AN ABSTRACT OF THE THESIS OF

<u>Nicole Towers</u> for the degree of <u>Master of Science</u> in <u>Food Science and</u> <u>Technology</u> presented on <u>June 13, 2013</u>. Title: <u>The Effectiveness of Dietary Learning on Hedonic Responses to a Novel,</u> <u>Initially Disliked Vegetable</u>

Abstract approved: _____

Juyun Lim

A previous study conducted in our laboratory demonstrated the critical role that retronasal odors play in vegetable liking and disliking. It remains unclear, however, why some individuals like certain vegetable odors, while others do not. A possible explanation is that some individuals have learned to like the odor of a vegetable that is initially described as unpleasant. The current study investigated the effectiveness of two dietary learning mechanisms (i.e. mere exposure and flavor-nutrient conditioning) on the hedonic responses of a novel and initially disliked vegetable. The effectiveness of learning was measured in both shortterm (approximately 3 weeks) and long-term (approximately 2 months) periods. A total of 47 subjects were screened based on several key criteria including novelty to the study vegetable (i.e., kale) and initial degree of liking/disliking to a kale juice sample. The subjects visited the lab five times over a two-week period while hungry, and consumed a 250mL serving of kale juice containing either carboxymethyl cellulose (CMC) (i.e. mere exposure group) or maltodextrin (i.e. flavor-nutrient conditioning group). Maltodextrin was used to increase the

energy content (i.e., 80 kcal) without adding a perceivable taste, while CMC was added to match the viscosity/mouthfeel of the maltodextrin containing samples without adding calories or a taste. The subjects rated their degree of liking/disliking of kale juice samples, as well as the perceived intensities of sweetness, bitterness, and vegetable odor, under nose-open and nose-closed conditions, before and after the conditioning period. Approximately two months later, the subjects returned and again made the same ratings. Results showed a significant increase in vegetable flavor liking for both conditioning groups (dependent t-test, p < 0.01) under the nose-open condition. Importantly, this increase in liking was stable even two months later. In support of the previous research, there was no significant increase in liking under the nose-closed condition. These results suggest that mere exposure (both with and without added calories) is effective in increasing the degree to which individuals like the flavor of a novel and initially disliked vegetable. In addition, the present results suggest that vegetable odors, but not necessarily tastes, are the driving force behind the increases in liking of an initially disliked vegetable.

© Copyright by Nicole Towers June 13, 2013 All Rights Reserved The Effectiveness of Dietary Learning on Hedonic Responses to a Novel, Initially Disliked Vegetable

> by Nicole Towers

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APPROVED:

Major Professor, representing Food Science and Technology

Head of the Department of Food Science and Technology

Dean of the Graduate School

I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

Nicole Towers, Author

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The Effectiveness of Dietary Learning on Hedonic Responses to a Novel, Initially Disliked Vegetable

Chapter 1

Introduction

Although there are a multitude of health benefits associated with the consumption of vegetable, consumption in the United States is far below the levels recommended by the United States Department of Agriculture (USDA) (Guenther, Dodd et al. 2006; Nebeling, Yaroch et al. 2007; Kimmons, Gillespie et al. 2009; CDC 2010). The health related benefits of vegetables are primarily due to the high contents of dietary fiber (Liu, Lee et al. 2001), vitamins (Dauchet, Amouyel et al. 2006), antioxidants (Bazzano, He et al. 2002), phytochemicals (Liu, Serdula et al. 2004), folic acid (Joshipura, Hu et al. 2001) and carotenoid compounds (Ziegler 1989). In order to educate consumers and increase awareness of the importance of vegetable consumption, the USDA recently launched a new campaign, titled 'My Plate' (HHS 2010). The 'My Plate' campaign is a revision of the food pyramid used until around 2005, and was designed as a clearer depiction of current dietary recommendations. Despite these on-going governmental efforts, studies have continued to show a consistent decline in vegetable consumption (Krebs-Smith, Cook et al. 1995; Casagrande, Wang et al. 2007).

While there are many socially related factors that also influence vegetable consumption (e.g. cost, convenience) (Reicks, Randall et al. 1994; Betts, Amos et al. 1995; Glanz, Basil et al. 1998; Roos, Talala et al. 2008), the majority of vegetable rejection has been linked to the 'bitter' taste of vegetables (Drewnowski 1997; Clark 1998; Drewnowski, Henderson et al. 1999; Drewnowski, Henderson et al. 2000; Engel, Baty et al. 2002; Turnbull and Matisoo-Smith 2002; Bell and Tepper 2006). However, studies investigating the impact of bitterness on vegetable hedonics and consumption tend to have conflicting data, suggesting that bitterness is not the only sensory factor involved in vegetable rejection. Conversely, a recent study investigating the role of odors in vegetable liking/disliking found that retronasal odors play a critical role in both vegetable liking and disliking (Lim and Padmanabhan 2012). Their study also generated the important question of *why* there are differences between individuals in terms of their degree of liking and disliking of vegetables. The authors postulated two potential explanations; individual differences in odor sensitivity and flavor learning through exposure (Lim and Padmanabhan 2012).

There has been a significant amount of research into the role that learning plays in dietary behavior. There are several major mechanisms through which dietary learning occurs. Those include mere exposure (i.e., multiple exposures without harm until neophobia decreases (Zajonc 1968)), flavor-flavor conditioning (i.e., a novel flavor paired with an already liked taste/flavor to increase liking to the novel flavor (Baeyens, Eelen et al. 1990)) and flavornutrient conditioning (i.e., a novel flavor paired with positive metabolic consequences, for example calories, which help form positive associations towards the novel flavor (Brunstrom 2005)). Accordingly, some researchers have used these mechanisms to improve disliking/rejection of vegetables (Birch, McPhee et al. 1987; Maier, Chabanet et al. 2007; Capaldi and Privitera 2008; Zeinstra, Koelen et al. 2009; Olsen, Ritz et al. 2012; de Wild, de Graaf et al. 2013). Unfortunately, many of these studies had some limitations in study design, which might have resulted in inconsistencies across studies.

One of the most notable limitations in some of the previous studies was the lack of attention to novelty of the test flavor. It has been demonstrated that test stimuli must be experienced as novel in order to see significant changes in liking (Zajonc 1968; Poelman and Delahunty 2011; Yeomans 2012). The second common limitation in study design is the use of sugar as part of the conditioning stimuli. Sugar offers not only calories, but also a generally liked sweet taste. This means that any positive results cannot be properly classified into either flavorflavor conditioning or flavor-nutrient conditioning.

The objective of this study was to investigate the effectiveness of mere exposure and flavor-nutrient conditioning on a novel and initially disliked vegetable juice with college students. These learning mechanisms have been mostly applied to children, who are generally faster leaners than adults (Birch,

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McPhee et al. 1987; Brunstrom 2005), but not in young adults. Since it has been shown that college students are not consuming enough vegetables (Richards, Kattelmann et al. 2006), this study directly targeted young, college students (i.e., mostly 18-25 year olds), to see whether these mechanisms also work in a population other than children. It is hypothesized that while mere exposure will increase liking to the novel vegetable, the increase should be even larger for flavor-nutrient conditioning since metabolic consequences are known to be a strong reinforcement (Ackroff 2008). Although, flavor-flavor conditioning has been shown to have positive effects on flavor learning (Capaldi and Privitera 2008), it was not considered in this study; adding other tastants such as sugar or salt would be counterproductive, as this study was specifically interested in increasing vegetable consumption as a means to improve health. Note that vegetables are generally not consumed on their own but are typically consumed as a part of a whole meal along with something of caloric value (e.g. complex carbohydrates). Therefore, the addition of calories in the form of maltodextrin simulates normal eating conditions, but without the risk of forming flavor-flavor associations. Importantly, factors that are known to be essential in dietary learning (i.e., hunger status, restricted eating) (Yeomans 2012) were also considered in this experiment.

Chapter 2

Literature Review

2.1. Vegetables and Health

2.1.1. Health of Americans and the Effect of Vegetables

2.1.1.1 Health of Americans

One of the most common health issues currently plaguing Americans is obesity (Ledikwe, Blanck et al. 2006). Obesity has been reported to be the second leading cause of preventable death, killing an approximate 300,000 Americans per year, surpassed only by smoking (Flegal, Williamson et al. 2004). According to a recent study by the U.S. Department of Health and Human Services (HHS), approximately 35.7% (~78 million) of adults and 16.9% (~12.5 million) of children and adolescents were obese in 2009-2010 (Ogden, Carroll et al. 2012). This is an increase from the 30.5% of adults (adolescents not reported) found in a similar study in 1999-2000 (Flegal, Carroll et al. 2002). This rise in prevalence is concerning because obesity is a major contributor to serious health conditions including; cardiovascular disease (Manson, Colditz et al. 1990; Eckel and Krauss 1998), diabetes (Mokdad, Ford et al. 2003; Lazar 2005) and cancer (Calle, Rodriquez et al. 2003; Calle and Kaaks 2004).

2.1.1.2 <u>Health Effects of Vegetables</u>

Vegetable consumption has been shown to be a significant contributor in the reduction of cardiovascular disease (Morris, Kritchevsky et al. 1994; Dwyer, Navab et al. 2001; Joshipura, Hu et al. 2001; Liu, Lee et al. 2001; Bazzano, He et al. 2002; Hung, Joshipura et al. 2004; Dauchet, Amouyel et al. 2006). This has been attributed to several key benefits such as high amounts of dietary fiber (Joshipura, Hu et al. 2001; Liu, Lee et al. 2001; Dauchet, Amouyel et al. 2006), vitamin content (Dauchet, Amouyel et al. 2006), antioxidant activity (Joshipura, Hu et al. 2001; Liu, Lee et al. 2001; Bazzano, He et al. 2002), phytochemicals (Liu, Serdula et al. 2004) and folic acid (folate) (Joshipura, Hu et al. 2001; Liu, Lee et al. 2001; Bazzano, He et al. 2002). Increased vegetable consumption has also been linked to the prevention of several cancers including those of the oral cavity (Block, Patterson et al. 1992; Steinmetz and Potter 1996; Key, Schatzkin et al. 2004; Key 2011), stomach (Block, Patterson et al. 1992; Steinmetz and Potter 1996; Van Duyn and Pivonka 2000; Key, Schatzkin et al. 2004; Key 2011), lung (Ziegler 1989; Block, Patterson et al. 1992; Steinmetz and Potter 1996; Astorg 1997; Van Duyn and Pivonka 2000; Key, Schatzkin et al. 2004; Hayes, Kelleher et al. 2008), esophagus (Block, Patterson et al. 1992; Steinmetz and Potter 1996; Key, Schatzkin et al. 2004; Key 2011) and prostate (Ziegler 1989; Van Duyn and Pivonka 2000; Hayes, Kelleher et al. 2008; Key 2011). The cancer prevention benefits have been attributed to the same properties as above (i.e. fiber

(Steinmetz and Potter 1996), vitamins (Steinmetz and Potter 1996; Key 2011), phytochemicals (Hayes, Kelleher et al. 2008)), as well as a general lack of macronutrients (e.g. low in calories) (Key, Schatzkin et al. 2004). Perhaps one of the most beneficial components of vegetables are carotenoids such as βcarotene and lycopene. Carotenoids have been linked with the prevention of several cancers (Ziegler 1989; Block, Patterson et al. 1992; Astorg 1997), decreases in cardiovascular disease (Morris, Kritchevsky et al. 1994; Dwyer, Navab et al. 2001), improved skin health (Stahl, Henrich et al. 2000) and improved eye health (Olmedilla, Granado et al. 2003; Semba and Dagnelie 2003). Incorporating vegetables into the diet is also particularly beneficial in obesity prevention (Epstein, Gordy et al. 2001; He, Hu et al. 2004; Rolls, Ello-Martin et al. 2004; Ledikwe, Blanck et al. 2006) and subsequently the reduction in the likelihood of other health problems, including a decreased risk of diabetes (Ford and Mokdad 2001; Liu, Serdula et al. 2004; Bazzano, Li et al. 2008; Carter, Gray et al. 2010). This is due to the decreased energy intake (i.e. consuming fewer calories) and the promotion of satiety associated with vegetables (Rolls, Ello-Martin et al. 2004).

Recognizing the significant impact of fruits and vegetables in human diets, the U.S. Department of Agriculture (USDA) currently recommends eating at least 2 servings of fruits and at least 3 servings of vegetables per day (HHS 2010). To further encourage balanced eating, the USDA recently launched the 'my plate'

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campaign. The campaign features a plate divided into color-coded sections labeled with each food category (fruits, vegetables, grain, protein and dairy), making it easier for Americans to visualize the recommended amount of each food category to consume. Based on 'MyPlate' (see Fig. 2.1), fruits and vegetables should make up approximately half of each plate. Despite these governmental efforts, many researchers have found a lack of sufficient quantities of produce in the typical American diet (Krebs-Smith, Cook et al. 1995; Subar, Heimendinger et al. 1995; Baker and Wardle 2003; Guenther, Dodd et al. 2006; Casagrande, Wang et al. 2007; Nebeling, Yaroch et al. 2007; Kimmons, Gillespie et al. 2009). For example, a study by the Centers for Disease Control and Prevention (CDC, 2009) found that only 32.5% of adults were meeting the recommendations for fruits and only 26.3% were meeting the recommendations for vegetables which is far below USDA targets (CDC 2010).



Figure 2.1. The "MyPlate" campaign launched June 2011 by the USDA, showing fruits and vegetables taking up half of each plate, making the recommendations easier to visualize. [Image source: <u>http://www.choosemyplate.gov]</u>

2.1.2 Factors Influencing Vegetable Consumption

Researchers have cited several factors associated with inadequate vegetable consumption (Anderson, Bybee et al. 2001; Richards, Kattelmann et al. 2006; Dehghan, Akhtar-Danesh et al. 2011). These factors can generally be divided into two categories; social and sensory factors.

2.1.2.1 Social Factors

Social factors influencing individuals food choices include demographical and socioeconomical backgrounds (Reicks, Randall et al. 1994; Roos, Talala et al. 2008), cost of foods (Betts, Amos et al. 1995; Glanz, Basil et al. 1998), convenience (e.g. time to shop for and prepare meals, ease of preparation and availability of foods) (Betts, Amos et al. 1995; Glanz, Basil et al. 1998), educational level of the consumer (Roos, Talala et al. 2008), weight concerns and weight management of the individual(s) particularly in relation to the nutritional factors of the foods themselves (Betts, Amos et al. 1995; Glanz, Basil et al. 1998). Social influences (e.g. peer pressure) are generally more obvious in children and adolescents than in adults, particularly in the development of food preferences (Birch, Zimmerman et al. 1980). The majority of these factors favor the consumption of food that is fast, easy and cheap over healthier options such as fruits and vegetables.

2.1.2.2 Sensory Factors

Sensory factors cited for vegetable dislike, and therefore, low vegetable consumption have primarily focused on bitter taste (Clark 1998; Engel, Baty et al. 2002). Studies have reported the strong bitter taste associated with vegetables as the main reason for vegetable disliking (Drewnowski 1997; Drewnowski, Henderson et al. 1999; Drewnowski and Gomea-Carneros 2000). Most researchers have used phenylthiocarbamide (PTC) and 6-n-propylthiouracil (PROP) taster status to predict vegetable rejection. PROP and PTC are bitter tasting compounds that researchers have used to classified subjects into one of three groups: individuals who do not taste PROP/PTC as bitter ('non'-tasters); individuals who taste these compounds as somewhat bitter ('medium'- tasters) and; individuals who taste them as extremely bitter ('super'-tasters) (Bartoshuk 1991; Bartoshuk, Duffy et al. 2002; Bartoshuk, Duffy et al. 2004). Some researchers attempted to correlate PTC or PROP taster status with vegetable acceptance/rejection and showed that PROP 'medium' and 'super'-tasters disliked "bitter"-tasting vegetables significantly more than 'non'-tasters (i.e., spinach (Turnbull and Matisoo-Smith 2002), broccoli (Keller, Steinmann et al. 2002; Bell and Tepper 2006) and Brussels sprouts (Drewnowski, Henderson et al. 1999)). However, other studies have found no significant effect of PTC or PROP taster status on vegetable rejection (Niewind, Krondl et al. 1988; Mattes and Labov 1989; Anliker, Bartoshuk et al. 1991; Kaminski, Henderson et al. 2000). The lack of agreement among studies may suggest that bitterness is not the only factor in vegetable acceptance and rejection (Lim and Padmanabhan 2012).

Despite the importance of retronasal odor on flavor in general (Keck and Finley 2004; Yeomans, Leitch et al. 2008; Yeomans 2010), the effect of odor on vegetable acceptance and liking has not been sufficiently investigated (Lim and Padmanabhan 2012). For example, research has found a strong odor associated with several vegetables, especially cruciferous vegetables (Maruyama 1970; Engel, Baty et al. 2002; Goff and Klee 2006); however these studies focus on the formation and identification of the volatile compounds with little information on the sensory attributes and their impact on disliking/rejection. Since the importance of odor in relation to liking has not be sufficiently studied, a recent study (Lim and Padmanabhan 2012) sought to determine the role that retronasal odors play in vegetable liking and disliking. In that study, subjects rated their degree of liking/disliking as well as the perceived intensities of relevant tastes (i.e.. sweetness and bitterness) and of retronasal odors of three "bitter" vegetables (asparagus, celery, Brussels sprouts) and one "sweet" vegetable (snap peas). Subjects rated all of these qualities both with their nose blocked and unblocked. The authors separated subjects into 'likers' and 'dislikers' for each vegetable based on their hedonic responses under the nose-open condition. They found that the degree to which the 'likers' liked and 'dislikers' disliked each vegetable was significantly less when retronasal odors of vegetables were

blocked. Unlike what has been suggested by other studies (Drewnowski 1997; Drewnowski and Gomea-Carneros 2000; Engel, Baty et al. 2002), there was no significant difference for bitterness intensity between 'likers' and 'dislikers'. More importantly, there was no significant difference in odor intensities between 'likers' and 'dislikers' in the nose-open condition. These results suggest that it was the quality of the odor (i.e., the characteristic or defining odor (Zheng 2008)), not the intensity that differentiated 'likers' from 'dislikers'.

One theory relating to vegetables and their flavor components states that vegetables have developed natural defense mechanisms that may be responsible for both the strong bitterness and odor (Feeny 1977; Wink 1988). Cruciferous vegetables from the Brassica family (e.g. cauliflower, cabbage, broccoli and kale) are especially healthy for humans due to the high quantities of phytochemicals, particularly isothiocyanates and glucosinolates (Talalay and Fahey 2001; Kristal and Lampe 2002; Keck and Finley 2004; Jahangir, Kim et al. 2009). Unfortunately, these compounds taste bitter and have an unpleasant sulfurous odor (which can occur through hydrolysis) (Feeny 1977; Engel, Baty et al. 2002). In general, humans instinctively avoid bitter foods (Capaldi and Privitera 2008) and, when combined with a strong, disliked odor, it is easy to understand why many people do not like vegetables.

Lim and Padmanabhan (2012) suggested two possible explanations for why some individuals like certain vegetables and others dislike them. The first

possibility is a difference in the perceived quality of the vegetable odor across individuals (O'Connell, Stevens et al. 1989; Stevens and O'Connell 1991; Malnic, Hirono et al. 1999; Keller, Zhuang et al. 2007). For example, a steroid derivative androstenone can smell fruity to some but urinous to others (Amoore 1970; Wysocki and Beauchamp 1984; Bremner, Mainland et al. 2003). Vegetables contain a multitude of different odor compounds, sometime several hundreds, and sensitivity to each compound can differ across individuals. Thus, an individual may reject a particular vegetable if they are sensitive to one or more of the specific compounds. The second possible explanation for differences in vegetable liking/disliking is that some individuals have learned to like the flavor of a particular vegetable. Leaning to like new foods has been reported in several dietary learning studies that demonstrated multiple dietary exposures lead to an increase in liking (Pliner 1982; Birch, McPhee et al. 1990).

2.2 Learning Mechanisms

There are several mechanisms, generally discovered and studied using animal models, through which learning may occur (Kalat and Rozin 1973; Best 1975; Pliner 1982; Sclafani and Ackroff 1994; Zeinstra, Koelen et al. 2009; Ackroff, Drucker et al. 2012), unfortunately, translating to human models from an animal model is very difficult. In general there are two categories of learning mechanisms: 1) those that occur through non-ingestion types of exposure (e.g. seeing new symbols until they become familiar) and 2) those that do involve ingestion (e.g. tasting a new food multiple times). Ingestion types of exposure are more relevant when considering food stimuli and there are two main types of mechanisms involving ingestion; mere exposure (tasting a new food several times) (Birch and Marlin 1982; Pliner 1982) and flavor conditioning (e.g. drinking a novel flavored drink sweetened with sugar) (Baeyens, Eelen et al. 1990; Brunstrom and Mitchell 2007).

2.2.1 Mere Exposure

Of the learning mechanism studied to date, mere exposure is the broadest category. In simple terms, this learning mechanism postulates that the more an individual is exposed to a new/foreign stimulus, the less novel it becomes and the more likely they are to grow to like it (Zajonc 1968; Birch and Marlin 1982; Pliner 1982). Mere exposure occurs through all five senses (i.e., auditory, visual, tactile, olfactory, and/or gustatory stimulation) in order to learn about new stimuli of all types (Pliner 1982; Birch, McPhee et al. 1990). However, each sensory modality may not be equal in their power to facilitate learning. For example, a person does not learn as much about a new object by only looking at it, as they do if they are also allowed to touch the object (Birch, McPhee et al. 1987). By providing more information through multiple sensory modalities, learning can be easier.

The learned safety mechanism is a special case within mere exposure that happens specifically when experiencing a new stimulus without negative consequences. An example of learned safety occurs when eating a new food for the first time and not getting sick afterwards. Learned safety can be faced every time a new stimulus is encountered; the experience can be positive, negative, or neutral. If the experience is negative, an individual is less likely to try that stimuli again; this is called learned aversion (Nachman and Jones 1974). Learned aversion generally produces a strong reaction, which is remembered after even one exposure (Kalat and Rozin 1973). Conversely, when experience are not harmful individuals are more likely to try the stimulus again, which is called learned safety (Siegel 1974; Best 1975; Birch, McPhee et al. 1987; Lubec, Monje et al. 2010). Unlike learned aversion, safety of a stimulus generally takes several exposures to be remembered (Kalat and Rozin 1973; Birch 1999). Learned safety is especially important when trying a new food, specifically in relation to the effect on the gastrointestinal tract (Kalat and Rozin 1973; Birch, McPhee et al. 1987). A person can learn to like new foods only if they actually ingest the food and experience no negative side effects (e.g. nausea or vomiting) over several exposures (Birch, McPhee et al. 1987).

Some dietary learning researchers have investigated the effects of merely exposing people to new stimuli (Zajonc 1968; Pliner 1982). Others have investigated the number of exposures that are necessary for a change in liking to

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occur (Birch and Marlin 1982; Maier, Chabanet et al. 2007; Olsen, Ritz et al. 2012) or which types of exposure (e.g. visual vs. taste) are effective in changing liking (Birch, McPhee et al. 1987; Osborne and Forestell 2012). Most of these studies use children, because they can often learn easier than adults (Birch, McPhee et al. 1987; Brunstrom 2005). Also, it has been speculated that by the age of five to six children have developed a more or less adult attitude towards what is and what is not food (Birch, McPhee et al. 1987). Therefore, it stands to reason that forming healthy eating habits (e.g. learning to like vegetables) at a young age could help in the development of lasting healthy eating behavior. However, using children as study subjects poses its own problems in study design and data collection/reliability (e.g. scale usage, cooperation, attention span, communication skills). Some of this research has looked at the difference between allowing a child to see a new vegetable (either physically or in a book) and having him or her actually taste the new vegetable (Birch, McPhee et al. 1987; Maier, Chabanet et al. 2007; Olsen, Ritz et al. 2012; Osborne and Forestell 2012). Birch et al (1987) specifically found that type of exposure matters; although visual exposure reduced the neophobia slightly, the decrease was significantly greater when the child actually consumed the vegetable. This is likely due to getting several types of exposures through consumption; visual, taste, odor and metabolic consequences, as opposed to only visual exposure.

2.2.2 Flavor Conditioning

Though there are many theories relating to how individuals begin to like specific flavors, two have attracted the most attention; 1) flavor-flavor conditioning and 2) flavor-nutrient conditioning (Yeomans, Leitch et al. 2008). In flavor-flavor conditioning, a novel flavor is repeatedly paired with an alreadyknown and already-liked flavor, which leads to an increased liking to the novel flavor (Baeyens, Eelen et al. 1990). An example of this would be having multiple exposures to a sweetened grapefruit juice that eventually led to an increased liking of a grapefruit flavor even when not sweetened (Capaldi and Privitera 2008). In contrast, flavor-nutrient conditioning occurs when a novel flavor is paired with something that provides a positive post-ingestive metabolic consequence. Positive metabolic consequences can include the effect of calories (i.e., feeling of satiety or fullness) or other pharmacological effects (e.g. caffeine or alcohol) (Pérez, Lucas et al. 1998; Brunstrom 2005; Brunstrom and Mitchell 2007). An example of flavor-nutrient learning is when experiencing coffee for the first time, generally it is too bitter to be enjoyed, but the bitterness becomes bearable as the effects of the caffeine become associated with the bitter taste of coffee.

When trying to separate the effects of the two conditions, it is important to use stimuli that are either 1) flavored without calories (flavor-flavor) or 2) caloric without flavor (flavor-nutrient). Unfortunately, many studies have used sugar, which is both a liked flavor and caloric, and are therefore not able to separate the two conditions (Zellner, Rozin et al. 1983; Baeyens, Crombez et al. 1995; Capaldi and Privitera 2008). Other investigators have used non-caloric sweeteners (e.g. saccharin) to effectively study flavor-flavor conditioning (Fanselow and Birk 1982). The potential problem of these non-caloric sweeteners, however, is that they tend to have undesirable off-flavors (e.g. bitterness, metallic sensation) (Gwak, Chung et al. 2012), which could negate any positive effect of their sweetness. Flavor-nutrient conditioning is a little more difficult to study, especially in humans, since most caloric substances have some sort of tastes (e.g. sugar). In rats, this problem can be readily avoided by giving a novel flavor orally while directly administering a nutrient into the gut intravenously (Sclafani and Ackroff 1994; Myers and Sclafani 2003; Ackroff 2008; Ackroff, Drucker et al. 2012). Since using gastric infusions is not feasible in humans, studies have used a non-sweet carbohydrate (e.g. maltodextrin) to increase the caloric value of a stimuli (Yeomans 2012).

Chapter 3

The Effectiveness of Dietary Learning on Hedonic Responses to a Novel, Initially Disliked Vegetable

3.1 Introduction

Vegetables are an important part of the human diet, yet most Americans consume far less than the recommended amount (CDC 2010). While odor quality has a significant impact on whether a vegetable is liked or not, it is not well understood why some people like the odor, while others do not (Lim and Padmanabhan 2012). One potential explanation is that 'likers' have learned to like the flavor of a specific vegetable. In fact, there are many theories regarding how learning affects human behavior to new stimuli of all kinds, such as: written words, symbols, abstract paintings, human faces, names, music, and, of course, food (Zajonc 1968; Pliner 1982; Zellner, Rozin et al. 1983; Birch 1999). Learning about new foods is a special case as it generally involves ingestion and therefore, metabolic consequences, which are not experienced with the other stimuli types.

Learning about new food flavors generally occurs through the basic 'mere exposure,' or one of the two types of flavor conditioning; 'flavor-flavor' and 'flavor-nutrient' conditioning. Mere exposure occurs when a novel flavor is consumed multiple times without negative side effects (e.g. nausea, vomiting) (Zajonc 1968). Flavor-flavor conditioning occurs when a novel flavor is paired with a well-know and well-liked taste/flavor (e.g. sweetness) (Baeyens, Eelen et al. 1990). Flavor-nutrient conditioning occurs when a novel flavor is paired with a positive metabolic or pharmacological consequence (e.g. calories, caffeine) (Brunstrom 2005). The effects of these mechanisms have been well established using animal models; in fact, approximately 95% of dietary-learning research uses animals such as rats and mice (Brunstrom 2004). However, clear evidence of how these mechanisms work in humans remains elusive, because it is difficult to translate results from animal models to those from human studies.

There are several key differences between human models and animal models, which attribute to the differences in outcomes. The first major difference is the way in which the nutrients in flavor-nutrient conditioning are delivered. When using animals, experimenters can inject the nutrients directly into the gut intravenously, but this procedure is not feasible for human studies. Humans must therefore ingest the nutrients orally, which limits the types of nutrients that can be used, as well as the strength of the metabolic consequences (Yeomans 2012). In addition, higher levels of calories generally have a taste, which introduces the confounding factor of flavor-flavor conditioning. The second major difference between animal and human models is prior experience, which relates directly to novelty of the flavor. It has been shown that the test stimuli must be experienced as novel in order for significant changes in liking to be observed (Poelman and Delahunty 2011; Yeomans 2012). Lab animals experience only what the experimenter allows them to experience, so that novelty to the test stimuli can be directly controlled and guaranteed. Unfortunately, humans have a wide range of previous experiences; therefore prior exposure (e.g. novelty) to test stimuli is difficult to achieve and cannot be guaranteed.

Through the many dietary learning studies involving humans, there has been little consensus on which mechanisms work and which do not (Yeomans 2012). For example, Capaldi and Privitera (2008) saw positive results when they studied college students and measured hedonic responses to cauliflower and broccoli, before and after conditioning. The test vegetables were sweetened via a sucrose solution coating. In another study, deWild et al (2013) saw an increase in intake of a high energy density vegetable soup in 2-4 years old children over multiple exposures, relative to a lower energy soup (de Wild, de Graaf et al. 2013). However, Zeinstra et al (2009) found no significant changes in preference or consumption for either a high or low energy vegetable drink in 7-8 years old children (Zeinstra, Koelen et al. 2009). Olsen et al (2012) also found no increases in liking to whole vegetables when presenting children with pairs of neutral, liked, or disliked vegetables (Olsen, Ritz et al. 2012). There are several possibilities to explain the discrepancies in results, including: level of novelty of the test stimuli, level of nutrients used for both high and low energy stimuli, the

number of exposures employed, and the amount of conditioning stimuli actually consumed. Also of a concern in some of the previous studies was the confounding factor of using stimuli that have both a taste and nutrients (i.e. sugar) (Capaldi and Privitera 2008), meaning any positive results cannot be properly classified as either flavor-flavor or flavor-nutrient conditioning.

Based on the recommendations of Yeomans (2012) there are four important factors to be considered and carefully controlled for in order to successfully study eating behavior and dietary-learning in humans: novelty, nutrient level, non-restricted eating and hunger state. Novelty is extremely important when learning about all kinds of new stimuli; a person has to experience a stimulus as new in some way in order to improve his/her liking of the stimulus (Poelman and Delahunty 2011; Yeomans 2012). It is also important to ensure conditioning stimuli contain a high enough level of nutrients to ensure the body recognizes the stimuli as caloric (Yeomans 2012). Next, the number of restricted eaters in a dietary-learning study should be minimized. Restricted eaters are individuals who consciously restrict all or portions of their daily diet. Unfortunately, when such restriction becomes a chronic habit, it can lead to undesirable outcomes such as eating disorders and even lack of normal bodily responses to food (Brunstrom 2005). If a person does not have normal bodily responses to food, their reactions will not necessarily be an accurate reflection of the nutritional conditioning experienced by non-restricted eaters (Brunstrom and Mitchell

2007; Yeomans 2012). Lastly, the hunger state of the subject is also an important factor. In order for the individuals to be receptive to the nutrient level of the stimuli, subjects must not be full (i.e. neutral or hungry) (Yeomans 2012).

The objective of this study was to investigate the effectiveness of two learning mechanisms (i.e., mere exposure/learned safety and flavor-nutrient conditioning) on hedonic responses (liking) of a novel vegetable (i.e., kale) through consumption in young adults. It is hypothesized that mere exposure will result in increases in flavor-liking of a novel and initially disliked vegetable. It is also hypothesized that the increase in flavor liking will be even larger for flavornutrient conditioning, as metabolic consequences are known to provide stronger reinforcements than mere exposures. Much of previous research has used children because kids are generally faster learners than adults. Also, it has been speculated that by the age of five to six children have developed a more or less adult attitude towards what is and what is not food (Birch, McPhee et al. 1987). Therefore introducing vegetables, and healthy eating habits in general, at a young age could help in the development of lasting healthy eating behavior. However, previous work has seen mixed results (e.g. some positive, some negative, some inconclusive). Also, it has been shown that young adults are not consuming enough vegetables (Richards, Kattelmann et al. 2006), therefore, this study was designed to study these effects in college students (i.e., targeting mostly 18-25 years old). Flavor-flavor conditioning was not included because the
addition of a liked flavor such as sweetness in the form of sugar would have been counterintuitive. This study was interested in increasing liking and intake to healthy vegetables, without also increasing intake to already over consumed sweet or salty foods. The degree of liking of a novel vegetable juice was compared before and after multiple conditioning sessions using a betweensubjects design. Subjects were separated into two groups and conditioned with fresh kale juice samples that contain either Carboxymethyl Cellulose (CMC) (i.e., without taste or added calories; mere exposure) or low-glucose maltodextrin (i.e., with added calories but no taste; metabolic consequences). Maltodextrin added calories without adding a perceivable taste, and CMC eliminated any confounding texture factors by matching sample viscosity. Additional important factors under consideration when selecting subjects included: kale novelty, initial kale dislike, maltodextrin taster status, BMI, non-restricted eating, and hunger state at each conditioning session.

3.2 Materials and Methods

3.2.1 Subjects

47 subjects (15M, 32F) between the ages of 18 and 32 years old (mean age= 21) successfully participated in the initial experiment. 33 of these 47 subjects returned to participate in the follow-up experiment (see Table 3.1 for subject demographics). The experimental protocol was approved by the Oregon State University Institutional Review Board. Subjects gave written informed consent

and were compensated for their participation.

| Table 3.1. Distribution and demographic information of subjects in each |
|--|
| conditioning group who participated in the initial and follow-up experiments |

| | Ν | Gender | Age ^a | BMI ^a | Restrained Eating ^{ab} | | |
|---|--------------------------------|---------|------------------|------------------|---------------------------------|--|--|
| Initial Experiment (N=47) | | | | | | | |
| ME* Group | 24 | 6M, 18F | 21.8 ± 3.6 | 24.6 ± 5.7 | 7.4 ± 1.7 | | |
| FNC** Group | 23 9M, 14F 20.9 ± 2.9 24.6 ± 4 | | 24.6 ± 4.4 | 7.1 ± 1.7 | | | |
| Follow-up Experiment (N=33) | | | | | | | |
| ME Group | 13 | 4M, 9F | 21.6 ± 2.9 | 23.6 ± 5.7 | 7.4 ± 1.7 | | |
| FNC Group | 20 | 8M, 12F | 20.8 ± 2.9 | 24.7 ± 4.6 | 6.9 ± 1.7 | | |
| Initial Experiment (N=47) ME* Group 24 6M, 18F 21.8 ± 3.6 24.6 ± 5.7 7.4 ± 1.7 FNC** Group 23 9M, 14F 20.9 ± 2.9 24.6 ± 4.4 7.1 ± 1.7 Follow-up Experiment (N=33) ME Group 13 4M, 9F 21.6 ± 2.9 23.6 ± 5.7 7.4 ± 1.7 FNC Group 20 8M, 12F 20.8 ± 2.9 24.7 ± 4.6 6.9 ± 1.7 | | | | | | | |

^a Mean ± SD.

^b assessed on a 4-16 point scale (≤9 was considered unrestrained)

*Mere Exposure

**Flavor Nutrient Conditioning

3.2.2 Subject Selection Criteria

3.2.2.1 Subject Screening by Email

The experiment was advertised via flyers posted around the Oregon State

University campus as a study to investigate human flavor perception. Individuals

who were interested were asked to fill out an email screener designed to identify

qualified participants (see Appendix A for email screener). A total of 370

volunteers responded. Of the 370 volunteers, 255 were not qualified for

participation based on initial screening criteria (see Table 3.2 for a breakdown of

disqualification criteria).

General subject inclusion criteria were as follows: all subjects were 1) non-

smoking, 2) non-pregnant, 3) free from deficits in taste and smell, 4) had no

known food allergies, 5) had no oral piercings or lesions, and 6) were not taking any prescription pain medications, all by self-report.

Two key criteria that were established specifically for dietary learning included: novelty to test stimuli (Poelman and Delahunty 2011; Yeomans 2012) and history of restrained eating (e.g. dieting) (Brunstrom 2005; Brunstrom and Mitchell 2007; Yeomans 2012). Novelty to the test vegetable was considered as consuming kale once every 6 months or less. In order to assess restrained eating habits, several questions from part II of The Three-Factor Eating Questionnaire (Stunkard and Messick 1985) were selected. Those questions reflected 4 diet and eating behavior aspects: 1) frequency of dieting; 2) weight fluctuation; 3) guilt after overeating; and 4) consciousness of calorie consumption. Each question was given 4 answer choices and assessed on a 1-4 point scale, for a possible total of 4-16 points (see Appendix B for original and modified questions). Individuals who had a total score of 10 points or more were considered to be restrained eaters and excluded from participation.

| Qualified Subjects | | 115 |
|---------------------------------|-----|-----|
| Disqualified Subjects | | |
| Not novel to kale | 162 | |
| Restricted Eater | 77 | 255 |
| Over desired Age (30 years old) | 11 | |
| Other | 3 | |
| Total | | 370 |

Table 3.2. Qualification distribution of all volunteer responses

3.2.2.2 In-person Screening

Of the 115 qualified volunteers, 87 attended the in person screening/pre-test session (full details in section 3.2.4.1 below). This screening session was designed to test whether subjects have an initial dislike to the test stimuli (i.e., kale). Therefore, the 37 subjects who rated the flavor of the kale juice at or above 'like slightly' on the Labeled Hedonic Scale, under the nose-open condition, were excluded from further testing. This left 50 of the 87 subjects who attended the in-person screening session to be deemed qualified for further participation. Over the course of testing 3 subjects chose to stop participation, leaving a total of 47 to successfully complete the experiment.

3.2.3 Stimuli

Kale is a leafy green member of the Brassica oleracea family. This family also includes other cruciferous vegetables such as: broccoli, cauliflower, Brussels sprouts and collard greens. Kale was selected as the test vegetable for several reasons. First off, for its sensory characteristics; kale has a fairly low bitter taste and a strong odor. Secondly, kale has a high likelihood of novelty with Americans. Third, kale is known for its health benefits (e.g. rich in vitamins and minerals and high amounts of phytochemicals (Talalay and Fahey 2001; Kristal and Lampe 2002; Keck and Finley 2004; Jahangir, Kim et al. 2009)). Lastly, kale was chosen for its moderate initial dislike (based on pilot studies). Fresh kale was purchased from a local supermarket and stored for a maximum of 3 days in an airtight container in the crisper drawer of a refrigerator (4-6°C) until used. On each testing day, kale was rinsed well and any yellow/brown pieces were removed before being processed through a commercial juicer (Omega Product, Model 8005 Nutrition Center, Harrisburg PA). Juicing was chosen as the preparation method in order to mask the identity of kale in an attempt to keep the subjects novel to kale and to facilitate consumption. Kale can be quite tough, especially when raw, juicing made it much easier to consume as well as allowed for the presentation of a larger overall amount of kale.

Conditioning samples consisted of 30% (v/v) kale juice concentrate (i.e. the undiluted juice extract) mixed with either 1) 0.5% (w/v) carboxymethyl cellulose (CMC, Carboxymethylcellulose sodium salt, Sigma-Aldrich, St. Louis MO) for the ME group or 2) 8% (w/v) maltodextrin (Star-Dri 1, Tate & Lyle, Decatur IL) for the FNC group (see table 3.3 for exact sample compositions). In order to facilitate measurement and to speed sample preparation a stock solution of 1% CMC (e.g. 5g CMC + 495mL dH₂O) was made weekly and stored under refrigeration until used. ME samples started with 125mL of the CMC stock plus an additional 50mL of dH₂O (total volume of 175mL). FNC samples were made by combining 20g of maltodextrin and approximately 100mL dH₂O in a 250mL flask, by mixing on a stir plate with a magnetic stir bar until all the maltodextrin was in solution. This mixture was measured and additional dH₂O was added until the total volume

measured 175mL. Conditioning samples were made fresh daily and contained 175mL of either the CMC or maltodextrin mixtures plus 75mL of kale concentrate, and had a total final volume of 250mL. Samples were placed in 8oz disposable cups fitted with lids. The concentration of maltodextrin was chosen to maximize a calorie load without evoking a noticeable taste, while that of CMC was selected to match the viscosity/mouthfeel of the maltodextrin containing samples without adding calories or a taste. The composition of the test samples were identical to that of the ME conditioning stimuli, and presented in 1oz plastic soufflé cups with lids. All stimuli were stored at 4-6°C and served directly from the refrigerator.

| | Sample for ME | Sample for FNC |
|-----------------------------|---------------|----------------|
| Energy (kcal/250mL) | 38* | 118 (38+80) |
| Kale Juice concentrate (mL) | 75 | 75 |
| Water (mL) | 175 | 165 |
| Maltodextrin (g) | 0 | 20 |
| Carboxymethylcellulose (g) | 0.125 | 0 |

Table 3.3. Energy and ingredients of conditioning sample per serving (250mL)

Juicing produces approx. 60% juice and 40% fiber per g whole kale. 1 serving of whole kale is 67g and 34 kcal (67.6= 40.2mL and 34*.6= 20.2 calories in the juice of 1 serving), each sample has 75mL of juice (75/40.2 = 1.87 servings per sample) therefore each sample has 20.2 calories per serving *1.87 servings = 38 calories in each sample.

3.2.4 Procedure

This study consisted of 8 separate sessions; screening/pre-test (session 1), conditioning (sessions 2-6), post-test (session 7), and follow-up test (session 8) as detailed below and displayed in Figure 3.1.

3.2.4.1 In-person Screening/Pre-Test (Session 1)

Subjects were asked to refrain from eating or drinking anything but water for at least 1 hour prior to their scheduled test sessions. The screening/pre-test session consisted of 3 test blocks: 1) a hedonic rating block, 2) an intensity rating block, and 3) a discrimination task block, in that order. Within the hedonic and intensity rating blocks, subjects had two tasting conditions: nose-open and closed. Nose clips were used in the nose-closed condition to block odor input. The discrimination task was performed in the normal tasting condition only (i.e., with nose-open). Given the two tasting conditions within each testing block and nature of the discrimination testing, subjects tasted a total of 13 samples ([2 hedonic conditions + 2 intensity conditions] + [3 discrimination trials x 3 samples per trial]). Subject also had their height and weight measured in order to calculate their Body Mass Index (BMI: kg/m₂) (NIH 1998).

Hedonic Rating Block. Prior to hedonic data collection, subjects were given instructions and allowed to practice using the Labeled Hedonic Scale (LHS; Appendix C) to express their degree of liking/disliking. The LHS (Lim, Wood et al. 2009; Lim 2011) is a bipolar category-ratio scale bounded by "Most Liked Sensation Imaginable" at the top and "Most Disliked Sensation Imaginable" at the bottom, with intermediate hedonic labels (i.e. like or dislike: slightly, moderately, very much, and extremely) empirically spaced as determined by their sematic magnitudes (Lim, Wood et al. 2009), with neutral at the midpoint. Verbal instructions were followed by practice ratings. Practice allowed subjects to use the scale within a broad context of hedonic sensations, not just taste and smell, by having subjects rate their liking/disliking of 15 remembered or imagined sensations (e.g. the feel of a massage, the smell of vomit) [see Appendix D for the full list of imagined sensations]. The scale was displayed on a computer monitor and subjects used the mouse to move a cursor along the scale to rate their degree of liking/disliking for each sensation.

Once sufficiently trained on the use of the LHS, actual testing began by having subjects rinse at least 3 times with deionized water. Subjects were instructed to wear a nose clip in order to block the airflow through the nose. They were then instructed to put the entire 10mL sample into their mouth and swish gently for three seconds, and then expectorate into a spit cup. Without removing the nose clip, subjects were asked to use the LHS to immediately rate 1) the degree of overall liking/disliking (i.e. the sample as a whole sensation) and 2) the degree of vegetable flavor liking/disliking. Note that while 'flavor' is generally intended to be inclusive (taste, odor and tactile sensations), it was explained to the subjects in a more restrictive manor (i.e., only the taste and odor). Ratings for overall liking were collected in order to allow the subjects to express their liking/disliking of the test stimuli for reasons other than vegetable flavor per se (e.g. color, texture, temperature), therefore these results were not included in the final data analysis. Once subjects made both ratings, they were allowed to remove the nose clip and given a one-minute break, during which they were asked to rinse at least 3 times with deionized water. After thoroughly rinsing, subjects were asked to perform the same task, without wearing the nose clip. All subjects tasted the stimuli with the nose clip first, in order to avoid any biases based on odor input. Once subjects completed both hedonic trials they moved on to the intensity block.

Intensity Rating Block. Subjects were first familiarized with and allowed to practice using the general Labeled Magnitude Scale (gLMS; see Appendix E) which allowed them to rate perceived stimulus intensity. The gLMS (Green, Shaffer et al. 1993; Green, Dalton et al. 1996; Bartoshuk, Duffy et al. 2002) is a category-ratio scale bounded by "No Sensation" at the bottom and "Strongest Imaginable Sensation of Any Kind" at the top, with intermediate labels (i.e. barely detectable, weak, moderate, strong, very strong) spaced quasilogarithmically as empirically determined by their semantic magnitudes (Green, Shaffer et al. 1993). Again, verbal instructions were followed by practice ratings of 15 remembered or imagined sensations (e.g. the burning sensation of a whole hot pepper, the sourness of a lemon) (see Appendix F for the complete list of imagined sensations) to give experience using the gLMS in the broad context of normal sensations beyond just taste and odor. This scale was also displayed on the computer screen and subjects used the mouse to move the cursor along the scale to make their ratings.

For the intensity rating task, subjects were asked to rate the perceived intensities of sweetness, bitterness and vegetable odor on 3 consecutive gLMS scales. Subjects were told to consider 'vegetable odor' as anything other than the sweet or bitter tastes of the sample. Other than the rating task itself, the tasting procedure was identical to the hedonic rating block, including completing all ratings first with a nose clip, followed by a break and rinsing, and then all ratings without the nose clip.

Discrimination Task. Research findings in our lab have shown that some individuals are able to taste maltodextrin which they describe as "somewhat sweet" or "cereal- or bread-like" (Lim and Lapis 2013). To identify those subjects who can perceive the taste of maltodextrin, subjects were asked to perform 3 trials of a discrimination (triangle) test. For each trial, subjects were presented with a set of 3-10mL samples labeled with 3-digit codes consisting of either: 2-ME and 1-FNC or 1-ME and 2-FNC samples. The subjects were instructed to taste each sample, from left to right, using the same tasting procedure as in the hedonic and intensity rating blocks and to pick the one that they thought was different from the other two samples. Subjects were given no information to the true difference between samples, therefore allowing them to use any cues they determined as important when making their decision. Subjects were given a one-minute break between sets, during which they rinsed at least 3 times with deionized water. All discrimination trials were performed without a nose clip.

Those subjects who could consistently detect the difference between the ME and FNC samples (i.e. 2 or 3 correct identification) were considered to be 'tasters' and automatically placed in the ME group. The remaining qualified subjects were assigned so that the two conditioning groups had approximately the same average; age, gender, BMI, restricted eating score, overall liking, vegetable flavor liking, vegetable odor intensity, bitterness intensity and sweetness intensity.

3.2.4.2 Conditioning (Sessions 2-6)

Each subject participated in five drop-in style sessions each lasting 5-10 minutes, within a two week period. Subjects were asked to refrain from eating or drinking anything but water for at least 3 hours prior to coming to the lab and were encouraged to participate when hungry. It has been shown that individuals are more responsive to the metabolic effects of stimuli when they are in a hungry state (Yeomans, Leitch et al. 2008), and that the effectiveness of dietary learning (particularly flavor-nutrient conditioning) may be dependent on hunger/satiety (Yeomans, Leitch et al. 2008; Yeomans 2012). In order to be sure that all subjects were participating in the conditioning sessions while they were hungry, at the beginning of each conditioning session they were asked to rate their degree of hunger on a Visual Analog Scale (VAS: 0 = 'Extremely Hungry'; 50 = 'Neutral/Comfortable'; 100 = 'Extremely Full'; see Appendix G) displayed on a computer monitor. There was no significant differences between groups for each conditioning session and all sessions had an average rating below 50 (i.e. neutral/comfortable) indicating subjects were, on average, hungry when they participated (see Table 3.4).

 Table 3.4 Mean Hunger Rating ± SD by conditioning session and group

| Group | Conditioning Session ^a | | | | | |
|-------|-----------------------------------|---------------|---------------|---------------|---------------|--|
| | 1 | 2 | 3 | 4 | 5 | |
| ME* | 31.96 ± 11.94 | 34.03 ± 12.39 | 37.16 ± 10.41 | 35.81 ± 10.99 | 35.08 ± 12.56 | |
| FNC** | 31.35 ± 9.92 | 37.24 ± 11.47 | 35.38 ± 11.80 | 36.29 ± 13.98 | 37.79 ± 12.42 | |
| 2 | | | | - | | |

^a Mean ± SD- assessed on a VAS (0 = 'Extremely Hungry'; 50 = 'Neutral/Comfortable'; 100 = 'Extremely Full')

*Mere Exposure

******Flavor Nutrient Conditioning

During each conditioning session, subjects were served a 250mL juice sample in an 8oz disposable cup. All samples were stirred well with a spoon before being given to the subject, in order to ensure no settling or separating had occurred. They were asked to consume the entire sample (though not necessarily in one gulp), within a 10 minute period. Once they had consumed the sample, subjects were not asked to make any ratings about the sample, and were instructed not to eat or drink anything besides water for at least the next hour and to report any unusual stomach problems (none were reported). It was assumed that the majority of the dietary learning took place during these conditioning sessions.

3.2.4.2 Post-Test (Session 7)

Within one week of their last conditioning session, subjects returned to the lab and repeated the same hedonic and intensity rating tasks as in the pre-test session.

3.2.4.3 Follow-Up Test (Session 8)

Approximately two months after their post-test session (54-77 days, mean=64 days), subjects again returned to the lab and repeated the same hedonic and intensity rating tasks as in the pre- and post-test sessions.



Figure 3.1 Study Schematic

3.2.5 Data Analysis

3.2.5.1 Data Preparation

Hedonic ratings made on the LHS range from -100 to +100, and intensity ratings made on the gLMS range from 0 to +100. Hedonic ratings tend to be normally distributed across subjects (Lim, Wood et al. 2009; Lim and Fujimaru 2010), while intensity ratings tend to be log-normally distributed (Green, Shaffer et al. 1993; Green, Dalton et al. 1996). Therefore the intensity, but not hedonic, ratings were log-transformed prior to data analysis.

3.2.5.2 Statistical Analysis

The effects of conditioning (pre- vs. post-test) on the hedonic ratings for each conditioning group were analyzed by a paired t-tests assuming equal variances. Paired t-tests were also performed to examine the long term stability of hedonic ratings (post- vs. follow-up test) for each conditioning group. Differences between pre-, post- and follow-up test sessions for sweetness, bitterness and vegetable odor intensity were also analyzed using a paired t-tests. In order to analyze the average change per group, the difference between post-test and pre-test ratings were calculated, averaged across groups, and then analyzed using an unpaired t-test assuming equal variances. The statistical significance criterion was maintained at the standard accepted level of P <0.05. All statistical analyses were performed using Statistica 9.0 (StatSoft Inc., Tulsa OK).

3.3 Results

Effect of Exposure and Flavor-Nutrient Conditioning on Liking; under the Nose-Open Condition. Figure 3.2 shows the mean vegetable flavor hedonic ratings obtained under the nose-open condition for both conditioning groups. The left panel of Fig 3.2 shows the pre- and post-test ratings for the ME group, while the right panel of Fig 3.2 shows the pre- and post-test ratings for the FNC group. A paired t-test for each group shows a significant increase (p<0.01) in liking to the vegetable flavor, indicating that liking to a novel, initially disliked vegetable can increase through mere exposure. In fact, Fig 3.3 shows that the majority of subjects (~85%) of subjects showed an increase in vegetable flavor liking (i.e., a positive change from pre-test to post-test). Only approximately 15% of subjects showed a decrease in vegetable flavor liking (i.e., a negative change from pre-test to post-test). This indicates that the majority of subjects did learn to like the vegetable juice to some degree.



Figure 3.2: Mean hedonic ratings ± S.E. of vegetable flavor liking for "Pre-Test" (white bars) and "Post-Test" (gray bars) for the two conditioning groups (ME; Mere Exposure vs. FNC; Flavor Nutrient Conditioning) under the "nose-open" condition. Asterisks indicate significant differences between the two testing days (paired *t*-test ** denote for P < 0.01). Letters on the right y-axis represent sematic labels of the LHS: DVM= dislike very much; DM= dislike moderately; DS= dislike slightly; LS= like slightly; LM= like moderately; LVM= like very much.

Figure 3.3 also shows that the pattern of degree of change in vegetable flavor

liking ratings under the nose-open condition was similar across the two groups.

This was confirmed using an unpaired t-test on the average change by group

which showed that no significant difference between groups (p>0.05). This

indicates that the addition of calories (i.e., 80 kcal) had no further effect beyond

mere exposure on vegetable flavor liking.



Figure 3.3: Change in vegetable flavor liking from Pre-Test to Post-Test for each of the two conditioning groups Mere Exposure (left panel) and Flavor Nutrient Conditioning (right panel) for each individual subject under the "nose-open" condition.

Effect of Exposure and Flavor-Nutrient Conditioning on Liking; under the Nose-

Closed Condition. Figure 3.4 shows the mean vegetable flavor hedonic ratings

obtained under the nose-closed condition for both conditioning groups. Paired t-

tests show that liking did not change significantly for either conditioning group

(p>0.05), suggesting vegetable flavor liking could not change without the input of

retronasal input.



Figure 3.4: Mean hedonic ratings ± S.E. of vegetable flavor liking for "Pre-Test" (white bars) and "Post-Test" (gray bars) for the two conditioning groups (ME; Mere Exposure vs. FNC; Flavor Nutrient Conditioning) under the "nose-closed" condition. Letters on the right y-axis represent sematic labels of the LHS: DVM= dislike very much; DM= dislike moderately; DS= dislike slightly; LS= like slightly; LM= like moderately; LVM= like very much.

Role of taste and odor on changes in liking. Figure 3.5 shows the mean log-

intensity ratings of vegetable odor under the nose-open condition and the intensity ratings of sweetness and bitterness under the nose-closed condition, measured during the "pre-" and "post-test" sessions. Note that odor intensity results are only displayed for the nose-open condition as the nature of the nose clips should have made the odor essentially nonexistent under the nose-closed condition. Note also that previous research (Padmanabhan 2011) has shown that taste intensities are rated significantly higher in the presence of olfactory cues due to being misattributed to bitterness. This led to the suggestion that taste intensity ratings should be examined only in the absence of retronasal odors; therefore the sweetness and bitterness results are displayed only for the noseclosed condition. In general, there was a trend for an increase in sweetness intensity and a decrease in both odor intensity and bitterness intensity from "pre-" to "post-test" sessions. However, paired t-tests revealed that perceived intensity ratings between the sessions were not significantly different for either group (p>0.05). The mean odor intensity was about "weak", more importantly the intensities of both sweetness and bitterness averaged between 'no sensation' and 'barely detectable'. Therefore, any observed changes in sweetness and bitterness intensity ratings were considered negligible.



Figure 3.5: Log means of intensity ratings ± S.E. of vegetable odor (top), sweetness (bottom right), and bitterness (bottom left) for the "Pre-Test" (white bars) and "Post-Test" (grey bars) for the two conditioning groups. Vegetable odor intensity ratings were made under the "nose-open" condition, sweetness and bitterness intensities ratings were made under the "nose-closed" condition. Letters on the right y-axis represent semantic labels of the gLMS: BD= barely detectable; W= weak, M= moderate, S= strong.

Long term effect of dietary learning. Figure 3.6 shows the mean vegetable

flavor hedonic ratings obtained approximately two months after the post-test session, under the nose-open condition. A paired t-test showed no significant change in liking (p > 0.05) between the ratings obtained during "post-test" and "follow-up" sessions for either group. The intensity ratings for odor, sweetness and bitterness were also not significantly different between the two sessions for either group (p>0.05) (results not shown). Overall, these results indicate that increased liking to an initially novel vegetable is stable even two months after the conditioning sessions.



Figure 3.6: Mean hedonic ratings ± S.E. of vegetable flavor liking for the long term effects of dietary conditioning for the "Post-Test" (gray bars) and "Follow-up Test" (black bars) for the two conditioning groups (ME; Mere Exposure vs. FNC; Flavor Nutrient Conditioning) under the "nose-open" condition. Letters on the right y-axis represent sematic labels of the LHS: DVM= dislike very much; DM= dislike moderately; DS= dislike slightly; LS= like slightly; LM= like moderately; LVM= like very much.

Chapter 4

Discussion

4.1 The Effects of Mere Exposure on Dietary Learning as it relates to a novel and disliked vegetable

The present results show that mere exposure can increase hedonic responses to the novel and initially disliked vegetable. Approximately 85% of the subjects had increased vegetable flavor liking from pre-test to post-test. This result is consistent with findings of previous studies, which have also seen positive results of exposure to novel fruits and/or vegetables (Birch and Marlin 1982; Pliner 1982; Birch, McPhee et al. 1987; Richards, Kattelmann et al. 2006; Maier, Chabanet et al. 2007). For example, Birch et al (1987) exposed children a variety of novel fruits a varied number of times (i.e., 20, 15, 10, 5, or 0 times per fruit) and found that the more exposures the child had, the more they preferred it in a paired preference test (Birch and Marlin 1982). Maier et al (2007) used infants (i.e., starting at 7 months old) and novel, initially disliked vegetables over multiple in-home exposures, and saw a drastic increase in consumption with more exposures.

However, other studies have seen little to no positive changes (Zeinstra, Koelen et al. 2009; Olsen, Ritz et al. 2012; Osborne and Forestell 2012). For example, Zeinstra et al (2009) presented children with novel vegetable juices, but saw no change in the liking across time (Zeinstra, Koelen et al. 2009). Olsen et al (2012) also saw no increase in vegetable liking in children, across multiple conditioning exposures (Olsen, Ritz et al. 2012). The inconsistencies in results may come from differences in experimental design across studies.

The first major difference in experimental design between studies is the type of exposure used. Some of these studies used non-consumption types of exposure (e.g. children's picture books of vegetables). These studies observed only minimal effects with regards to increases in liking and therefore potential consumption.

Perhaps the most important factor when studying learning, particularly dietary learning, is novelty of the flavor. Learning can only occur when stimuli are sufficiently novel (Poelman and Delahunty 2011; Yeomans 2012). Yet, many of the previous studies (Zeinstra, Koelen et al. 2009; Olsen, Ritz et al. 2012) have used non-novel stimuli (e.g. cucumber, carrot, tomato). This oversight may have been a large contributing factor to the lack of significant changes in hedonic responses seen in these studies.

Individuals who conscientiously restrict all or part of their diet are considered 'restricted eaters'. It has been demonstrated that restricted eaters may have abnormal reactions to dietary learning (Brunstrom 2005). This is particularly true with regard to the influence of nutrients (Brunstrom and Mitchell 2007; Yeomans 2012). Many of the previous studies failed to test for, or take into account, restricted eaters (Zeinstra, Koelen et al. 2009; Olsen, Ritz et al. 2012; Osborne and Forestell 2012). Again, this oversight may have significantly decreased potential effects of dietary learning.

Another key factor not considered for many of the previous studies is hunger status (Olsen, Ritz et al. 2012; Osborne and Forestell 2012). It has been shown that hunger state may have a large impact on the ability of humans to learn, especially when the stimuli are nutritious (Yeomans 2012). Only a few of the previous studies took into account hunger state of their subjects. This was generally done by asking the subjects to taste the new food immediately before lunch (Birch, McPhee et al. 1990; Zeinstra, Koelen et al. 2009; de Wild, de Graaf et al. 2013). However, some of these studies were followed by food within 30 minutes (Birch, McPhee et al. 1990; de Wild, de Graaf et al. 2013), which confounded the potential effects of the test stimuli.

All of the factors discussed above became critical control points during this experiment and were carefully controlled in order to maximize the chances of success. The exposure was consumption based and should have been a sufficient number to see an increase in liking. Subjects indicated their level of familiarity with the test vegetable (kale) before being invited to participate, in order to ensure it was novel to all subjects. All subjects answered questions regarding their diet and eating behaviors, which helped to limit the number of restricted eaters. Lastly, each conditioning session was preceded by a measure of hunger level, so that subjects were always consuming the juice while hungry or near neutral (i.e., not hungry) (see Table 3.4).

4.2 The Effects of Nutrient Conditioning on Dietary Learning as it relates to a novel and disliked vegetable

The present results show that flavor-nutrient conditioning can increase hedonic responses to the novel and initially disliked vegetable juice. However, in opposition to the original hypothesis, the addition of calories did not show any *additional* increase in liking beyond the effects of mere exposure. This results is consistent with a very recent and similar study that saw the same degree of increase in both a low and high energy stimuli (de Wild, de Graaf et al. 2013). For the current study, this lack of additional increase may be a direct result of insufficient calories (i.e., only 80 kcal/250mL sample) of the flavor-nutrient conditioning stimuli. Therefore, the role of flavor-nutrient conditioning in learning to like a new vegetable is inconclusive.

The current and deWild et al (2013) results are in stark contrast to those found in many of the previous studies using flavor-nutrient conditioning in animal models (Sclafani and Ackroff 1994; Myers and Sclafani 2003; Ackroff, Drucker et al. 2012). Animal models generally find very strong positive effects of adding nutrients to novel flavors, especially in rats and mice. However, animal models are vastly different from human models and translating the experimental design from one model to the other is very difficult. Therefore, comparisons between the results of those studies and the current one are somewhat tentative.

While there have been some studies using human models, there are only a few studies that can serve as reasonable comparisons to the current one. For example, Yeomans et al (2008) used novel flavored sorbet and compared liking and intake under several conditions; nothing added, sweetened without added calories (i.e., aspartame), caloric without added sweetness (i.e., maltodextrin), and sweetened and caloric (i.e., sucrose). They found that liking increased only under the sucrose sweetened condition, potentially indicating that liking increases only when flavor-flavor and flavor-nutrient conditioning co-occur (Yeomans, Leitch et al. 2008). One of the main differences between the Yeomans study and the current one is the stimuli; they used fruit based stimuli vs. the current vegetable based stimuli. Using fruit based stimuli is a common practice (Zellner, Rozin et al. 1983; Baeyens, Eelen et al. 1990; Birch, McPhee et al. 1990; Capaldi and Privitera 2007; Yeomans, Leitch et al. 2008; Hogenkamp, Mars et al. 2010) but cannot be directly compared to the current study as fruits are usually sweet and humans generally innately like sweet foods. Other studies using human models and vegetable stimuli have either studied only mere exposure (Maier, Chabanet et al. 2007; Olsen, Ritz et al. 2012; Osborne and Forestell 2012) or have failed to account for mere exposure, that is, only accounting for the

effects of flavor-flavor and/or flavor-nutrient conditioning (Capaldi and Privitera 2008; Zeinstra, Koelen et al. 2009). This means that any increases in results (e.g. liking, preference, consumption) cannot be correctly attributed to the flavor conditioning vs. the effects of exposure.

4.3 The Importance of Odor on Dietary Learning

While the current study showed a significant improvement in vegetable flavor liking for both conditioning groups, this effect was seen only under the nose-open condition. Meanwhile, there was no significant improvement for vegetable flavor liking under the nose-closed condition. It is also important to note that the intensity of the odor, under the nose-open condition did not change significantly between test session ('pre-', 'post-' and 'follow-up' tests). This contrast between nose-open and nose-closed conditions strongly supports the previous findings (Lim and Padmanabhan 2012) that odor quality is a primary driver of vegetable acceptance/rejection.

More importantly, the present results provide strong evidence that learning could not occur in the absence of retronasal odor input. It has been reported that there are thousands of different odors recognized by the human olfactory system (Liu, Cai et al. 2006). Therefore, it stands to reason that learning would be based primarily on one of the near infinite number of possible unique volatile compounds or combination of volatile compounds that lead to the signature odor of foods. The subjects could only learn to like the new vegetable when they were able to identify it through the presence of its signature odor. This leads to the conclusion that not only is odor the primary driver of vegetable liking, is also the driving force behind learning to like new flavors.

It is also important to discuss the potential role that taste has on learning. Both tastes measured in this experiment (e.g. sweetness and bitterness) showed no significant changes in intensity between the three test sessions ('pre-test', 'post-test' and 'follow-up'). Furthermore, the intensity of both sweetness and bitterness averaged at or below 'barely detectable' for all test sessions (see Figure 3.9). This indicates that they were of little importance in the overall perception of the vegetable juice. This led to the conclusion that these elements were not the important factors in learning, and was not the driving force behind the observed increases in liking. This contradicts previous research that has shown that the 'bitter' taste of vegetables is the driving factor behind consumer rejection (Drewnowski 1997; Drewnowski and Gomea-Carneros 2000; Turnbull and Matisoo-Smith 2002). It is possible the importance of bitterness seen in the previous experiments were a consequence of taste-smell confusion (Rozin 1982; Lim and Johnson 2011). Alternatively, these studies generally asked for taste intensity ratings only under the nose un-blocked condition and did not measure odor intensity. This may have led to 'halo- dumping' as described by Clark and

Lawless (Clark and Lawless 1994) and further explored by Green et al (Green, Nachtigal et al. 2011).

4.4 The Long Term Effects of Dietary Learning

The final important finding of the current study observes the lasting positive effects of dietary conditioning. Results between 'post-test' and 'follow-up' showed no significant change for vegetable flavor liking, or the intensities of sweetness, bitterness or vegetable odor. These findings are consistent with previous research, which has shown stable ratings at two, six and even nine months after conditioning (Maier, Chabanet et al. 2007; Olsen, Ritz et al. 2012; de Wild, de Graaf et al. 2013). This aligns with humans' everyday lives. By recalling prior experiences (regardless of how many months ago they had the experience), individuals are able to expand their dietary horizons and add variety.

4.5 Potential Study Limitation

This study resulted in several useful conclusions. Mere exposure and flavornutrient conditioning were both able to increase liking ratings to the novel and initially disliked vegetable to the same degree. Previous research on the importance of retronasal odor in vegetable flavor liking was supported. And the importance of retronasal odor in learning was established. However, there is one potential limitation to this study.

The primary limitation relates directly to the level of nutrients included in the flavor-nutrient condition stimuli (i.e., 80 kcal/250mL). A recent study in our lab found that some people can taste maltodextrin and generally describe it as 'sweet,' 'cereal- like' or 'bread-like' (Lim and Lapis 2013). Therefore, the current study deliberately chose a level of maltodextrin that did not invoke any taste to most people. This led to using only 20g/250mL (i.e., 80 kcal) of maltodextrin, as any further increase in maltodextrin content resulted in a taste and therefore introduced the confounding factor of flavor-flavor conditioning. It has been shown that the presence of the added nutrients should not be obvious to subjects, in any subtle sensory difference including taste or viscosity, as failure to adequately disguise the nutrients may confound conclusions drawn about changes in flavor evaluation (Yeomans 2012). Unfortunately, the level chosen in this study may have been too low to cause the metabolic consequences necessary for flavor-nutrient conditioning to occur, as an 80 kcal drink would be unlikely to cause feelings of satiety for some subjects.

Chapter 5

Conclusion

Vegetables are an essential part of the human diet as there are many beneficial components in vegetables. These components include high contents of dietary fiber, vitamins, and minerals, which can lend to the reduction of known major health concerns (e.g. diabetes, obesity). However, the majority of Americans are not consuming nearly enough vegetables (CDC 2010). There are several important factors involved in food choice. For example, time-, cost-, and health-related factors can contribute significantly to decisions regarding which foods to consume (Betts, Amos et al. 1995). More importantly, the sensory components (e.g. sweetness, bitterness and retronasal odor) are essential in food choice. Vegetables are commonly described as having a 'bitter' taste (Drewnowski 1997; Drewnowski and Gomea-Carneros 2000). Humans instinctively avoid bitter tastes (Capaldi and Privitera 2008) therefore, bitterness is often the reason cited for vegetable dislike, and subsequent low consumption. Vegetables are also known to have strong odors, particularly sulfurous odors (Maruyama 1970; Engel, Baty et al. 2002). As such, retronasal odors have recently been shown to be the major contributing factor of vegetable dislike (Lim and Padmanabhan 2012). It therefore stands to reason that vegetable 'likers' may have learned to like specific vegetable flavors.

There are several ways through which humans learn to like new foods. Two of the key mechanisms are mere exposure and flavor conditioning. The objective of this thesis was to investigate the effectiveness of these two mechanisms on a novel and disliked vegetable in young adults. This experiment measured changes in vegetable flavor liking, along with sweetness, bitterness and vegetable odor intensities after conditioning. Results suggest that both mere exposure and flavor-nutrient conditioning were able to increase liking to the new and initially disliked vegetable flavor. Approximately 85% of subjects tested showed a positive change in vegetable flavor liking between the pre- and posttest sessions. Unlike what was hypothesized, however, that the addition of calories did not provide any additional increase in liking beyond mere exposure. This lack of additional increase was likely due to the low calorie content (i.e., 80 kcal) lending to insufficient metabolic reinforcement, leaving the role of additional calories in vegetable learning to be somewhat inconclusive. Another important finding of the current study was that there were significant increases in liking under the nose-open condition, but no significant changes in any of the measured qualities under the nose-closed condition. These results lend further support to the importance of retronasal odor in vegetable liking. More importantly, this supports the hypothesis that retronasal odor is the driving force behind dietary learning. These results, and previous, imply that it is possible to learn to like initially disliked vegetables through multiple exposures. More

exposures are potentially reducing neophobia, leading to neutral or even positive feelings (and therefore higher consumption) towards new vegetables.

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Appendices

Appendix A

Screener- Human Flavor Perception Study

We are conducting research to understand human flavor perception. The first step is to identify subjects who will be qualified to participate in the study.

- The procedure involves filling out the screener below. It will take approximately 5-10 minutes.
- Filling out the screener has no direct benefit to you.
- We will do our best to keep your information confidential. All information from qualified subjects will be stored in the password-protected E-mail account that only the investigators have access to. All information from ineligible subjects will be deleted immediately from the email account.
- Participation in filling out the screener is voluntary, and you may discontinue participation at any time.
- One of the risks in filling out the screener is potential breach of confidentiality. Information sent by email can be intercepted, corrupted, lost, destroyed, arrive late or incomplete, or contain viruses. Thus, security and confidentiality of information sent by email cannot be guaranteed, and we may accidentally disclose information that may identify you.

If you have any question about this study, please contact any of the following:

- Dr. Juyun Lim (Principal Investigator: juyun.lim@oregonstate.edu)
- Nicole Babb (Graduate Research Assistant: nicole.babb2@oregonstate.edu)
- OSU Taste Perception Lab (<u>taste.study@oregonstate.edu</u>)

You may skip questions that you do not want to answer. However, please note that if you don't answer all questions, we may not be able to determine if you can be part of the study.

Name: Gender: Age: Email address: Daytime phone #: Best way to reach you: phone, email, or either

- 1. Are you a smoker?
- 2. Are you pregnant?
- 3. Are you taking any prescription medication? If so, what general type?
- 4. Do you have any taste deficits or other oral disorders (e.g. burning mouth syndrome)?
- 5. Do you currently have any oral lesions, canker sores, or piercings of the tongue, lip, or cheek?
- 6. Do you have a history of food allergy? If so, what kind?
- 7. Do you have any other allergy? If so, what kind?
- 8. Do you have any dietary restrictions (e.g., vegetarian, gluten-free)?
- 9. Are you willing to consume foods that you dislike?
- 10. Please indicate your general availability (Monday-Friday) this term.
 - MONDAY (9 am -6 pm):
 - TUESDAY (9 am 6 pm):
 - WEDNESDAY (9 am 6 pm):
 - THURSDAY (9 am 6 pm):
 - FRIDAY (9 am 6 pm):
- 11. Are you able to commit to coming into the lab 3 times a week (on a drop-in basis, anytime between 10:00am and 6:00pm, approximately 10 minutes per time)?

12. How often do you CONSUME the following vegetables?

| | At least once a week | At least once a month | Once every two to three months | Once every six months | Once a year | Once or twice in lifetime | Never (or never heard of) |
|------------------------|---|---------------------------------------|--|--------------------------------|--------------------------|--|---------------------------------------|
| Asparagus | | | | | | | |
| Beets | | | | | | | |
| Broccoli | | | | | | | |
| Carrots | | | | | | | |
| Celery | | | | | | | |
| Endive | | | | | | | |
| Kale | | | | | | | |
| Okra | | | | | | | |
| Radishes | | | | | | | |
| Spinach | | | | | | | |
| Sweet Potatoes or Yams | | | | | | | |

13. How much do you LIKE the following vegetables?

| | Like | Neutral | Dislike | Unsure |
|------------------------|------|---------|---------|--------|
| Asparagus | | | | |
| Beets | | | | |
| Broccoli | | | | |
| Carrots | | | | |
| Celery | | | | |
| Endive | | | | |
| Kale | | | | |
| Okra | | | | |
| Radishes | | | | |
| Spinach | | | | |
| Sweet Potatoes or Yams | | | | |

(Continue on next page)

- A. How often do you diet?
 - a. Never
 - b. Sometimes
 - c. Usually
 - d. Always
- B. Would a weight fluctuation of 5 pounds affect your food choices? (in terms of amount of or kinds of foods you eat)
 - a. Not at all
 - b. Slightly
 - c. Moderately
 - d. Extremely
- C. Do you feel guilty after overeating?
 - a. Never
 - b. Sometimes
 - c. Usually
 - d. Always
- D. How conscious are you about how many calories or how much food you consume?
 - a. Not at all
 - b. Slightly
 - c. Moderately
 - d. Extremely

Thank you for filling out the screener. We will contact you and set up and appointment ONLY IF you qualify.

Appendix B

Original questions from Three-Factor Eating Questionnaire and modified

| Original Question | Modified Question | | | |
|---|--------------------------------------|--|--|--|
| 37: How often are you dieting in a conscious effort to control your | 1: How often do you diet? | | | |
| | 1 Never | | | |
| 1- Rarely | 1- Never | | | |
| 2- Sometimes | 2- Sometimes | | | |
| 3- Usually | 3- Usually | | | |
| 4- Always | 4- Always | | | |
| 38: Would a weight fluctuation of 5 | 2: Would a weight fluctuation of 5 | | | |
| lbs. affect the way you live your life? | pounds affect your food choices? (in | | | |
| | terms of amount of or kinds of foods | | | |
| | you eat) | | | |
| 1- Not at all | 1- Not at all | | | |
| 2- Slightly | 2- Slightly | | | |
| 3- Moderately | 3- Moderately | | | |
| 4- Very much | 4- Extremely | | | |
| 40: Do your feelings of guilt about | 3: Do you feel guilty after | | | |
| overeating help you control your food | overeating? | | | |
| intake? | | | | |
| 1- Never | 1- Never | | | |
| 2- Rarely | 2- Sometimes | | | |
| 3- Often | 3- Usually | | | |
| 4- Always | 4- Always | | | |
| 42: How conscious are you of what | 4: How conscious are you about | | | |
| you are eating? | how many calories or how much food | | | |
| | you consume? | | | |
| 1- Not at all | 1- Not at all | | | |
| 2- Slightly | 2- Slightly | | | |
| 3- Moderately | 3- Moderately | | | |
| 4- Extremely | 4- Extremely | | | |

questions used for subject classification

Appendix C





Appendix D

Imagined sensations used for LHS scale training

- 1. The taste of plain bread
- 2. The taste of a soggy potato chip
- 3. The feel of pure silk
- 4. The sound of fingernails dragging across a blackboard
- 5. The taste of your favorite chocolate
- 6. The feel of a massage
- 7. The smell of clean laundry
- 8. The smell of vomit
- 9. The taste of water
- 10. The smell of a rose
- 11. Stinging eyes from cutting an onion
- 12. The feel of coarse sandpaper
- 13. The smell of bad body odor
- 14. The feel of a minor scratch
- 15. The taste of room temperature soda

Appendix E

The general Labeled Magnitude Scale (gLMS)

– Strongest imaginable

- Very strong

- Strong

– Moderate

– Weak

Barely detectable

Appendix F

Imagined sensations used for gLMS scale training

- 1. The sweetness of milk
- 2. The coolness of sipping an ice cold beverage
- 3. The warmth of sipping lukewarm water
- 4. The bitterness of celery
- 5. The burning sensation of eating a whole hot pepper
- 6. The burning sensation of cinnamon gum
- 7. The sourness of a lemon
- 8. The sweetness of a banana
- 9. The heat of sipping boiling hot tea
- 10. The touch sensation of a pill on your tongue
- 11. The weight of a feather in your hand
- 12. The coolness of a peppermint candy
- 13. The pain from biting your tongue
- 14. The bitterness of black coffee
- 15. The bitterness of fresh spring water

Appendix G

The Hunger Visual Analog Scale (VAS)

