII

Perspectives on the Current Findings and Insights for Future Research

This research explored the many aspects of metallic flavor in drinking water through applications of sensory science, medicine, health, and the environment. Characterization of flavor metals in drinking water is a multifaceted issue. Flavor producing metals, such as iron, copper, and zinc are essential nutrients, can be toxic, and are known to produce unpleasant tastes and flavor attributes in water as well as other food and/or beverage media. On the other hand, their occurrence in drinking water can add nutritional quality to water as a healthy beverage resource. Another important issue associated with metallic flavor is the variations in human perception. The population variability must consider the ranges of sensitivities among healthy individuals as well as those with specific diseases and/or conditions that may influence their sensory abilities. As illustrated in Figure 8.1, understanding all these variables and issues requires a collaborative and holistic approach in order to address the many factors that may influence human consumption and resulting impacts on health. In this regard, insights gained through this research provide additional perspectives for further scientific explorations, as outlined below:

***On the aspects of sensory science**:* one important and unique finding is the observation that human sensitivity to metallic flavor can diminish by age. Unlike, the five basic tastes of bitter, salty, sweet, sour, and umami, little is known about the influence of age on metallic flavor perception by humans. Given the fact that humans are routinely exposed to trace metals through multiple routes and specifically drinking water, the issue of sensitivity can have important implications on health. This issue can be further explored through additional research to find answer to questions such as:

- To what extent do smell functions influence age-associated sensitivity to metallic flavors?

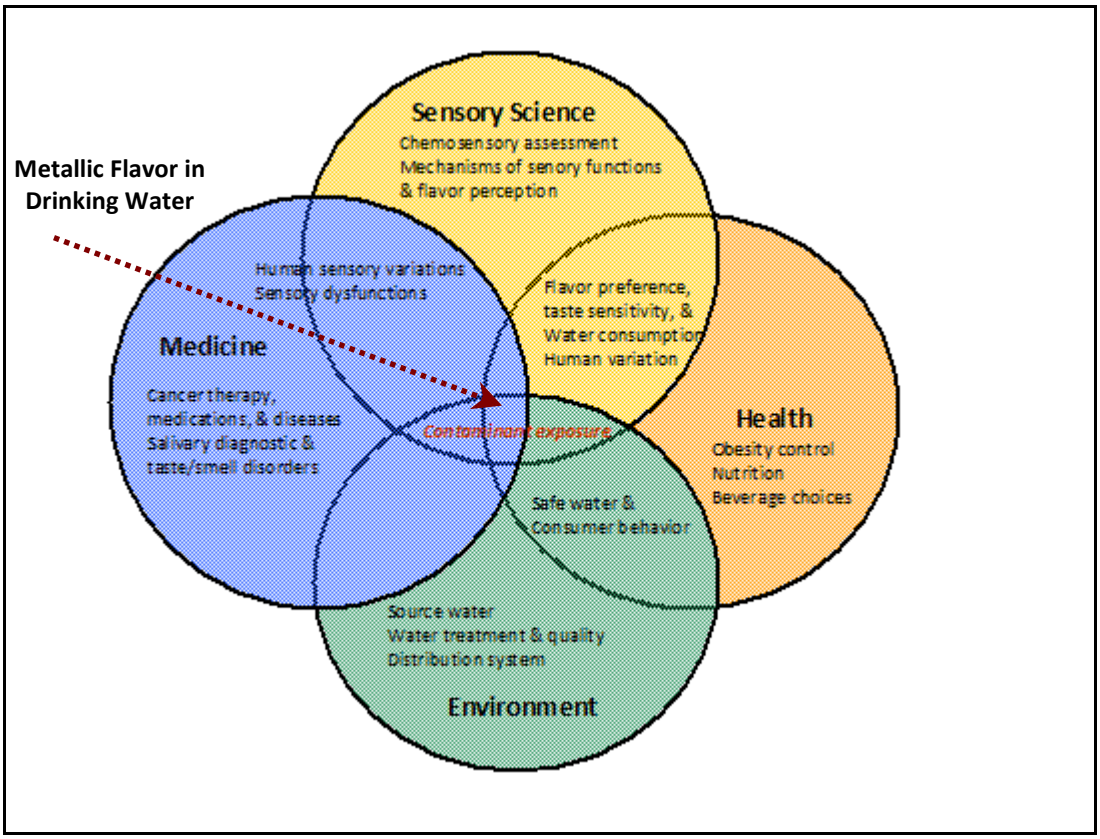


Figure 8.1: Characterization of metallic flavor in drinking water, *The Big Picture*.

- How are the orthonasal and retronasal odor perceptions related in terms of metallic flavors? Would the retronasal sensory threshold for a person insensitive to metallic flavor be equal, or close to that of their overall flavor threshold?

- Are individuals insensitive to metallic flavor anosmic to specific odorants associated with metallic flavors?

- How prevalent is insensitivity to metallic flavor? Is it mostly associated with elderly populations? Are individuals taking medications at a greater of risk of insensitivity to metallic flavor, since they may develop taste dysfunctions?

- Since beverages such as orange juice, wine, and alcoholic drinks, can contain relatively high levels of trace metals, namely copper and iron, how does human sensitivity to metallic flavor vary in these beverages compared to plain water?

Does intake of these beverages make one less sensitive to metallic flavor perception?

***On the health aspects**:* with regard to water consumption for health, one important and novel finding is that diminished sensitivity to metallic flavor may influence total water intake among elderly populations who are most susceptible to dehydration. Additionally, reduced intake of water may result in increased intake of less healthy beverage choices. Insufficient water intake can lead to chronic dehydration, which in turn can lead to fatigue and risk of developing diseases. Dehydration can also result in reduced salivary flow, which in turn can influence taste perception, thus having a circular effect on health status. Further research in exploring the underlying causes of declining drinking water consumption behavior among the elderly can be helpful in finding answers to questions such as:

- Are there any relationships between basic taste sensitivity and beverage intake behavior among the elderly? Does diminished sensitivity to metallic flavor influence beverage choices? Can beverage intake levels and choices under- or over- expose the elderly to trace metal nutrients that have been implicated in numerous diseases of aging?

- Are there factors other than taste sensitivity influencing drinking water behavior among healthy elderly adults?

***On the aspects of medicine**:* this study was the first to use salivary lipid oxidation (SLO) as an indirect measure of iron-induced oxidative stress response, associated with exposure to oral metallic stimuli. Our findings indicate that SLO can possibly be used as a novel assessment tool to study chemosensory functions in malignant glioma patients over the course of their treatment, in addition to the traditionally used self-reported taste and smell assessment questionnaires. However, due to the wide between-subjects variability, the use of SLO measures may require grouping of patients based on their initial chemosensory assessment. With this consideration, the use of a higher alpha level (0.08 – 0.1) may be more reasonable when planning statistical

evaluations for future clinical trials. With regard to potential biomarkers, study of other salivary biomarkers, such as proteins, electrolytes, pH, and metals may be useful. Although in this regard our results did not show considerable variations in such salivary parameters over the course of the cancer treatment; however, specific biomarkers, such as salivary proteins may provide clues on the causes of taste and smell disorders since some salivary proteins have implications on metallic flavor perception due to their metal-binding abilities.

***On the aspects of environment**:* with consideration of health-implicated relevance of trace metal nanoparticles in the environment, this research took first steps in assessing sensory properties of zerovalent iron nanoparticles (nZVI) through novel application of in-vitro salivary lipid oxidation as an indirect measure of metallic flavor perception by humans. While limited in scope, the preliminary research findings provide many insights for future research in this area:

- The findings from this research indicate that oral exposure to nZVI would likely evoke a weaker metallic sensation than that of ferrous iron; this can be predictive of a diminished detection of metallic flavor by humans when exposed to nZVI. This finding has important implications in terms of potential for human overexposure; while our research focused on iron nanoparticles, not typically considered to be a concern for toxic exposure to humans, similar in-vitro studies with toxic metals such as copper would be beneficial in assessing the potential for human sensory detection of copper nanoparticles through oral intake.

- Additionally, since metal nanoparticles in drinking water distribution systems would most likely be present in conjunction with other trace metal nanoparticles, such as copper and lead, in-vitro studies using combination of these metal nanoparticles would be of interest.

Appendix A

IRB Approval Documentation

VirginiaTech

Office of Research Compliance
Institutional Review Board
2000 Kraft Drive, Suite 2000 (0497)
Blacksburg, Virginia 24060
540/231-4606 Fax 540/231-0959
e-mail irb@vt.edu
Website: www.irb.vt.edu

MEMORANDUM

DATE: June 15, 2011

TO: Andrea M. Dietrich, Susan E. Duncan, Susan Mirlohi, Heather Vereb, Alexandra Gerling, Conor Gallagher

FROM: Virginia Tech Institutional Review Board (FWA00000572, expires May 31, 2014)

PROTOCOL TITLE: Mechanisms of Metallic Flavor From Drinking Water

IRB NUMBER: 06-395

Effective July 14, 2011, the Virginia Tech IRB Chair, Dr. David M. Moore, approved the continuation request for the above-mentioned research protocol.

This approval provides permission to begin the human subject activities outlined in the IRB-approved protocol and supporting documents.

Plans to deviate from the approved protocol and/or supporting documents must be submitted to the IRB as an amendment request and approved by the IRB prior to the implementation of any changes, regardless of how minor, except where necessary to eliminate apparent immediate hazards to the subjects. Report promptly to the IRB any injuries or other unanticipated or adverse events involving risks or harms to human research subjects or others.

All investigators (listed above) are required to comply with the researcher requirements outlined at http://www.irb.vt.edu/pages/responsibilities.htm (please review before the commencement of your research).

PROTOCOL INFORMATION:
Approved as: **Expedited, under 45 CFR 46.110 category(ies) 3, 7**
Protocol Approval Date: **7/14/2011 (protocol's initial approval date: 7/14/2006)**
Protocol Expiration Date: **7/13/2012**
Continuing Review Due Date*: **6/29/2012**
*Date a Continuing Review application is due to the IRB office if human subject activities covered under this protocol, including data analysis, are to continue beyond the Protocol Expiration Date.

FEDERALLY FUNDED RESEARCH REQUIREMENTS:
Per federally regulations, 45 CFR 46.103(f), the IRB is required to compare all federally funded grant proposals / work statements to the IRB protocol(s) which cover the human research activities included in the proposal / work statement before funds are released. Note that this requirement does not apply to Exempt and Interim IRB protocols, or grants for which VT is not the primary awardee.

The table on the following page indicates whether grant proposals are related to this IRB protocol, and which of the listed proposals, if any, have been compared to this IRB protocol, if required.

Date*	OSP Number	Sponsor	Grant Comparison Conducted?
3/21/2007	06170502	Institute for Public Health & Water	Not Required (not federally funded)

*Date this proposal number was compared, assessed as not requiring comparison, or comparison information was revised.

If this IRB protocol is to cover any other grant proposals, please contact the IRB office (irbadmin@vt.edu) immediately.

cc: File
OSP

Comprehensive Cancer Center of Wake Forest University

CCCWFU # 97408

Changes in Oral Mucosal Oxidative Stress Responses in Patients with Newly Diagnosed Malignant Brain Tumors Treated with Combined Modality Therapy

You are being asked to participate in a research study. In order to decide whether or not you should agree to be part of this research study, you should understand enough about its risks and benefits to make an informed judgment. This process is known as informed consent.

This consent form gives detailed information about the research study that the investigator will discuss with you. Once you understand the study, you will be asked to sign this form if you wish to participate. You will have a copy to keep as a record.

PURPOSE OF THE RESEARCH STUDY:

The purpose of this study is to see if there is a change in the chemical reactions that happen inside the mouth and back of the nasal cavity in cancer patients when they receive a treatment such as radiation and chemotherapy.

DESCRIPTION OF THE RESEARCH PROCEDURES:

You have been selected to participate in this study because you have a newly diagnosed, recurrent or progressive malignant brain tumor. You have to be older than 18 years of age to participate in this study. Dr. Lesser will identify possible patients in his brain tumor clinic. Personnel from Virginia Tech Department of Civil and Environmental Engineering will oversee collection of saliva sample, perform the saliva analysis and tabulate the results of each sample per standard procedures.

You will be asked to provide a saliva sample at the beginning of the trial before you are started on any treatment. Additional saliva samples will be collected at 3, 6 and 10 weeks after you have started treatment. Also saliva samples will be collected 1, 3 and 6 months later after your treatment is completed. You will be asked to donate saliva twice at each collection, one will serve as a control, and the other will be after sampling the metal salt solution. Also at each time to understand the metallic sensation you will be asked to put on nose clips before tasting the metal salt solution. You will also fill out two short questionnaires every time.

The samples are not being used to treat your condition. The results of the test will not be available to you.

DURATION OF PLANNED STUDY:

We will analyze 15 patients. It will take approximately 12 months to finish the study.

PRIVACY:

Your research and hospital records are confidential. Your identity, personal and confidential information will NOT be divulged to any persons. Your medical record number will be used by the investigators in charge of this study to identify your saliva sample information. In order to compare your sample with other patients' samples, the following information will be recorded: your age, sex and previous treatment you received for your cancer.

BENEFITS:

You will not benefit directly from this study. This study will not affect your treatment plan. No information obtained by this study will be made available to you. There is the potential for this study to result in an improved understanding of metallic flavor perceived by cancer patients caused by therapy. You will not receive compensation for participation in this study.

RISKS:

The risks of participating in this study are minimal and are only the discomfort associated with producing a saliva sample.

By signing this consent form you give to Wake Forest University School of Medicine your saliva sample for the advancement of science and will relinquish all rights and privileges obtained from analysis and experimental work on your cancer tissue or information obtained.

For more information about risks and side effects, contact Dr. Glenn Lesser at 336-716-9527.

ALTERNATIVES:

You do not need to participate in this study to be treated. Your alternative is to choose not to participate in this study.

FINANCIAL COST:

There will be no additional charges to you for participating in this study. You will receive a bill for doctor or clinic visits, hospitalization, tests and evaluations, therapy, and/or surgery that is part of the customary treatment of your disease. You or your insurance carrier will be responsible for these costs. Financial compensation for such things as lost wages, disability or discomfort due to an injury or directly resulting from your disease or from the treatment you receive is not available. You will not give up any of your legal rights by participating in this study as a patient at North Carolina Baptist Hospital, Inc. However, North Carolina Baptist Hospital, Inc. will not provide compensation.

RIGHT TO REFUSE OR WITHDRAW:

The choice to enter or not to enter this study is yours. You are in a position to make a decision if you understand what the doctor has explained and what you have read about the research study. If you decide not to participate, all usual and customary treatment will be made available without prejudice. You have the right to withdraw consent at any time prior to or during the procedure.

Appendix B

Taste and Smell Questionnaire for Cancer Patients

Taste and Smell Questionnaire

Patient #_____ Date_____

Taste Complaints: Please rate

Questions	Insignificant	Mild	Moderate	Severe	Incapacitating
I have noticed a change in my sense of taste					
A food tastes different than it used to					
I have a persistent bad taste in my mouth					
Drugs interfere with my sense of taste					
I would rate my abnormal sense of taste as					

Taste Complaints: Answer "yes" or "no"

Questions	Yes	No	If "Yes" then:
I am experiencing an abnormal sensitivity to salt			Salt tastes: Stronger or Weaker
I am experiencing an abnormal sensitivity to sweet			Sweet tastes: Stronger or Weaker
I am experiencing an abnormal sensitivity to sour			Sour tastes: Stronger or Weaker
I am experiencing an abnormal sensitivity to bitter			Bitter tastes: Stronger or Weaker

Smell Complaints: Please rate

Questions	Insignificant	Mild	Moderate	Severe	Incapacitating
I have noticed a change in my sense of smell					
A food smells different than it used to					
Specific drugs interfere with my sense of smell					
I would rate my abnormal sense of smell as					

Smell Complaints: Answer "yes" or "no"

Questions	Yes	No	If "Yes" then:
I have an abnormal sensitivity to odors			Odors are: Stronger___ or Weaker ___

Appendix C

Salivary Analysis Data on Cancer Patients and Healthy Subjects

Group ID	Time (Weeks)	Treatment	Subject ID	Gender	Age	OLO (uM/L) - Control	OLO (uM/L) - Ferrous	Total Protein (g/L) - Control	Total Protein (g/L) - Ferrous	OLO/ Total Protein (uM/g) - Control	OLO/ Total Protein (uM/g) - Ferrous	Delta OLO (uM)	Delta OLO/ Total Protein (uM/g)	CSC Score (#, 0 - 16)	Oral pH - Control	Oral pH - Ferrous
CP	0	0 [None]	C003	F	55	0.31	0.23	0.50	0.32	0.62	0.70	0.08	0.09	1	5.5	5.5
CP	3	1 [CMT]	C003	F	55	0.28	0.75	1.08	1.27	0.26	0.59	0.47	0.33	7	6.5	5.5
CP	6	1 [CMT]	C003	F	55	0.03	0.01	0.62	0.62	0.05	0.02	0.02	0.03	8		6.0
CP	18	3 [Chemo]	C003	F	55	0.60	0.46	1.56	1.45	0.38	0.32	0.14	0.07		6.0	
CP	30	3 [Chemo]	C003	F	55	0.60	0.46	1.56	1.45	0.38	0.32	0.14	0.07	0	6.0	7.0
CP	0	0 [None]	C005	M	62	0.06	0.35	0.62	0.44	0.09	0.80	0.29	0.70	0	7.5	8.0
CP	3	1 [CMT]	C005	M	62	2.29	1.79	2.91	1.62	0.79	1.10	0.50	0.32	0	6.0	6.5
CP	6	1 [CMT]	C005	M	62	0.21	1.07	1.33	1.08	0.16	0.99	0.86	0.83	3	6.0	6.5
CP	10	2 [Post CMT]	C005	M	62	0.24	0.38	1.51	1.03	0.16	0.37	0.14	0.21	2	6.5	7.0
CP	18	3 [Chemo]	C005	M	62	0.44	1.57	4.29	2.08	0.10	0.76	1.13	0.65	3		7.0
CP	30	3 [Chemo]	C005	M	62	0.52	1.32	1.69	1.24	0.31	1.06	0.80	0.76	0	6.5	7.5
CP	0	0 [None]	C006	F	20	0.27	0.43	0.62	0.80	0.43	0.54	0.16	0.11	0	8.0	7.5
CP	3	1 [CMT]	C006	F	20	0.10	0.15	0.72	1.13	0.14	0.13	0.05	0.01	3	6.0	7.5
CP	6	1 [CMT]	C006	F	20	0.21	0.24	1.45	1.03	0.14	0.23	0.03	0.09	0	7.0	7.0
CP	10	2 [Post CMT]	C006	F	20	0.01	0.03	0.78	0.38	0.01	0.08	0.02	0.07	1	6.5	
CP	18	3 [Chemo]	C006	F	20	0.52	1.26	0.34	0.38	1.55	3.31	0.74	1.75	0	6.5	7.0
CP	30	3 [Chemo]	C006	F	20	0.08	0.77	1.32	0.69	0.06	1.13	0.70	1.07	2	7.0	7.0
CP	0	0 [None]	C007	F	65	6.75	6.54	1.11	1.23	6.06	5.32	0.21	0.75	0	6.0	5.5
CP	6	1 [CMT]	C007	F	65	0.14	0.14	0.82	1.01	0.17	0.14	0.00	0.03	8	6.0	5.0
CP	10	2 [Post CMT]	C007	F	65	0.34	2.95	0.32	0.10	1.06	29.50	2.61	28.44	3	5.5	6.5
CP	18	3 [Chemo]	C007	F	65	0.52	21.32	0.50	0.52	1.04	41.00	20.80	39.96	6		6.5
CP	30	3 [Chemo]	C007	F	65	0.35	0.57	0.21	0.23	1.71	2.49	0.21	0.78	8	6.0	6.0
CP	0	0 [None]	C008	M	79	1.28	4.41	1.33	0.98	0.96	4.50	3.13	3.54	4		5.5
CP	3	1 [CMT]	C008	M	79	3.94	6.65	0.87	1.20	4.53	5.54	2.71	1.01	0	5.0	6.0
CP	6	1 [CMT]	C008	M	79	5.46	1.14	1.02	0.99	5.35	1.15	4.32	4.20	3	5.5	7.5
CP	10	2 [Post CMT]	C008	M	79	0.99	12.24	0.80	0.50	1.24	24.48	11.25	23.24	0	5.0	6.5
CP	0	0 [None]	C010	F	69	0.67	0.56	2.42	2.14	0.28	0.26	0.11	0.01	0	7.5	
CP	3	1 [CMT]	C010	F	69	7.20	9.77	2.69	2.74	2.68	3.57	2.57	0.89		6.5	7.0
CP	6	1 [CMT]	C010	F	69	0.22	0.67	1.94	1.09	0.11	0.61	0.45	0.50	4	5.5	6.5
CP	10	2 [Post CMT]	C010	F	69	0.65	0.98	0.81	0.41	0.80	2.39	0.33	1.59	4	6.0	6.5
CP	18	3 [Chemo]	C010	F	69	0.87	0.87	1.70	0.74	0.51	1.18	0.00	0.67	5	6.0	
CP	30	3 [Chemo]	C010	F	69	0.25	5.09	2.17	3.08	0.11	1.65	4.84	1.54	1	7.0	
CP	0	0 [None]	C011	M	75	3.70	6.00	3.49	1.41	1.06	4.26	2.30	3.20	6	5.5	6.0
CP	3	1 [CMT]	C011	M	75	0.05	1.08	0.59	0.23	0.08	4.70	1.03	4.61	7	6.0	7.0
CP	6	1 [CMT]	C011	M	75	0.24	0.61	0.33	0.23	0.73	2.65	0.37	1.92	5	7.0	
CP	10	2 [Post CMT]	C011	M	75	0.02	0.92	0.35	0.10	0.06	9.20	0.90	9.14	5	6.0	7.0
CP	18	3 [Chemo]	C011	M	75	1.06	3.18	0.23	0.22	4.53	14.21	2.13	9.68	4		7.0
CP	30	3 [Chemo]	C011	M	75	1.61	2.58	2.94	2.88	0.55	0.90	0.97	0.35	0	6.0	
CP	0	0 [None]	C012	M	42	1.22	7.98	1.72	2.38	0.71	3.35	6.76	2.64	5	5.5	7.0
CP	3	1 [CMT]	C012	M	42	1.25	1.46	2.40	1.22	0.52	1.20	0.21	0.68	9	5.0	7.0
CP	6	1 [CMT]	C012	M	42	0.65	0.53	0.82	0.53	0.79	1.00	0.12	0.21	9		7.0
CP	10	2 [Post CMT]	C012	M	42	0.92	2.37	3.57	5.83	0.26	0.41	1.45	0.15	5	6.5	
CP	18	3 [Chemo]	C012	M	42	1.32	2.63	2.02	2.78	0.65	0.95	1.31	0.29	9	6.0	
CP	30	3 [Chemo]	C012	M	42	2.58	1.16	2.91	2.11	0.89	0.55	1.42	0.34	0		
CP	0	0 [None]	C013	F	54	0.08	0.35	0.66	0.78	0.12	0.45	0.27	0.33	1	6.0	7.0
CP	3	1 [CMT]	C013	F	54	0.41	0.59	0.26	0.30	1.58	1.97	0.18	0.39	1	6.0	6.5
CP	6	1 [CMT]	C013	F	54	0.52	3.53	1.82	2.17	0.28	1.63	3.02	1.35	9	6.5	7.0
CP	10	2 [Post CMT]	C013	F	54	1.19	2.70	0.89	0.34	1.35	7.98	1.51	6.63	11	6.5	
CP	30	3 [Chemo]	C013	F	54	0.42	0.56	1.98	1.70	0.21	0.33	0.14	0.12	12		
CP	0	0 [None]	C014	M	58	0.05	0.45	1.12	0.77	0.04	0.58	0.40	0.54	2	7.5	7.0
CP	3	1 [CMT]	C014	M	58	0.10	0.12	0.23	0.27	0.43	0.44	0.02	0.01	0	7.5	7.5
CP	6	1 [CMT]	C014	M	58	0.23	0.44	1.97	0.89	0.12	0.49	0.21	0.38	0	6.5	
CP	10	2 [Post CMT]	C014	M	58	0.38	0.86	3.63	2.71	0.10	0.32	0.48	0.21	0		
CP	18	3 [Chemo]	C014	M	58	0.52	0.70	1.80	1.28	0.29	0.55	0.18	0.26	1	6.5	
CP	30	3 [Chemo]	C014	M	58	0.19	0.35	0.49	0.43	0.38	0.83	0.16	0.44	0	6.5	
CP	0	0 [None]	C015	M	64	0.43	0.60	1.95	0.86	0.22	0.70	0.17	0.48	0	6.0	6.0
CP	6	1 [CMT]	C015	M	64	0.68	0.76	1.45	1.48	0.47	0.51	0.08	0.05	15	6.5	6.5
CP	0	0 [None]	C016	M	54	0.10	0.15	0.30	0.10	0.33	1.50	0.05	1.17	0		
CP	3	1 [CMT]	C016	M	54	1.53	1.10	1.80	0.46	0.85	2.40	0.44	1.55	12	6.5	6.5
CP	6	1 [CMT]	C016	M	54	0.01	0.66	0.93	1.11	0.01	0.60	0.65	0.59	5	6.0	
CP	10	2 [Post CMT]	C016	M	54	0.52	6.21	0.25	0.32	2.10	19.40	5.69	17.31			
CP	0	0 [None]	C017	M	35	0.20	0.65	0.10	0.33	2.00	1.97	0.45	0.03	0	6.5	6.0
CP	3	1 [CMT]	C017	M	35	0.62	1.59	2.67	2.18	0.23	0.73	0.97	0.49		7.5	6.5
CP	10	2 [Post CMT]	C017	M	35	1.06	1.96	1.18	1.79	0.90	1.09	0.90	0.20	1		6.0
CP	18	3 [Chemo]	C017	M	35	0.23	2.05	1.66	2.34	0.14	0.88	1.82	0.74	0		6.5
CP	30	3 [Chemo]	C017	M	35	0.49	0.63	1.19	0.96	0.41	0.66	0.15	0.25	0		6.5
CP	0	0 [None]	C020	F	50	0.37	0.58	1.53	1.68	0.24	0.35	0.21	0.10	1		6.0
CP	3	1 [CMT]	C020	F	50	1.84	1.15	1.94	1.52	0.95	0.75	0.69	0.19	4	6.5	7.0
CP	10	2 [Post CMT]	C020	F	50	0.76	1.47	0.96	0.81	0.80	1.82	0.71	1.02	3	6.0	5.5
CP	0	0 [None]	C021	M	72	0.23	0.69	2.04	1.42	0.11	0.48	0.46	0.37	0	7.0	7.5
CP	3	1 [CMT]	C021	M	72	0.25	0.34	1.14	1.24	0.22	0.27	0.09	0.05		6.5	6.5
CP	6	1 [CMT]	C021	M	72	2.76	3.03	2.90	2.12	0.95	1.43	0.28	0.48	4		6.5
CP	10	2 [Post CMT]	C021	M	72	0.77	0.84	2.11	3.04	0.37	0.28	0.07	0.09	6		
CP	18	3 [Chemo]	C021	M	72	0.31	0.49	1.08	0.42	0.29	1.16	0.18	0.87	12		
CP	0	0 [None]	C022	M	66	0.32	0.88	0.44	0.14	0.72	6.26	0.56	5.54	7	6.5	
CP	3	1 [CMT]	C022	M	66	0.12	0.39	0.21	0.23	0.57	1.65	0.26	1.07	9	6.5	6.5
CP	6	1 [CMT]	C022	M	66	0.05	0.27	0.43	0.44	0.11	0.60	0.22	0.49	3	6.5	7.0
CP	10	2 [Post CMT]	C022	M	66	0.08	0.58	0.42	0.66	0.20	0.88	0.49	0.68	9		
CP	30	3 [Chemo]	C022	M	66	0.53	1.27	2.46	2.14	0.21	0.59	0.75	0.38			7.0
CP	0	0 [None]	C023	F	46	1.01	1.04	3.22	2.84	0.31	0.37	0.04	0.05	0		5.5
CP	3	1 [CMT]	C023	F	46	5.54	4.95	2.64	2.04	2.10	2.43	0.59	0.33	3	6.5	5.5
CP	6	1 [CMT]	C023	F	46	1.85	3.38	2.74	2.64	0.68	1.28	1.53	0.61	0	6.0	6.5
CP	10	2 [Post CMT]	C023	F	46	0.62	1.32	0.67	1.70	0.92	0.78	0.71	0.14	0		5.5
CP	18	3 [Chemo]	C023	F	46	0.82	1.12	2.15	0.70	0.38	1.61	0.30	1.23			6.5
CP	30	3 [Chemo]	C023	F	46	1.10	1.50	2.64	2.04	0.42	0.74	0.40	0.32		6.0	6.5

CP = Cancer Patients

Group ID	Time (Weeks)	Treatment	Subject ID	Gender	Age	OLO (uM/L) - Control	OLO (uM/L) - Ferrous	Total Protein (g/L) - Control	Total Protein (g/L) - Ferrous	OLO/ Total Protein (uM/g) - Control	OLO/ Total Protein (uM/g) - Ferrous	Delta OLO (uM)	Delta OLO/ Total Protein (uM/g)	Oral pH - Control	Oral pH - Ferrous
HS	0	0 [None]	H001	F	42	0.071	1.943	2.061	1.676	0.034	1.159	1.872	1.125	7	6.5
HS	3	0 [None]	H001	F	42	0.162	1.943	0.291	1.676	0.557	1.159	1.781	0.603	7	7
HS	6	0 [None]	H001	F	42	0.050	0.722	0.862	0.959	0.057	0.753	0.673	0.695	7	7
HS	10	0 [None]	H001	F	42	0.371	0.722	2.450	1.372	0.151	0.526	0.351	0.375	6.5	7
HS	18	0 [None]	H001	F	42	0.188	0.977	0.941	0.835	0.200	1.170	0.789	0.970	6.5	6.5
HS	30	0 [None]	H001	F	42	0.269	0.700	2.544	2.205	0.106	0.317	0.431	0.212	8	8
HS	0	0 [None]	H002	F	21	0.031	0.760	1.980	1.056	0.016	0.720	0.729	0.704	7	7
HS	3	0 [None]	H002	F	21	0.184	0.760	0.643	1.056	0.286	0.720	0.576	0.433	6	6.2
HS	6	0 [None]	H002	F	21	0.247	0.654	0.831	0.612	0.297	1.069	0.407	0.771	6.3	6
HS	10	0 [None]	H002	F	21	0.125	0.747	0.281	0.517	0.445	1.445	0.622	1.000	6.5	6.5
HS	18	0 [None]	H002	F	21	0.143	0.212	0.455	0.438	0.314	0.484	0.069	0.170	7.5	7.5
HS	30	0 [None]	H002	F	21	0.230	0.613	1.957	2.695	0.118	0.227	0.383	0.110	7	7
HS	0	0 [None]	H004	M	26	0.130	0.630	0.890	0.871	0.146	0.723	0.500	0.577	5.5	5.5
HS	3	0 [None]	H004	M	26	0.290	0.630	0.363	0.871	0.799	0.723	0.340	0.076	6.5	7
HS	6	0 [None]	H004	M	26	0.155	0.385	0.340	0.390	0.456	0.987	0.230	0.531	7	7.5
HS	10	0 [None]	H004	M	26	0.010	1.340	0.469	1.660	0.021	0.807	1.330	0.786	7.5	7.5
HS	18	0 [None]	H004	M	26	0.264	0.368	1.051	1.173	0.251	0.314	0.104	0.063	6.5	6.5
HS	30	0 [None]	H004	M	26	0.108	0.212	0.315	0.180	0.343	1.178	0.104	0.835	7.2	7
HS	0	0 [None]	H005	F	53	0.149	0.473	2.153	0.646	0.069	0.732	0.324	0.663	6.8	6.5
HS	3	0 [None]	H005	F	53	0.510	0.473	0.760	0.646	0.671	0.732	0.037	0.062	6	6
HS	6	0 [None]	H005	F	53	0.122	0.533	0.778	0.842	0.157	0.633	0.411	0.476	6.5	6.5
HS	10	0 [None]	H005	F	53	0.162	1.484	0.256	1.270	0.633	1.169	1.322	0.536	6.5	6.3
HS	18	0 [None]	H005	F	53	0.108	0.421	0.826	0.831	0.131	0.507	0.313	0.376	7	6.5
HS	30	0 [None]	H005	F	53	0.125	0.369	0.753	0.888	0.166	0.416	0.244	0.250	6	6
HS	0	0 [None]	H006	F	52	0.297	0.230	0.908	0.990	0.327	0.232	0.067	0.095	5.5	6
HS	3	0 [None]	H006	F	52	0.265	0.230	1.185	0.990	0.224	0.232	0.035	0.009	6.5	7
HS	6	0 [None]	H006	F	52	0.090	0.212	0.702	0.646	0.128	0.328	0.122	0.200	6.5	6.2
HS	10	0 [None]	H006	F	52	0.073	0.459	0.399	0.590	0.183	0.778	0.386	0.595	7	7
HS	18	0 [None]	H006	F	52	0.157	0.175	0.900	1.676	0.174	0.104	0.018	0.070	7	7
HS	30	0 [None]	H006	F	52	0.108	0.212	0.624	0.444	0.173	0.477	0.104	0.304	7	6.5
HS	0	0 [None]	H007	M	68	0.722	0.467	0.724	2.286	0.997	0.204	0.255	0.793	7	7.5
HS	3	0 [None]	H007	M	68	0.389	0.467	1.230	2.286	0.316	0.204	0.078	0.112	6.5	6.6
HS	6	0 [None]	H007	M	68	0.047	0.722	0.733	0.905	0.064	0.798	0.675	0.734	7	7
HS	10	0 [None]	H007	M	68	0.212	0.449	0.826	0.256	0.257	1.754	0.237	1.497	7.2	7
HS	18	0 [None]	H007	M	68	0.047	0.193	0.686	0.729	0.069	0.265	0.146	0.196	7	7.5
HS	30	0 [None]	H007	M	68	0.010	0.590	0.710	1.230	0.014	0.480	0.580	0.466	7	6.8
HS	0	0 [None]	H008	F	40	0.541	1.150	1.592	1.150	0.340	1.000	0.609	0.660	6.5	6.5
HS	3	0 [None]	H008	F	40	0.768	1.150	1.388	1.150	0.554	1.000	0.382	0.446	7	7
HS	6	0 [None]	H008	F	40	0.197	0.341	0.736	0.312	0.268	1.093	0.144	0.825	7	7.2
HS	10	0 [None]	H008	F	40	0.707	0.351	1.326	0.258	0.533	1.360	0.356	0.827	7	7
HS	18	0 [None]	H008	F	40	0.449	0.952	0.326	0.305	1.377	3.121	0.503	1.744	6.4	6.3
HS	30	0 [None]	H008	F	40	0.309	0.421	1.090	0.803	0.283	0.524	0.112	0.241	7	7
HS	0	0 [None]	H009	M	54	0.031	0.516	0.796	0.877	0.039	0.588	0.485	0.549	7.5	7.5
HS	3	0 [None]	H009	M	54	0.166	0.516	0.689	0.877	0.242	0.588	0.350	0.347	5.5	5.2
HS	6	0 [None]	H009	M	54	0.056	0.516	0.355	0.750	0.158	0.688	0.460	0.530	6.8	6.8
HS	10	0 [None]	H009	M	54	0.230	0.520	0.719	0.653	0.320	0.796	0.290	0.476	7.2	7
HS	18	0 [None]	H009	M	54	0.089	0.582	0.432	0.482	0.206	1.207	0.493	1.001	7.5	7.5
HS	30	0 [None]	H009	M	54	0.266	2.381	1.333	1.214	0.200	1.961	2.115	1.762	7	7
HS	0	0 [None]	H011	M	59	0.227	5.043	0.791	1.019	0.287	4.949	4.816	4.662	6.5	6.5
HS	3	0 [None]	H011	M	59	0.212	5.043	1.090	1.019	0.194	4.949	4.831	4.754	6.5	6.7
HS	6	0 [None]	H011	M	59	0.206	0.419	0.614	0.576	0.336	0.727	0.213	0.392	7	7
HS	10	0 [None]	H011	M	59	0.073	0.299	0.399	0.669	0.183	0.447	0.226	0.264	7	7
HS	18	0 [None]	H011	M	59	0.029	0.339	0.500	0.719	0.058	0.471	0.310	0.413	6.5	6.5
HS	30	0 [None]	H011	M	59	0.295	0.330	1.133	1.081	0.260	0.305	0.035	0.045	7.5	7
HS	0	0 [None]	H015	M	57	0.085	0.485	0.837	0.452	0.102	1.073	0.400	0.971	6.5	6
HS	3	0 [None]	H015	M	57	0.108	0.485	0.500	0.452	0.216	1.073	0.377	0.857	6.5	6.2
HS	6	0 [None]	H015	M	57	0.348	0.703	1.186	0.767	0.293	0.917	0.355	0.623	6.2	6
HS	10	0 [None]	H015	M	57	0.177	0.317	0.775	0.955	0.228	0.332	0.140	0.104	6.5	6.8
HS	18	0 [None]	H015	M	57	0.084	0.376	0.619	0.667	0.136	0.564	0.292	0.428	7	6.8
HS	30	0 [None]	H015	M	57	0.170	0.720	0.890	0.681	0.191	1.057	0.550	0.866	6.5	6.5
HS	0	0 [None]	H016	M	75	0.227	2.217	1.485	0.814	0.153	2.724	1.990	2.571	6.5	6.5
HS	3	0 [None]	H016	M	75	0.340	2.217	1.370	0.814	0.248	2.724	1.877	2.475	6.5	6.6
HS	6	0 [None]	H016	M	75	0.277	1.626	0.657	0.633	0.422	2.569	1.349	2.147	7	6.8
HS	10	0 [None]	H016	M	75	0.295	0.299	0.919	0.669	0.321	0.447	0.004	0.126	6.8	6.8
HS	18	0 [None]	H016	M	75	0.162	0.305	1.320	0.651	0.123	0.469	0.143	0.346	6.8	7
HS	30	0 [None]	H016	M	75	0.241	0.490	0.514	1.148	0.469	0.427	0.249	0.042	6.5	6.5
HS	0	0 [None]	H020	F	72	0.177	0.376	0.455	1.524	0.389	0.247	0.199	0.142	6.5	6.4
HS	3	0 [None]	H020	F	72	0.155	0.376	1.244	1.524	0.125	0.247	0.221	0.122	6.5	6.7
HS	6	0 [None]	H020	F	72	0.047	0.485	0.519	0.800	0.091	0.606	0.438	0.516	6.3	6.5
HS	10	0 [None]	H020	F	72	0.241	0.612	1.676	0.843	0.144	0.726	0.371	0.582	7	6.5
HS	18	0 [None]	H020	F	72	0.521	1.418	0.581	0.277	0.897	5.119	0.897	4.222	6.6	6.3
HS	30	0 [None]	H020	F	72	0.157	0.594	0.419	0.414	0.375	1.435	0.437	1.060	7.5	7.5
HS	0	0 [None]	H021	F	75	0.197	2.837	1.364	0.667	0.144	4.253	2.640	4.109	6	6.5
HS	3	0 [None]	H021	F	75	0.175	2.837	0.914	0.667	0.191	4.253	2.662	4.062	6	6.3
HS	6	0 [None]	H021	F	75	0.558	1.360	1.057	0.900	0.528	1.511	0.802	0.983	6.5	7
HS	10	0 [None]	H021	F	75	0.206	2.176	0.771	1.162	0.267	1.873	1.970	1.605	6.5	6.5
HS	18	0 [None]	H021	F	75	0.206	1.129	0.395	1.186	0.522	0.952	0.923	0.430	5.5	5.5
HS	30	0 [None]	H021	F	75	0.120	0.594	0.700	0.957	0.171	0.621	0.474	0.449	7	6.5
HS	0	0 [None]	H022	F	84	0.162	0.594	0.418	0.414	0.388	1.435	0.432	1.047	7.4	7
HS	3	0 [None]	H022	F	84	0.029	0.594	0.729	0.414	0.040	1.435	0.565	1.395	7	7
HS	6	0 [None]	H022	F	84	0.357	0.594	0.767	0.914	0.465	0.650	0.237	0.184	7.2	7.2
HS	10	0 [None]	H022	F	84	0.330	0.348	1.538	1.438	0.215	0.242	0.018	0.027	6.5	6
HS	18	0 [None]	H022	F	84	0.359	0.664	0.249	0.411	1.442	1.616	0.305	0.174	7.2	7
HS	30	0 [None]	H022	F	84	0.157	0.995	1.176	1.657	0.134	0.600	0.838	0.467	7	7
HS	0	0 [None]	H023	M	75	0.341	0.923	1.873	1.200	0.182	0.769	0.582	0.587	6.5	7
HS	3	0 [None]	H023	M	75	0.28	0.923	1.652	1.200	0.169	0.769	0.643	0.600	7.5	7.5
HS	6	0 [None]	H023	M	75	0.157	0.576	0.343	0.395	0.458	1.458	0.419	1.001	6.5	6.2
HS	10	0 [None]	H023	M	75	0.241	0.703	0.729	0.305	0.331	2.305	0.462	1.974	5	5
HS	18	0 [None]	H023	M	75	0.348	0.490	0.795	0.624	0.438	0.785	0.142	0.348	7.5	7.5
HS	30	0 [None]	H023	M	75	0.12	0.813	1.314	0.852	0.091	0.954	0.693	0.863	6	6
HS	0	0 [None]	H027	F	66	0.138	0.521	1.025	1.957	0.135	0.266	0.383	0.132	7	7
HS	3	0 [None]	H027	F	66	0.485	0.995	0.995	1.657	0.487	0.600	0.510	0.113	7	7
HS	6	0 [None]	H027	F	66	0.084	1.615	0.443	0.386	0.190	4.184	1.531	3.994	6.5	7
HS	10	0 [None]	H027	F	66	0.277	0.756	0.095	0.876	2.916	0.863	0.479	2.053	6.8	7
HS	18	0 [None]	H027	F	66	0.084	1.240	0.338	0.290	0.249	4.276	1.156	4.027	7.5	7.2
HS	30	0 [None]	H027	F	66	0.029	0.613	0.957	1.767	0.030	0.347	0.584	0.317	7	6.8
HS	0	0 [None]	H028	M	77	0.341	0.995	0.263	1.657	1.297	0.600	0.654	0.696	6.5	6.6
HS	3	0 [None]	H028	M	77	0.108	0.521	0.702	1.957	0.154	0.266	0.413	0.112	6.8	6.8
HS	6	0 [None]	H028	M	77	0.284	0.959	1.662	1.910	0.171	0.502	0.675	0.331	6.5	6.2
HS	10	0 [None]	H028	M	77	0.419	0.774	1.452	2.519	0.289	0.307	0.355	0.019	7	7
HS	18	0 [None]	H028	M	77	0.277	0.738	0.814	0.767	0.340	0.962	0.461	0.622	6.5	7
HS	30	0 [None]	H028	M	77	0.084	2.837	0.605	0.667	0.139	4.253	2.753	4.115	7	7
HS	0	0 [None]	H029	F	71	0.31	0.613	1.150	1.767	0.270	0.347	0.303	0.077	6.4	6
HS	3	0 [None]	H029	F	71	0.084	0.613	1.071	1.767	0.078	0.347	0.529	0.268	6	6
HS	6	0 [None]	H029	F	71	0.193	0.448	1.929	1.590	0.100	0.282	0.255	0.182	5	5
HS	10	0 [None]	H029	F	71	0.241	0.632	0.514	0.310	0.469	2.039	0.391	1.570	7.5	7.8
HS	18	0 [None]	H029	F	71	0.011	0.722	0.548	0.848	0.020	0.851	0.711	0.831	6.5	6.3
HS	30	0 [None]	H029	F	71	0.157	0.923	0.100	0.367	1.570	2.515	0.766	0.945	6	6
HS	0	0 [None]	H032	F	46	0.162	0.813	0.284	0.852	0.570	0.954	0.651	0.384	6.5	6.5
HS	3	0 [None]	H032	F	46	0.175	0.813	1.776	0.852	0.099	0.954	0.638	0.856	6	6
HS	6	0 [None]	H032	F	46	0.241	0.348	0.881	1.214	0.274	0.287	0.107	0.013	6.5	7
HS	10	0 [None]	H032	F	46	0.262	1.377	1.362	0.695	0.192	1.981	1.115	1.789	6.5	6.3
HS	18	0 [None]	H032	F	46	0.348	0.809	0.238	0.295	1.462	2.742	0.461	1.280	7	7
HS	30	0 [None]	H032	F	46	0.120	0.368	1.051	1.173	0.115	0.314	0.248	0.199	6.5	6.7
HS	0	0 [None]	H033	F	52	0.377	0.594	1.075	0.957	0.351	0.621	0.217	0.270	7	6.5
HS	3	0 [None]	H033	F	52	0.157	0.594	1.195	0.957	0.131	0.621	0.437	0.489	8	7.5
HS	6	0 [None]	H033	F	52	0.419	0.809	0.233	0.295	1.798	2.742	0.390	0.944	6	6
HS	10	0 [None]	H033	F	52	0.056	0.720	0.432	1.086	0.130	0.663	0.664	0.533	7	7
HS	18	0 [None]	H033	F	52	0.295	1.377	0.452	0.976	0.653	1.411	1.082	0.758	7.5	7.5
HS	30	0 [None]	H033	F	52	0.510	1.607	1.709	1.439	0.298	1.117	1.097	0.819	6.5	6.2
HS	0	0 [None]	H034	F	51	0.348	0.809	0.238	0.295	1.462	2.742	0.461	1.280	7	7
HS	3	0 [None]	H034	F	51	0.120	0.813	1.314	0.852	0.091	0.954	0.693	0.863	6.5	6.5
HS	6	0 [None]	H034	F	51	0.558	1.360	1.057	0.900	0.528	1.511	0.802	0.983	7	7
HS	10	0 [None]	H034	F	51	0.241	1.377	0.729	0.695	0.331	1.981	1.136	1.651	7.5	7.5
HS	18	0 [None]	H034	F	51	0.341	0.718	1.873	1.640	0.182	0.438	0.377	0.256	6	6.3
HS	30	0 [None]	H034	F	51	0.155	0.385	0.340	0.390	0.456	0.987	0.230	0.531	6.5	6.7
HS	0	0 [None]	H035	F	60	0.295	1.377	0.452	0.976	0.653	1.411	1.082	0.758	6	6
HS	3	0 [None]	H035	F	60	0.120	0.594	0.700	0.957	0.171	0.621	0.474	0.449	6.5	6.5
HS	6	0 [None]	H035	F	60	0.241	0.348	0.881	1.214	0.274	0.287	0.107	0.013	7.5	7.5
HS	10	0 [None]	H035	F	60	0.206	0.720	0.771	1.086	0.267	0.663	0.514	0.396	6	6
HS	18	0 [None]	H035	F	60	0.197	0.521	1.364	1.675	0.144	0.311	0.324	0.167	6.5	6.5
HS	30	0 [None]	H035	F	60	0.197	0.341	0.736	0.312	0.268	1.093	0.144	0.825	6.5	6.6

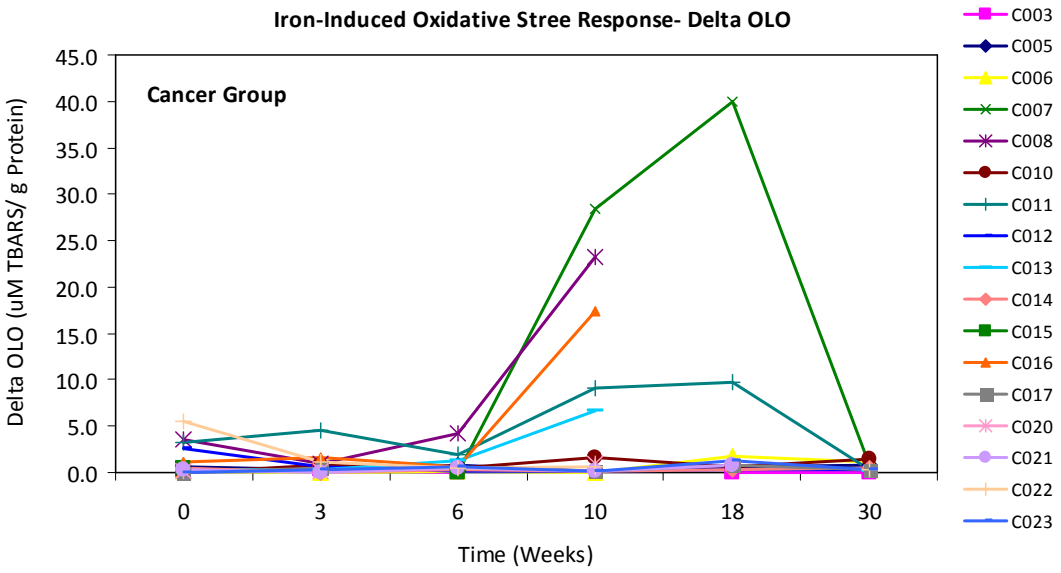
HS = Healthy Subjects

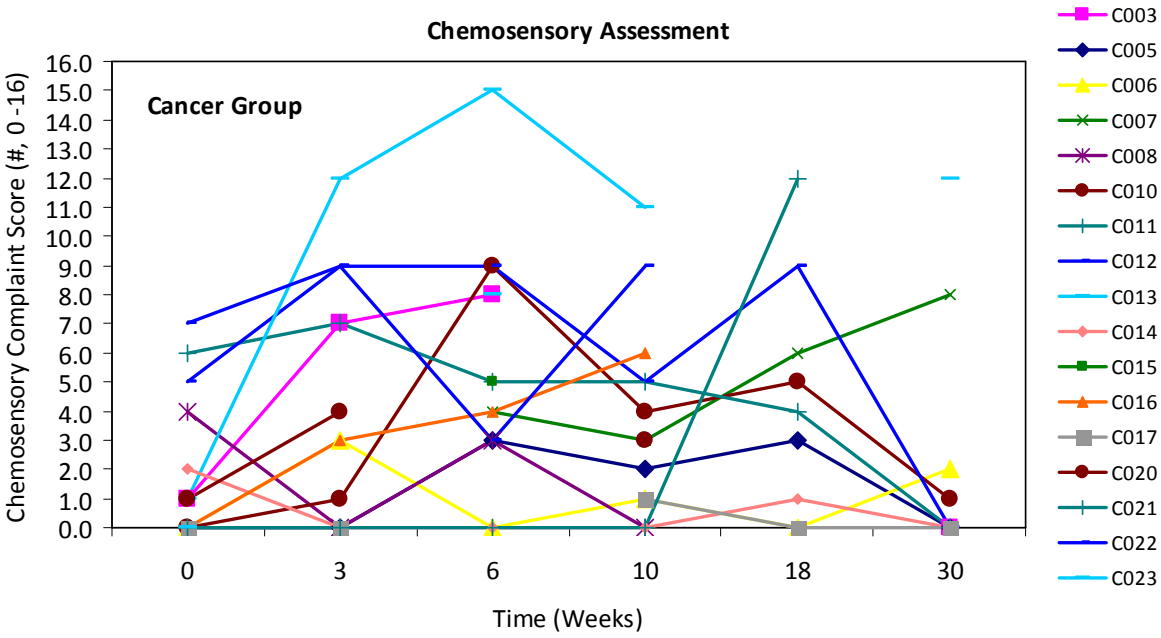
TSA Group	Subject ID	Group ID	Treatment	Gender	Age	Time (Weeks)	23Na (ppm)	25Mg (ppm)	31P (ppm)	34S (ppm)	35Cl (ppm)	39K (ppm)	43Ca (ppm)	54Fe (ppm)	65Cu (ppm)	66Zn (ppm)	54Fe (ppm) - After Ferrous Rinse
CG1	C005	CP	0 [None]	M	62	0	70.76	2.74	208.332	311.36	929.76	1504.624	102.112	0.95	0.003	0.353	2.80
CG1	C006	CP	0 [None]	F	20	0	105.444	3.93	102.518	257.52	437.28	529.974	38.565	0.3	0.055	1.115	2.22
CG1	C014	CP	0 [None]	M	58	0	178.04	18.10	391.172	395.56	942.56	1767.664	139.036	0.74	0.023	0.293	1.73
CG1	C017	CP	0 [None]	M	35	0	185.12	7.74	149.848	382.64	621.68	871.332	68.388	0.5	0.124	0.402	1.50
CG1	C020	CP	0 [None]	F	50	0	144.684	2.74	71.278	185.38	306.08	342.374	20.505	0.37	0.264	0.473	1.69
CG2	C003	CP	0 [None]	F	55	0	325.40	11.85	303.22	228.13	1089.60	1654.11	104.59	0.12	0.004	0.405	3.40
CG2	C007	CP	0 [None]	F	65	0	90.60	8.13	287.81	547.28	875.76	1379.02	72.33	0.85	0.124	0.380	2.58
CG2	C010	CP	0 [None]	F	69	0	182.00	4.74	248.06	301.56	996.96	1694.10	77.95	0.10	0.034	0.170	0.95
CG2	C013	CP	0 [None]	F	54	0	119.24	7.81	190.94	284.80	536.56	1201.30	48.87	0.16	0.052	0.182	1.00
CG2	C015	CP	0 [None]	M	64	0	178.04	18.10	391.17	395.56	942.56	1767.66	139.04	0.43	0.023	0.293	1.64
CG2	C016	CP	0 [None]	M	54	0	185.12	7.74	149.85	382.64	621.68	871.33	68.39	0.10	0.124	0.402	2.24
CG2	C021	CP	0 [None]	M	72	0	144.68	2.74	71.28	285.38	306.08	342.37	20.51	0.73	0.264	0.047	3.80
CG2	C023	CP	0 [None]	F	46	0	348.56	15.87	230.85	335.84	1173.36	1470.46	70.16	0.22	0.137	0.381	1.08
CG3	C008	CP	0 [None]	M	79	0	316.71	10.63	390.34	769.07	114.31	1979.38	109.94	0.16	0.284	0.417	2.40
CG3	C011	CP	0 [None]	M	75	0	147.88	7.64	227.18	149.90	904.46	1370.22	89.85	0.43	0.480	0.471	2.58
CG3	C012	CP	0 [None]	M	42	0	178.04	18.10	391.17	395.56	942.56	1767.66	139.04	0.22	0.023	0.293	0.95
CG3	C022	CP	0 [None]	M	66	0	185.12	7.74	149.85	382.64	621.68	871.33	68.39	0.10	0.124	0.402	1.00
CG1	C005	CP	1 [CMT]	M	62	3	152.92	20.79	559.01	476.36	782.56	1300.06	190.72	0.76	0.045	0.967	1.89
CG1	C006	CP	1 [CMT]	F	20	3	85.42	1.60	62.41	183.84	163.76	360.16	30.18	0.40	0.050	0.647	2.96
CG1	C014	CP	1 [CMT]	M	58	3	221.48	6.97	82.74	173.08	486.16	562.10	23.31	0.20	0.095	1.191	1.01
CG1	C017	CP	1 [CMT]	M	35	3	148.40	2.58	40.58	166.53	276.73	190.37	14.68	0.65	0.140	0.264	2.22
CG1	C020	CP	1 [CMT]	F	50	3	209.14	11.19	249.17	355.40	568.00	1110.02	85.73	0.32	0.112	1.032	1.27
CG2	C003	CP	1 [CMT]	F	55	3	142.56	4.83	118.27	224.86	243.36	656.56	37.52	0.20	0.120	0.406	1.94
CG2	C010	CP	1 [CMT]	F	69	3	123.84	10.21	292.01	662.64	704.88	1312.13	78.71	0.25	0.011	0.091	1.73
CG2	C013	CP	1 [CMT]	F	54	3	159.04	7.68	225.53	216.08	260.84	1014.53	82.35	0.89	0.197	0.391	3.03
CG2	C016	CP	1 [CMT]	M	54	3	526.84	18.92	316.18	827.64	1146.66	1313.22	102.11	0.94	0.110	0.565	2.16
CG2	C021	CP	1 [CMT]	M	72	3	248.40	2.58	40.58	166.53	176.73	190.37	14.68	0.65	0.021	0.264	2.84
CG2	C023	CP	1 [CMT]	F	46	3	155.48	3.48	113.51	159.54	185.26	453.36	28.60	0.64	0.029	0.234	2.13
CG3	C008	CP	1 [CMT]	M	79	3	407.09	9.52	173.96	250.44	223.96	1113.77	51.76	0.56	0.002	0.435	2.24
CG3	C011	CP	1 [CMT]	M	75	3	127.04	3.30	113.05	275.08	851.36	948.22	129.52	0.58	0.192	0.551	3.80
CG3	C012	CP	1 [CMT]	M	42	3	369.76	8.41	331.00	608.21	733.55	1200.83	78.28	0.46	0.044	0.438	2.08
CG3	C022	CP	1 [CMT]	M	66	3	148.40	2.58	40.58	266.53	276.73	190.37	46.40	0.65	0.081	0.264	2.04
CG1	C005	CP	1 [CMT]	M	62	6	184.66	7.65	197.29	183.88	377.96	822.96	45.86	0.40	0.016	0.451	0.88
CG1	C006	CP	1 [CMT]	F	20	6	337.24	5.25	165.21	212.04	717.76	1070.46	67.96	0.93	0.007	0.575	2.55
CG1	C014	CP	1 [CMT]	M	58	6	127.84	12.58	232.78	410.44	591.66	1059.42	55.29	0.37	0.071	1.17	0.89
CG2	C007	CP	1 [CMT]	F	65	6	153.20	1.69	183.13	391.00	710.96	1183.42	50.11	0.30	0.014	0.474	1.85
CG2	C010	CP	1 [CMT]	F	69	6	323.31	8.78	283.09	455.25	845.28	1328.74	91.61	1.01	0.117	0.426	3.42
CG2	C013	CP	1 [CMT]	F	54	6	183.60	13.65	364.96	416.16	998.36	1886.16	116.35	0.58	0.126	0.408	1.97
CG2	C015	CP	1 [CMT]	F	64	6	178.88	7.14	166.69	193.00	472.08	1141.73	78.55	0.77	0.190	0.192	1.69
CG2	C016	CP	1 [CMT]	M	54	6	127.84	12.58	232.78	410.44	591.66	1059.42	55.29	0.37	0.125	0.173	3.65
CG2	C021	CP	1 [CMT]	M	72	6	340.04	14.75	355.78	837.84	804.86	1405.62	103.59	0.69	0.020	0.418	1.87
CG2	C023	CP	1 [CMT]	M	46	6	269.92	13.04	371.36	499.36	1005.52	1947.51	110.70	0.69	0.120	0.428	1.29
CG3	C008	CP	1 [CMT]	M	79	6	317.57	11.19	228.36	287.44	160.20	987.77	79.44	0.79	0.218	0.220	1.73
CG3	C011	CP	1 [CMT]	M	75	6	114.48	6.95	104.12	350.92	412.76	674.56	50.51	0.62	0.243	0.262	3.03
CG3	C012	CP	1 [CMT]	M	42	6	278.68	9.94	173.05	341.44	593.68	710.13	50.11	0.30	0.023	0.185	1.16
CG3	C022	CP	1 [CMT]	M	66	6	348.56	15.87	230.85	335.84	1173.36	1470.46	70.16	0.22	0.137	0.250	1.84
CG1	C005	CP	2 [Post CMT]	M	62	10	208.32	10.70	364.52	343.00	557.16	1488.56	135.59	0.25	0.060	0.968	2.96
CG1	C006	CP	2 [Post CMT]	F	20	10	190.40	8.21	264.88	248.52	603.56	1252.96	89.11	0.21	0.063	0.418	1.01
CG1	C014	CP	2 [Post CMT]	M	58	10	186.80	5.50	92.73	240.44	604.88	759.33	41.03	0.55	0.014	1.110	1.85
CG1	C017	CP	2 [Post CMT]	M	35	10	485.20	15.97	312.77	534.24	900.08	1248.13	85.51	1.13	0.081	0.319	3.20
CG1	C020	CP	2 [Post CMT]	F	50	10	127.16	12.95	519.56	864.88	548.52	1899.64	93.94	0.64	0.080	0.350	1.97
CG2	C003	CP	2 [Post CMT]	F	55	10	208.00	3.51	145.50	203.08	601.76	842.50	84.51	0.36	0.032	0.400	0.97
CG2	C007	CP	2 [Post CMT]	F	65	10	183.64	5.63	190.77	253.08	613.28	778.53	52.35	0.20	0.029	0.384	0.89
CG2	C010	CP	2 [Post CMT]	F	69	10	127.16	12.95	519.56	864.88	1548.52	1899.64	93.94	0.64	0.039	0.357	3.31
CG2	C013	CP	2 [Post CMT]	M	54	10	233.72	3.80	185.76	258.64	550.76	1022.56	95.51	0.61	0.094	0.413	2.13
CG2	C016	CP	2 [Post CMT]	M	54	10	164.52	11.32	180.42	184.60	649.76	996.50	68.99	0.36	0.059	0.456	2.53
CG2	C021	CP	2 [Post CMT]	M	72	10	274.52	6.48	296.94	360.68	1032.56	1667.30	70.19	0.30	0.160	0.096	1.61
CG2	C023	CP	2 [Post CMT]	F	46	10	190.40	8.21	264.88	248.52	603.56	1252.96	89.11	0.16	0.063	0.418	1.36
CG3	C008	CP	2 [Post CMT]	M	79	10	171.88	2.70	65.38	172.96	206.72	366.58	17.64	0.93	0.039	0.256	2.53
CG3	C011	CP	2 [Post CMT]	M	75	10	186.80	5.50	92.73	240.44	604.88	759.33	41.03	0.74	0.014	0.110	1.61
CG3	C012	CP	2 [Post CMT]	M	42	10	485.20	15.97	312.77	534.24	900.08	1248.13	85.51	1.13	0.081	0.319	2.36
CG3	C022	CP	2 [Post CMT]	M	66	10	78.42	2.61	54.66	195.14	162.16	192.03	14.66	0.16	0.026	0.221	1.30
CG1	C005	CP	3 [Chemo]	M	62	18	134.96	12.00	392.77	304.88	824.08	1449.33	139.31	0.25	0.143	0.367	2.22
CG1	C006	CP	3 [Chemo]	F	20	18	203.84	5.66	113.88	336.84	490.06	643.82	70.05	0.20	0.050	0.250	1.27
CG1	C014	CP	3 [Chemo]	M	58	18	331.77	10.85	306.93	134.88	707.84	1364.25	90.94	0.53	0.071	0.679	1.92
CG1	C017	CP	3 [Chemo]	M	35	18	189.10	9.06	210.38	356.12	364.08	774.17	45.67	0.89	0.049	0.416	1.82
CG2	C003	CP	3 [Chemo]	F	55	18	331.77	10.85	306.93	134.88	707.84	1364.25	90.94	0.53	0.071	0.579	1.30
CG2	C007	CP	3 [Chemo]	F	65	18	203.84	5.66	113.88	336.84	490.06	643.82	70.05	0.20	0.020	0.200	1.02
CG2	C010	CP	3 [Chemo]	F	69	18	134.96	12.00	392.77	304.88	824.08	1449.33	139.31	0.42	0.14	0.04	2.84
CG2	C021	CP	3 [Chemo]	M	72	18	177.26	6.64	146.42	232.92	487.68	644.97	40.17	0.70	0.07	0.26	1.32
CG3	C011	CP	3 [Chemo]	M	75	18	129.18	6.46	315.57	199.84	824.48	2030.50	83.36	0.74	0.045	0.259	2.55
CG3	C012	CP	3 [Chemo]	M	42	18	189.10	9.06	210.38	356.12	364.08	774.17	45.67	0.89	0.049	0.316	2.84
CG3	C022	CP	3 [Chemo]	M	66	18	203.84	5.66	113.88	336.84	490.06	643.82	70.05	0.20	0.041	0.380	1.02
CG1	C005	CP	3 [Chemo]	M	62	30	219.84	4.15	174.88	148.96	647.26	1147.62	80.37	0.30	0.113	0.791	2.72
CG1	C006	CP	3 [Chemo]	F	20	30	164.30	4.01	207.98	146.86	430.48	730.77	57.67	0.10	0.043	0.400	0.87
CG1	C014	CP	3 [Chemo]	M	58	30	143.74	3.34	54.76	187.39	330.91	291.90	23.34	0.02	0.004	0.590	0.29
CG1	C017	CP	3 [Chemo]	M	35	30	143.79	2.73	53.43	188.38	144.71	334.30	25.80	0.47	0.004	0.341	1.85
CG2	C003	CP	3 [Chemo]	F	55	30	285.24	14.89	336.58	447.24	1162.86	1601.62	115.13	0.43	0.050	0.100	2.11
CG2	C007	CP	3 [Chemo]	F	65	30	144.68	11.12	273.78	458.64	583.46	1286.82	108.35	1.00	0.120	0.500	1.69
CG2	C010	CP	3 [Chemo]	F	69	30	236.84	9.68	243.98	564.64	777.46	1408.62	80.75	0.57	0.080	0.165	2.62
CG2	C013	CP	3 [Chemo]	M	54	30	219.84	4.15	174.88	148.96	647.26	1147.62	80.37	0.06	0.113	0.591	1.02
CG2	C023	CP	3 [Chemo]	F	46	30	449.37	8.47	253.73	847.88	886.44	1424.25	82.38	0.42	0.043	0.376	2.54
CG3	C011	CP	3 [Chemo]	M	75	30	203.84	5.66	113.88	336.84	490.06	643.82	70.05	0.20	0.080	0.250	1.32
CG3	C012	CP	3 [Chemo]	M	42	30	166.70	6.84	129.09	179.64	674.36	924.96	46.50	0.73	0.065	0.376	2.62

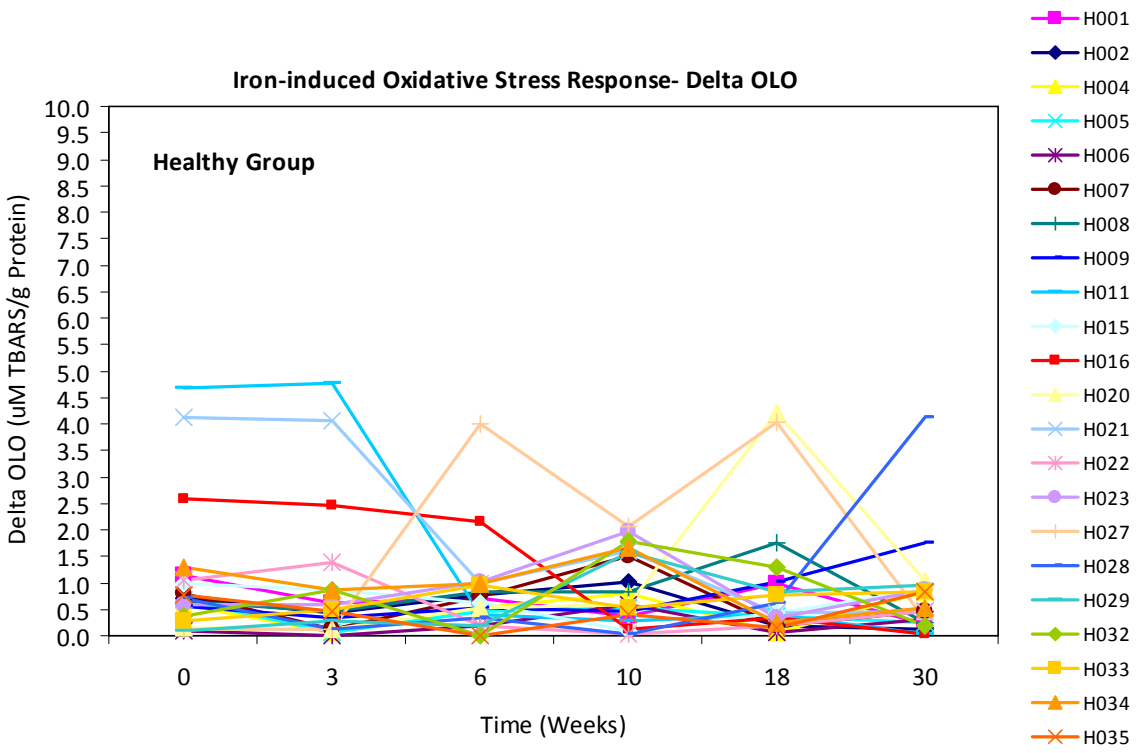
CP = Cancer Patients

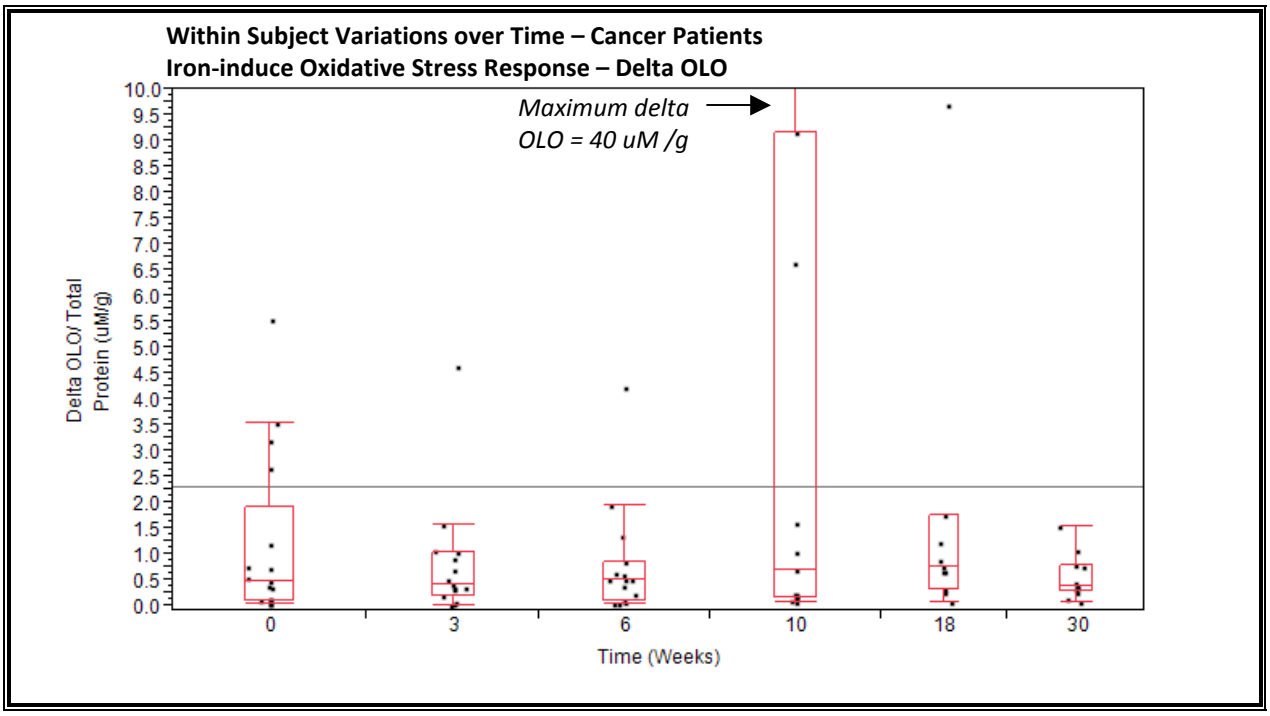
Group ID	Subject ID	Treatment	Gender	Age	Time (Weeks)	23Na (ppm)	25Mg (ppm)	31P (ppm)	34S (ppm)	35Cl (ppm)	39K (ppm)	43Ca (ppm)	54Fe (ppm)	65Cu (ppm)	66Zn (ppm)	54Fe (ppm) - After Ferrous Rinse
HS	H001	0 [None]	F	42	0	98.63	7.40	311.58	232.58	685.57	1082.37	125.99	1.41	0.060	1.352	3.669
HS	H002	0 [None]	F	21	0	138.25	12.49	98.32	173.69	421.95	732.36	52.70	1.99	0.133	0.824	2.768
HS	H004	0 [None]	M	26	0	228.61	5.85	177.52	186.09	566.35	852.76	73.34	1.36	0.047	0.644	3.091
HS	H008	0 [None]	F	40	0	313.91	2.98	98.62	175.45	763.56	630.35	50.30	1.47	0.023	0.362	3.476
HS	H009	0 [None]	M	54	0	251.67	7.16	228.06	230.06	766.77	923.57	67.23	1.65	0.106	0.847	3.902
HS	H005	0 [None]	F	53	0	230.95	4.66	101.10	143.98	780.37	706.37	53.59	1.41	0.014	0.566	3.875
HS	H006	0 [None]	F	52	0	175.48	4.08	209.20	113.78	394.20	1021.18	110.33	0.85	0.031	0.574	3.640
HS	H007	0 [None]	M	68	0	189.43	4.40	136.18	112.38	607.17	833.57	61.95	1.52	0.030	0.705	2.864
HS	H011	0 [None]	M	59	0	113.51	3.93	85.90	160.94	470.77	517.17	61.35	1.70	0.053	0.486	4.046
HS	H015	0 [None]	M	57	0	141.85	5.20	150.76	297.13	504.75	736.36	112.38	1.34	0.073	0.566	3.581
HS	H016	0 [None]	M	75	0	120.23	4.97	121.90	125.74	559.57	727.17	127.71	1.50	0.128	0.714	3.065
HS	H020	0 [None]	F	72	0	96.23	3.52	123.02	147.86	444.77	677.17	64.23	1.35	0.067	0.982	3.266
HS	H027	0 [None]	F	66	0	124.71	2.25	133.54	60.01	403.96	603.15	36.40	1.32	0.022	0.311	3.373
HS	H022	0 [None]	F	84	0	183.25	3.53	272.44	413.58	787.59	1297.55	60.49	1.69	0.046	0.445	3.011
HS	H029	0 [None]	F	71	0	92.89	4.89	232.76	373.54	699.19	1012.35	61.61	1.62	0.099	0.835	3.312
HS	H028	0 [None]	M	77	0	181.33	7.47	167.26	313.11	529.59	740.79	67.71	1.81	0.022	0.740	2.979
HS	H023	0 [None]	M	75	0	220.85	12.55	319.48	514.38	878.39	1109.15	111.97	1.30	0.183	1.096	2.739
HS	H021	0 [None]	F	75	0	107.90	5.89	78.22	167.15	340.71	317.79	67.17	1.53	0.033	0.953	3.139
HS	H032	0 [None]	F	46	0	309.17	8.64	128.16	387.38	789.59	671.55	65.53	1.88	0.084	0.780	3.821
HS	H033	0 [None]	F	52	0	232.78	5.92	158.74	198.19	886.79	802.39	62.29	1.96	0.043	0.954	3.679
HS	H034	0 [None]	F	51	0	150.90	3.68	141.53	113.78	394.20	505.01	47.64	1.02	0.082	0.750	3.649
HS	H035	0 [None]	F	60	0	180.52	2.80	68.56	197.53	294.80	463.58	33.59	0.26	0.029	0.445	1.960
HS	H001	0 [None]	F	42	3	151.15	6.73	307.38	273.21	701.56	969.55	105.74	1.47	0.092	0.695	4.892
HS	H002	0 [None]	F	21	3	152.09	3.38	173.72	207.85	533.55	900.36	80.06	1.82	0.014	0.327	4.084
HS	H004	0 [None]	M	26	3	89.59	3.12	123.66	241.50	568.77	811.97	51.11	1.50	0.025	0.636	4.436
HS	H008	0 [None]	F	40	3	238.45	6.67	163.00	350.54	612.39	675.95	62.89	1.89	0.030	0.591	4.868
HS	H009	0 [None]	M	54	3	195.67	4.46	152.42	139.13	796.76	766.75	66.26	1.35	0.031	0.356	3.0934
HS	H005	0 [None]	F	53	3	264.88	3.96	193.29	244.90	815.20	1097.18	117.26	2.09	0.034	0.550	4.496
HS	H006	0 [None]	F	52	3	207.03	3.70	116.58	146.41	596.76	612.75	49.14	2.26	0.061	0.451	4.712
HS	H007	0 [None]	M	68	3	127.31	4.36	140.34	234.73	483.56	608.75	83.46	1.53	0.020	0.494	2.774
HS	H011	0 [None]	M	59	3	173.03	4.07	216.78	96.61	633.16	999.15	59.58	1.35	0.070	0.549	4.384
HS	H015	0 [None]	M	57	3	81.15	2.55	135.46	114.97	383.48	585.15	40.50	1.34	0.273	0.809	4.912
HS	H016	0 [None]	M	75	3	207.12	3.76	154.05	125.34	665.20	723.98	60.06	1.88	0.011	0.689	4.044
HS	H020	0 [None]	F	72	3	75.00	5.40	247.17	235.86	425.20	812.38	65.18	2.13	0.015	0.726	4.4
HS	H027	0 [None]	F	66	3	85.37	4.42	140.30	204.59	464.79	563.59	58.31	1.62	0.004	0.384	4.476
HS	H022	0 [None]	F	84	3	83.97	3.38	132.30	205.23	526.79	626.79	37.09	1.59	0.004	0.242	3.188
HS	H029	0 [None]	F	71	3	180.29	13.95	290.06	389.95	782.39	1049.99	132.11	1.82	0.565	0.750	4.496
HS	H028	0 [None]	M	77	3	83.93	5.93	197.10	267.31	449.99	711.59	51.47	1.19	0.035	0.594	4.236
HS	H023	0 [None]	M	75	3	104.85	5.01	145.74	318.47	445.59	637.59	68.07	1.52	0.038	0.572	3.904
HS	H021	0 [None]	F	75	3	90.14	6.70	30.81	182.79	242.35	160.15	76.69	1.70	0.120	0.952	4.864
HS	H032	0 [None]	F	46	3	296.60	7.33	80.41	250.82	618.00	425.58	37.30	1.31	0.052	0.436	4.032
HS	H033	0 [None]	F	52	3	240.34	6.62	197.46	176.31	903.99	877.19	66.53	1.48	0.012	0.384	3.34
HS	H034	0 [None]	F	51	3	94.88	1.28	36.02	94.90	242.21	203.14	16.38	0.08	0.006	0.493	3.936
HS	H035	0 [None]	F	60	3	118.01	1.19	29.95	179.30	211.06	144.74	10.31	0.21	0.039	0.955	3.128
HS	H001	0 [None]	F	42	6	194.29	9.28	483.96	478.38	919.99	1356.35	117.65	1.68	0.148	0.900	3.956
HS	H002	0 [None]	F	21	6	142.31	2.66	141.38	131.98	372.33	764.77	92.91	1.49	0.050	0.843	4.428
HS	H004	0 [None]	M	26	6	165.46	5.03	252.84	219.75	673.20	853.59	66.95	1.46	0.025	0.413	3.072
HS	H008	0 [None]	F	40	6	174.78	4.94	161.64	203.95	691.20	741.59	70.59	1.46	0.031	0.380	4.132
HS	H009	0 [None]	M	54	6	200.74	5.93	227.37	259.64	673.30	1137.54	103.64	0.20	0.011	0.910	2.756
HS	H005	0 [None]	F	53	6	195.56	3.82	143.61	103.34	615.60	733.18	46.78	1.93	0.013	0.241	3.70
HS	H006	0 [None]	F	52	6	93.21	3.56	125.94	286.55	478.79	609.99	90.19	1.59	0.003	0.348	4.54
HS	H007	0 [None]	M	68	6	102.54	3.68	143.70	162.31	640.39	717.19	51.89	1.41	0.020	0.438	2.82
HS	H011	0 [None]	M	59	6	97.08	3.62	115.29	113.58	373.00	561.98	48.78	1.16	0.040	0.318	4.72
HS	H015	0 [None]	M	57	6	300.97	3.64	151.93	103.08	611.04	857.25	63.24	1.27	0.016	0.227	3.77
HS	H016	0 [None]	M	75	6	149.03	4.68	233.33	623.68	578.44	1218.65	87.86	0.45	0.015	0.111	3.59
HS	H020	0 [None]	F	72	6	93.54	5.61	137.20	370.23	415.20	526.39	53.43	1.74	0.043	0.748	4.33
HS	H027	0 [None]	F	66	6	237.90	13.77	340.84	403.59	1064.00	1295.19	140.83	2.27	0.058	0.746	4.35
HS	H022	0 [None]	F	84	6	100.74	5.08	142.28	196.75	524.80	678.39	53.87	1.54	0.008	0.507	3.62
HS	H029	0 [None]	F	71	6	203.53	4.15	112.78	213.63	612.39	622.39	43.55	1.63	0.001	0.365	3.92
HS	H028	0 [None]	M	77	6	100.74	4.43	201.44	215.75	514.00	781.59	52.67	1.65	0.233	1.373	4.62
HS	H023	0 [None]	M	75	6	107.02	6.44	115.40	236.75	368.92	437.19	63.43	1.63	0.083	0.544	4.45
HS	H021	0 [None]	F	75	6	102.63	3.32	72.25	125.38	195.44	342.25	51.48	0.95	0.053	0.520	3.18
HS	H032	0 [None]	F	46	6	237.22	6.06	103.42	227.35	584.39	512.79	53.13	1.67	0.228	0.437	4.60
HS	H033	0 [None]	F	52	6	299.17	5.09	159.15	147.26	647.64	1122.45	68.74	0.23	0.035	1.114	3.97
HS	H034	0 [None]	F	51	6	175.48	4.08	209.20	187.39	330.91	1021.18	110.33	0.85	0.031	0.574	2.73
HS	H035	0 [None]	F	60	6	189.43	4.40	136.18	112.38	607.17	833.57	61.95	1.52	0.030	0.705	4.58
HS	H001	0 [None]	F	42	10	135.68	8.69	449.33	301.78	831.20	1328.78	113.34	1.17	0.044	0.508	3.32
HS	H002	0 [None]	F	21	10	173.27	2.04	153.10	188.25	376.88	812.35	83.22	1.30	0.012	0.628	4.42
HS	H004	0 [None]	M	26	10	189.44	6.27	170.18	189.94	532.66	832.22	71.75	0.40	0.003	0.105	3.96
HS	H008	0 [None]	F	40	10	178.70	1.63	57.07	67.26	488.20	410.21	28.76	0.89	0.018	1.18	4.79
HS	H009	0 [None]	M	54	10	157.26	6.06	174.00	213.92	530.46	794.62	55.93	1.10	0.020	0.546	4.24
HS	H005	0 [None]	F	53	10	189.57	4.46	198.10	260.83	246.67	1175.98	105.87	0.61	0.023	0.532	3.93
HS	H006	0 [None]	F	52	10	250.04	7.75	198.18	297.04	760.06	1125.22	126.13	1.39	0.056	0.343	2.49
HS	H007	0 [None]	M	68	10	109.52	4.75	143.36	157.56	558.26	858.42	52.75	0.91	0.011	0.780	4.46
HS	H011	0 [None]	M	59	10	136.54	6.92	154.66	391.44	510.86	649.42	126.91	0.23	0.052	0.120	3.26
HS	H015	0 [None]	M	57	10	160.94	3.13	152.06	127.66	491.66	854.62	60.83	1.23	0.057	0.954	3.64
HS	H016	0 [None]	M	75	10	71.95	2.59	121.69	148.68	291.04	733.25	41.70	1.11	0.073	0.407	3.31
HS	H020	0 [None]	F	72	10	149.18	3.90	90.74	225.51	290.19	372.99	31.96	1.69	0.027	0.628	4.31
HS	H027	0 [None]	F	66	10	107.53	5.54	261.33	589.88	373.24	1084.85	51.36	1.65	0.061	0.261	4.60
HS	H022	0 [None]	F	84	10	77.23	2.31	75.81	112.20	229.64	524.45	34.78	1.54	0.996	0.804	3.67
HS	H029	0 [None]	F	71	10	273.17	7.37	276.33	740.48	593.44	1307.05	94.62	1.50	0.075	0.570	4.54
HS	H028	0 [None]	M	77	10	140.11	3.78	158.09	101.56	358.04	743.45	54.74	1.28	0.044	0.114	4.22
HS	H023	0 [None]	M	75	10	187.23	11.51	229.13	151.20	387.64	924.05	88.80	1.40	0.105	0.359	4.42
HS	H021	0 [None]	F	75	10	209.77	3.49	72.35	225.56	389.64	563.65	29.26	0.72	0.125	0.270	3.15
HS	H032	0 [None]	F	46	10	215.97	3.90	199.53	252.88	582.64	1199.85	98.80	0.82	0.04	0.483	3.89
HS	H033	0 [None]	F	52	10	379.53	5.13	144.07	379.58	968.82	812.57	65.51	1.32	0.024	0.197	4.37
HS	H034	0 [None]	F	51	10	337.73	3.53	103.31	317.18	844.22	710.57	54.67	1.21	0.051	0.121	3.93
HS	H035	0 [None]	F	60	10	172.05	4.76	119.79	307.98	371.82	578.97	41.23	0.84	0.156	0.382	2.66
HS	H001	0 [None]	F	42	18	99.80	12.26	328.97	257.58	560.00	947.58	95.90	1.20	0.040	0.497	3.81
HS	H002	0 [None]	F	21	18	118.60	20.26	374.38	320.64	612.06	1116.82	110.67	1.26	0.028	0.475	3.75
HS	H004	0 [None]	M	26	18	93.18	12.88	84.11	114.50	307.60	375.41	25.64	1.49	0.014	0.400	4.79
HS	H008	0 [None]	F	40	18	251.44	10.08	383.58	359.44	678.06	1331.62	84.19	1.13	0.028	0.477	3.92
HS	H009	0 [None]	M	54	18	215.97	2.26	199.53	252.88	582.64	1199.85	98.80	0.52	0.039	0.483	3.67
HS	H005	0 [None]	F	53	18	261.37	6.64	167.21	453.88	539.04	919.85	49.76	1.58	0.036	0.410	3.02
HS	H006	0 [None]	F	52	18	141.13	5.74	109.45	477.88	325.64	654.65	87.66	1.76	0.028	0.323	4.02
HS	H007	0 [None]	M	68	18	87.15	5.00	164.85	129.38	347.44	863.65	49.90	0.20	0.102	0.534	2.30
HS	H011	0 [None]	M	59	18	102.99	6.65	92.27	145.26	195.84	509.45	41.46	0.14	0.072	0.507	2.60
HS	H015	0 [None]	M	57	18	172.05	4.76	119.79	307.98	371.82	578.97	41.23	0.84	0.156	0.382	2.57
HS	H016	0 [None]	M	75	18	151.87	2.98	90.45	210.04	305.22	478.37	32.47	0.76	0.057	0.530	3.13
HS	H020	0 [None]	F	72	18	169.55	3.46	116.19	281.24	619.42	604.57	54.77	0.30	0.042	0.492	2.35
HS	H027	0 [None]	F	66	18	116.11	2.67	94.41	174.12	471.02	519.77	45.65	1.13	0.129	0.497	3.17
HS	H022	0 [None]	F	84	18	186.51	3.58	107.93	325.38	460.42	609.17	42.45	1.11	0.024	0.505	4.35
HS	H029	0 [None]	F	71	18	112.70	4.25	152.01	259.58	671.58	850.86	58.41	1.03	0.041	1.111	3.32
HS	H028	0 [None]	M	77	18	143.07	6.94	214.81	394.18	681.02	924.17	75.87	0.94	0.029	0.156	2.19
HS	H023	0 [None]	M	75	18	268.93	9.99	247.01	470.58	1021.02	1221.37	112.41	0.77	0.050	0.348	2.91
HS	H021	0 [None]	F	75	18	102.01	5.23	182.75	301.38	455.42	783.77	45.83	0.40	0.063	0.292	2.82
HS	H032	0 [None]	F	46	18	289.13	5.04	226.21	393.38	862.62	1107.17	63.93	0.25	0.071	0.250	1.86
HS	H033	0 [None]	F	52	18	141.41	5.43	175.03	422.98	565.42	852.17	73.21	0.36	0.152	0.319	2.47
HS	H034	0 [None]	F	51	18	113.51	3.93	85.90	160.94	470.77	517.17	61.35	1.70	0.05	0.49	2.59
HS	H035	0 [None]	F	60	18	141.85	5.20	150.76	297.13	504.75	736.36	112.38	1.34	0.07	0.57	4.15
HS	H001	0 [None]	F	42	30	152.64	7.66	157.23	142.38	377.80	513.41	49.62	0.96	0.014	1.180	2.34
HS	H002	0 [None]	F	21	30	230.44	3.04	123.38	179.98	510.46	658.42	52.83	0.30	0.030	0.420	2.54
HS	H004	0 [None]	M	26	30	289.13	5.04	226.21	393.38	862.62	1107.17	63.93	1.19	0.071	0.250	3.18
HS	H008	0 [None]	F	40	30	107.90	5.89	78.22	167.15	340.71	317.79	67.17	1.53	0.033	1.180	3.95
HS	H009	0 [None]	M	54	30	118.50	2.35	114.45	139.68	335.10	630.60	45.36	1.65	0.006	0.130	3.80
HS	H005	0 [None]	F	53	30	321.90	8.39	351.90	347.70	1036.50	1384.50	91.32	1.54	0.048	0.413	3.75
HS	H006	0 [None]	F	52	30	124.83	4.13	132.18	147.87	507.60	585.60	53.52	1.50	0.047	0.162	3.28
HS	H007	0 [None]	M	68	30	175.21	4.30	143.97	205.26	543.90	708.90	49.95	1.28	0.009	0.189	3.73
HS	H011	0 [None]	M	59	30	143.01	11.21	294.93	430.80	663.90	1158.00	91.35	1.40	0.003	0.254	4.05
HS	H015	0 [None]	M	57	30	213.84	5.63	238.89	304.80	785.70	1035.60	63.27	1.16	0.020	0.397	4.16
HS	H016	0 [None]	M	75	30	183.96	5.54	177.30	254.52	575.40	792.30	56.49	1.52	0.056	0.207	4.13
HS	H020	0 [None]	F	72	30	161.22	12.38	197.16	225.84	617.10	798.60	88.35	1.91	0.052	0.424	4.53
HS	H027	0 [None]	F	66	30	155.88	4.19	86.58	163.56	494.70	469.50	73.68	1.54	0.003	1.960	4.03
HS	H022	0 [None]	F	84	30	239.88	4.41	100.65	251.91	635.40	644.70	51.39	1.03	0.261	0.216	3.94
HS	H029	0 [None]	F	71	30	249.81	6.21	197.49	182.25	1000.50	977.70	72.21	1.37	0.030	0.237	2.94
HS	H028	0 [None]	M	77	30	93.00	5.15	132.48	135.76	552.80	691.60	57.84	1.66	0.014	0.776	4.22
HS	H023	0 [None]	M	75	30	151.16	2.81	127.28	195.96	533.60	836.40	60.60	1.74	0.002	0.104	4.42
HS	H021	0 [None]	F	75	30	69.64	4.78	137.80	158.76	587.20	926.40	51.64	1.76	0.001	0.182	3.46
HS	H032	0 [None]	F	46	30	161.40	4.40	124.28	105.52	371.12	566.40	42.68	1.60	0.001	0.443	4.04
HS	H033	0 [None]	F	52	30	228.68	5.78	131.92	100.00	503.60	549.60	32.80	1.71	0.002	0.207	3.95
HS	H034	0 [None]	F	51	30	161.84	4.92	118.12	160.12	472.40	580.80	47.52	0.48	0.001	0.583	2.86
HS	H035	0 [None]	F	60	30	234.32	5.64	183.08	119.60	532.40	780.80	44.48	1.02	0.001	0.159	3.72

HS = Healthy Subjects

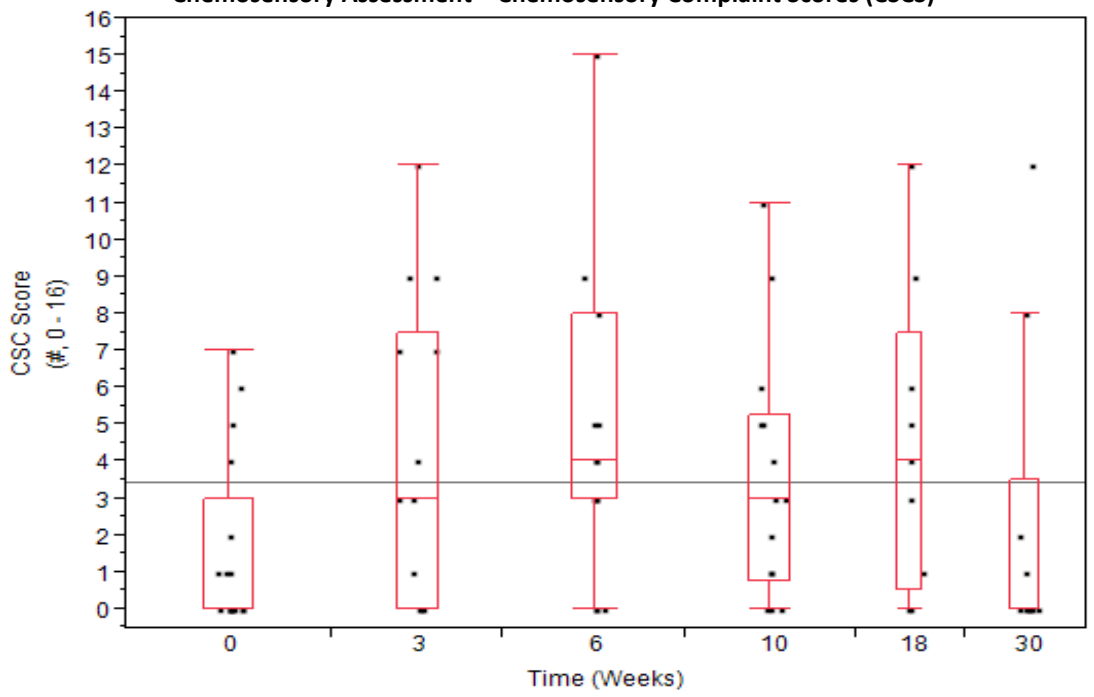

Iron-Induced Oxidative Stree Response- Delta OLO
Cancer Group
Delta OLO (uM TBARS/ g Protein)
45.0
40.0
35.0
30.0
25.0
20.0
15.0
10.0
5.0
0.0
0
3
6
10
18
30
Time (Weeks)
C003
C005
C006
C007
C008
C010
C011
C012
C013
C014
C015
C016
C017
C020
C021
C022
C023


Chemosensory Assessment
Cancer Group
Chemosensory Complaint Score (#, 0 -16)
16.0
15.0
14.0
13.0
12.0
11.0
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
0
3
6
10
18
30
Time (Weeks)
C003
C005
C006
C007
C008
C010
C011
C012
C013
C014
C015
C016
C017
C020
C021
C022
C023

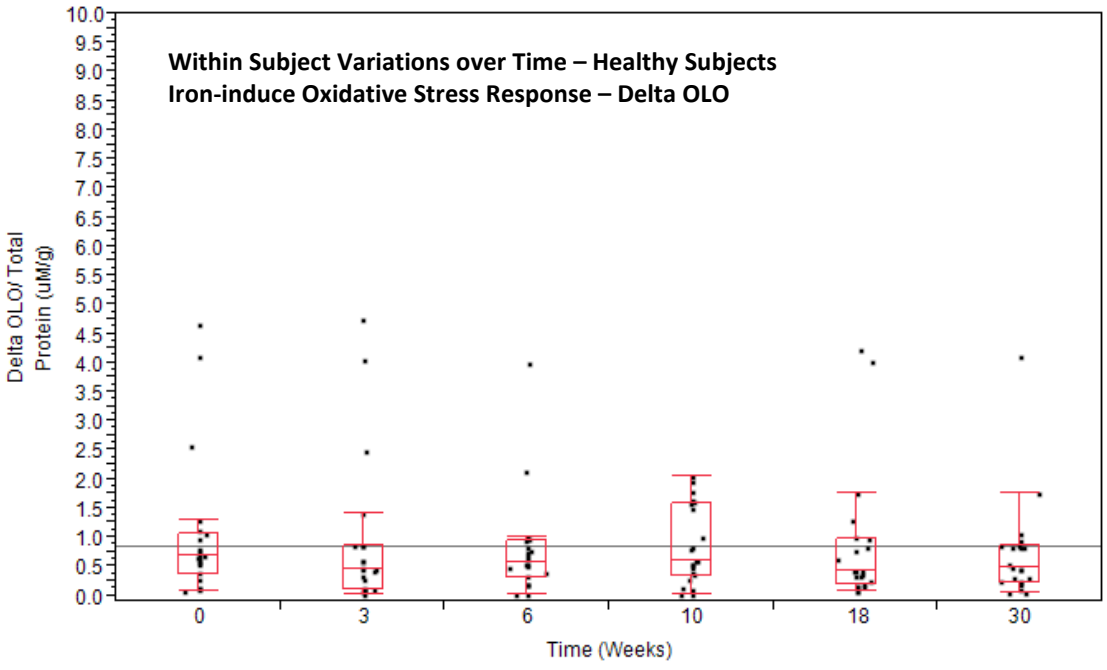

Iron-induced Oxidative Stress Response- Delta OLO
Healthy Group
Delta OLO (uM TBARS/g Protein)
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0
3
6
10
18
30
Time (Weeks)
H001
H002
H004
H005
H006
H007
H008
H009
H011
H015
H016
H020
H021
H022
H023
H027
H028
H029
H032
H033
H034
H035


Within Subject Variations over Time – Cancer Patients
Iron-induce Oxidative Stress Response – Delta OLO
Maximum delta
OLO = 40 uM /g
Delta OLO/ Total Protein (uM/g)
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0
3
6
10
18
30
Time (Weeks)

Within Subject Variations over Time – Cancer Patients
Chemosensory Assessment – Chemosensory Complaint Scores (CSCS)



Within Subject Variations over Time – Healthy Subjects
Iron-induce Oxidative Stress Response – Delta OLO



Appendix D

Beverage Consumption Data on Healthy Subjects

Time (Weeks)	Subject ID	Age	Age Group	Gender	Water (fl oz)	SSB (fl oz)	Avg daily Intake - Other Beverages (fl oz.)	Total Beverage (fl oz)	Avg daily Kcal - SBB	Avg daily Kcal - Other Beverages	Avg daily Kcal - All	Drinking Water Preference	BET Flavor Threshold (mg/L Fe^{+2})
0	H014	19	19 - 39	F	24	16	18.3	58.3	131.2	208.3	339.5	Tap	0.003
0	H004	26	19 - 39	M	24	2.3	0	26.3	31.2	0	31.2	Tap	
0	H010	25	19 - 39	F	36	8.6	53.1	97.7	105.1	227	332.1	Other	
0	H012	29	19 - 39	F	24	2.6	6.5	33.1	35.6	75.7	111.3	Tap	
0	H013	29	19 - 39	F	12	1.7	16.9	30.6	22.8	220.5	243.3	Tap	
0	H003	31	19 - 39	M	48	18.3	20.8	87.1	154.1	179.3	333.4	Well	
0	H008	40	40 - 59	F	24	1.1	33.8	58.9	11.4	157.6	169	None	0.003
0	H009	54	40 - 59	M	20	37.7	2.9	60.6	344.1	50.5	394.6	Tap	0.007
0	H001	42	40 - 59	F	16	0	32	48	0	158	158	Tap	0.071
0	H031	42	40 - 59	F	48	1.1	5.8	54.9	15.2	92.1	107.3	Bottled	8.77
0	H032	46	40 - 59	F	36	8	20.6	64.6	106.4	282.9	389.3	None	0.58
0	H005	53	40 - 59	F	16	9.7	16.6	42.3	109.2	144.1	253.3	Well	
0	H034	51	40 - 59	F	36	0	8	44	0	141.4	141.4	Other	0.077
0	H033	52	40 - 59	F	24	5.7	15.7	45.4	57.1	70.3	127.4	Well	0.077
0	H006	52	40 - 59	F	16	22.9	29.4	68.3	202	58.9	260.9	Tap	0.032
0	H015	57	40 - 59	M	16	4.3	49.7	70	57	245	302	Well	0.354
0	H011	59	40 - 59	M	24	4.3	23.1	51.4	49	196.2	245.2	Well	
0	H035	60	60 - 84	F	12	1.4	14.9	28.3	11.7	50	61.7	None	0.077
0	H027	66	60 - 84	F	24	24	9.7	57.7	196.8	136.1	332.9	None	0.071
0	H007	68	60 - 84	M	8	0	41.1	49.1	0	95	95	Tap	5
0	H029	71	60 - 84	F	16	5.4	2.9	24.3	59.2	32.7	91.9	Tap	0.158
0	H020	72	60 - 84	F	12	1.1	48.6	61.7	16.3	634.5	650.8	Tap	0.158
0	H017	73	60 - 84	F	16	0	24	40	0	170.4	170.4	Well	3.536
0	H016	75	60 - 84	M	36	20	14.9	70.9	164	655.1	819.1	None	0.354
0	H024	76	60 - 84	F	24	0	41.4	65.4	0	359.6	359.6	Tap	0.354
0	H025	76	60 - 84	M	8	5.7	14.9	28.6	64.3	110.1	174.4	Tap	14.14
0	H023	75	60 - 84	M	24	2.9	4.5	31.4	38	304.4	342.4	Tap	14.14
0	H028	77	60 - 84	M	24	6	18.9	48.9	65.1	335.4	400.5	Other	3.536
0	H026	78	60 - 84	M	12	2.9	21.7	36.6	40.9	88.8	129.7	Tap	1.768
0	H019	82	60 - 84	F	24	10	22.9	56.9	138.7	146.3	285	Tap	7.071
0	H018	84	60 - 84	F	24	4.3	24	52.3	60	170.4	230.4	None	0.158
0	H022	84	60 - 84	F	8	0.57	9.73	18.3	8	136.8	144.8	Tap	14.14
0	H030	82	60 - 84	F	8	9.4	9.5	26.9	91.7	116.9	208.6	Tap	14.14
18	H004	26	19 - 39	M	24	1.1	4	29.1	15.2	74.8	90	Tap	
18	H012	29	19 - 39	F	24	2.3	7.4	33.7	32.7	109.2	141.9	None	
18	H013	29	19 - 39	F	24	4.3	15.4	43.7	35.1	205.8	240.9	Tap	
18	H008	40	40 - 59	F	20	0	25.7	45.7	0	167	167	None	
18	H009	54	40 - 59	M	24	36.3	-2.6	57.7	323.7	25.2	348.9	Tap	
18	H001	42	40 - 59	F	8	1.4	29.7	39.1	11.7	115.3	127	Tap	
18	H031	42	40 - 59	F	36	2.9	5.7	44.6	38	51.5	89.5	Bottled	
18	H032	46	40 - 59	F	36	8	20	64	106.4	224.4	330.8	None	
18	H005	53	40 - 59	F	16	9.7	16.6	42.3	109.2	144.1	253.3	Well	
18	H034	51	40 - 59	F	36	0	12	48	0	73.5	73.5	Other	
18	H033	52	40 - 59	F	24	0	12.9	36.9	0	19.8	19.8	Well	
18	H006	52	40 - 59	F	16	18.3	28.8	63.1	161.6	58.7	220.3	Tap	
18	H015	57	40 - 59	M	24	16	40.9	80.9	131.2	254.6	385.8	Well	
18	H011	59	40 - 59	M	16	9.1	34	59.1	104.5	349.2	453.7	Well	
18	H035	60	60 - 84	F	8	6	25.7	39.7	66.7	94.3	161	None	
18	H027	66	60 - 84	F	24	16	6.9	46.9	131.2	103.3	234.5	None	
18	H007	68	60 - 84	M	8	0	52.6	60.6	0	93.3	93.3	Tap	
18	H029	71	60 - 84	F	16	8.6	8.5	33.1	114	66.4	180.4	Tap	
18	H020	72	60 - 84	F	12	2.6	32.5	47.1	35.6	302.5	338.1	Tap	
18	H024	76	60 - 84	F	24	4.3	8.6	36.9	52.3	117.3	169.6	Tap	
18	H023	75	60 - 84	M	24	1.4	12.6	38	19	222.1	241.1	Tap	
18	H028	77	60 - 84	M	24	8.3	13.4	45.7	93.7	249.7	343.4	Other	
18	H026	78	60 - 84	M	8	0	29.7	37.7	0	182.5	182.5	Tap	
18	H019	82	60 - 84	F	12	1.1	23.5	36.6	15.2	170.6	185.8	Tap	
18	H022	84	60 - 84	F	8	1.4	23.2	32.6	20	222.3	242.3	Tap	
30	H004	26	19 - 39	M	16	1.7	12	29.7	23.2	74.8	98	Tap	
30	H012	29	19 - 39	F	24	2.6	7.1	33.7	35.6	75.9	111.5	None	
30	H013	29	19 - 39	F	24	1.7	12.9	38.6	22.8	174.7	197.5	Tap	
30	H008	40	40 - 59	F	24	1.1	25.8	50.9	11.4	81.6	93	None	
30	H009	54	40 - 59	M	20	3.3	32.1	55.4	295.1	59.2	354.3	Tap	
30	H001	42	40 - 59	F	8	4	10	22	32.8	39.6	72.4	Tap	
30	H031	42	40 - 59	F	48	4.3	1.4	53.7	57	25.2	82.2	Bottled	
30	H032	46	40 - 59	F	36	8	19.4	63.4	106.4	415.7	522.1	None	
30	H005	53	40 - 59	F	16	8.3	22.3	46.6	90.2	195.6	285.8	Well	
30	H034	51	40 - 59	F	36	8	12	56	80	187.2	267.2	Other	
30	H033	52	40 - 59	F	24	0	22.9	46.9	0	56.5	56.5	Well	
30	H006	52	40 - 59	F	16	22.3	29.4	67.7	194.4	59	253.4	Tap	