PROP Taster Status and its Influence on
Dietary Intake and Plasma Concentrations of α-Tocopherol

By Yvonne Koelliker

A thesis submitted to the
Graduate School – New Brunswick
Rutgers, The State University of New Jersey
In partial fulfillment of requirements
For the degree of
Master of Science
Graduate Program in Food Science
Written under the direction of
Professor Beverly J. Tepper
And approved by

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New Brunswick, NJ
May, 2010
ABSTRACT OF THE THESIS

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Foods rich in Vitamin E (α-tocopherol), such as green, leafy vegetables and vegetable oils, are known to be an important part of a healthy diet, as Vitamin E has been shown to reduce the risk of cardiovascular disease through a variety of mechanisms. However, often these foods contain phytonutrients which impart a bitter taste, making them less acceptable to consumers. Sensitivity to 6-n-propylthiouracil (PROP) may be a marker for the selection of Vitamin E-rich foods. Some studies have demonstrated that PROP non-tasters (NT) showed a higher acceptance of bitter fruits, vegetables and vegetable oils than PROP tasters. The aim of this study was to relate PROP sensitivity to dietary intake and plasma concentrations of α-tocopherol. Healthy, non-vegetarian females, ages 21-44 years, who did not take dietary supplements, were classified as NT (n=30), MT (n=33), and ST (n=30) based on the PROP paper disk method. The subjects provided three, 24-hour diet recalls, collected using NDS-R. Eating attitudes were measured using the Dutch Eating Behavior Questionnaire. One fasting blood sample was collected from each subject and analyzed for α-tocopherol. There were no differences in the consumption of Vitamin E-rich foods or total α-tocopherol across taster groups. When the subjects were
divided by restrained eating, non-restrained NT had higher intakes of α-tocopherol than
the other groups (p=0.05). There were no differences among PROP taster groups for
plasma α-tocopherol status. However, when the subjects were divided by “higher” and
“lower” plasma concentrations of α-tocopherol, differences were seen in the “higher”
group, where NT had significantly higher concentrations of α-tocopherol in their plasma
than MT or ST (p=0.05). Regressions were performed to determine the factors
contributing to “higher” and “lower” plasma concentrations. Among those subjects with
“higher” levels, dairy intake, energy intake, and PROP taster status were found to
influence plasma concentrations. These data suggest that PROP taster status influences
the consumption and plasma concentrations of α-tocopherol. Further research should
examine long-term intake of α-tocopherol and its relationship to plasma status, and the
health implications of this relationship.
ACKNOWLEDGEMENTS

The author would like to thank Professor Beverly Tepper for her guidance, support, and advice with this research project, as well as her professional guidance in the field of sensory research. She would also like to thank her committee, Drs. Quadro, Schaffner, and Simon for their help and support. Additionally, she would like to thank her friends and family for their constant support and unwavering friendship, especially Brian Greene, Dawn Cacia, and Phil Wood. She would also like to thank members of the Sensory Lab at Rutgers University, past and present, for their help with this, and other research, and for their friendship especially Katherine Nolen, Lisa Belzer, Nelson Serrano-Bahri, Joey Donovan, and Reuben Gutierrez. Also, thank you to the numerous individuals who helped with various stages of the project, especially Drs. Burgess and Shapses, Amal Morgane, Diandian Shen, and Cara Welch. Finally the author would like to thank the professors, staff, and students of Cook campus for making Rutgers such an enjoyable place to work for the past eight years.
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1.1 Antioxidant Function and Epidemiological Importance

Fruits and vegetables have long been known to be an important part of a healthy diet, due to largely to the non-nutritive phytochemicals, such as antioxidants and anti-cancer compounds, found in these foods, as well as the high fiber and low fat content. The impact of fruit and vegetable intake on disease risk has been studied extensively, especially with regards to widely prevalent diseases, such as cardiovascular disease, cancer, and stroke [1-3]. These studies have found that intake of fruits and vegetables is associated with decreased risk for these, and other, diseases, largely due to the non-nutritive, phytochemicals present in these foods providing antioxidant and anti-inflammatory effects. Although a large variety of health-promoting compounds are found in fruits and vegetables, one of the most researched class of compounds are antioxidants, in particular the major antioxidants, Vitamins A (β-carotene), C, and E (α-tocopherol).

These antioxidant compounds help lower the risk for these diseases in many ways, including but not limited to, prevention of free radical damage, interception of harmful, reactive species, and repair of damaged cells [4]. Antioxidants can prevent reactive oxygen species (ROS), such as the peroxyl and hydroxyl radicals, as well as reactive nitrogen species (RNS) from forming, thus reducing the substantive damage and oxidative stress they can cause in the body. These reactive species can come from the environment and cellular functions, among other places, and can cause damage to cells and cellular components, leading to increased disease risk. ROS and RNS can be
neutralized (intercepted) by antioxidants, as these compounds have complex resonance structures, which allow the even distribution of the charge from the radical species [5]. Antioxidants, especially smaller molecules, can also serve as building blocks for enzymes involved in complex systems which can repair damaged body components, such as DNA and cell membranes. Antioxidants are produced within the body, as well, however dietary intake of these compounds is necessary to augment the protective effect of these compounds [6].

The major antioxidants vitamins (Vitamins A, C, and E) have been studied extensively and have been shown to impart a wide range of health benefits, from anti-carcinogenic to anti-inflammatory characteristics. Vitamins A and E have been associated with protection against various diseases, as well as diseases associated with aging, such as cataracts [7]. A study by Kontush showed significantly lower levels of $\alpha$-carotene and $\gamma$-tocopherol in patients with coronary heart diseases, suggesting lipophilic antioxidants contribute to heart health [8]. A study in obese children showed lowered levels of $\alpha$-tocopherol and $\beta$-carotene levels, making them more prone to oxidative stress [9]. The direct effects of Vitamin C on disease prevention have been inconclusive, but Vitamin C had been shown to be important biologically, as an antioxidant by recycling Vitamin E back to its active antioxidant form for reuse in the body [10]. The effect of $\alpha$-tocopherol intake on a number of diseases will be discussed later.

1.2 Antioxidant and Other Health-Promoting Compounds in Food

The major antioxidants are found in a variety of foods, most notably fruits and vegetables. The major sources of $\beta$-carotene in the U.S. diet include dark-colored
vegetables (carrots and dark, green leafy vegetables) and fruits, oily fruits, and red palm oil [11]. Vitamin C consumption is largely due to citrus fruit and juice, tomato and tomato-based product, and potato intake, while additional sources include Brussels sprouts, cauliflower, broccoli, cabbage, and spinach [12]. Specific sources of α-tocopherol will be discussed later, but they include dark, green leafy vegetables and vegetable oils [12]. Aside from these major antioxidants, a number of other antioxidants are present in foods, including lycopene (from tomatoes and tomato products) and other carotenoids, such as α-carotene and β-cryptoxanthin (from carrots, dark, green leafy vegetables, and fruit).

There are many different types of health-promoting compounds found in foods, aside from these major, antioxidant compounds. One example, phytoestrogens, compounds found in berries and soy products, are beneficial in that they bind to hormone receptors and can reduce the risk of hormone-dependent cancers [13]. Another class of compounds, anthocyanins, pigments found in many fruits and vegetables have been shown to reduce cancer risk, promote cardiovascular health, and to have antioxidant and anti-inflammatory effects [14]. These flavonoids, found in fruit such as grapes, red wine, and blueberries, have been shown to impart these benefits on people consuming them [15, 16]. Many different classes of these compounds exist, some of which are bitter or are in foods containing other bitter-tasting compounds [17]. The glucosinolate compounds in cruciferous vegetables have been shown to have anti-carcinogenic properties, while the bitter, antioxidant polyphenols in red wine have been related to heart health [18, 19]. Green tea, which contains epigallocatechin gallate (EGCG) and other
antioxidant catechins, has been linked with a number of health promoting activities, including cancer prevention and heart health [20, 21].

Unfortunately, many of the foods containing the highest levels or the most potent concentrations of antioxidants, also contain the highest levels of bitter compounds, and, in many cases, the antioxidant compounds are bitter themselves, which can lead to reduced acceptance and intake in those more sensitive to bitter tastes (Table 1). In cruciferous vegetables (cabbage, radish, horseradish), the glucosinolate compounds and their breakdown products, isothiocyanates, contribute a bitter or pungent taste, thought to be mainly due to the nitrogen-carbon-sulfur bond [22]. In addition, limonen and naringin in these fruits lends a bitter taste to the flesh. Caffeic acid imparts bitterness to coffee, as do the catechins and tea polyphenols found in green and black teas. In addition, polyphenols and flavonoids in red wine impart an astringent taste to these foods, while the ethanol also lends a burning sensation to the beverage. The same is true for beer, in which ethanol contributes to the burning sensation, while the beverage also has a bitter taste from the isohumalones (from hops) [23]. Due to these evolutionary developments, bitter tastes and trigeminal sensations are often deterrents to eating certain foods and can influence an individuals’ preference for foods, resulting in reduced intake of beneficial foods. While experience and exposure to these foods containing bitter and other strong-tasting compounds can lead to acceptance and increased intake of these beneficial foods, taste genetics can play an important role in whether or not these types of foods will be consumed. Individuals more sensitive to the bitter or strongly-flavored compounds in these foods may consume less of them, thereby reducing their intake of health-promoting antioxidant compounds.
Table 1: Beneficial compounds, action, and taste qualities

<table>
<thead>
<tr>
<th>Compound</th>
<th>Food</th>
<th>Activity</th>
<th>Sensory Qualities</th>
</tr>
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<tbody>
<tr>
<td>β-carotene</td>
<td>Leafy vegetables</td>
<td>Antioxidant</td>
<td>Bitter</td>
</tr>
<tr>
<td>α-tocopherol</td>
<td>Leafy vegetables, oils</td>
<td>Antioxidant</td>
<td>Bitter</td>
</tr>
<tr>
<td>Vitamin C</td>
<td>Citrus, broccoli</td>
<td>Antioxidant</td>
<td>Bitter</td>
</tr>
<tr>
<td>Carotenoids</td>
<td>Leafy vegetables</td>
<td>Antioxidant</td>
<td>Bitter</td>
</tr>
<tr>
<td>Polyphenols</td>
<td>Wine, tea</td>
<td>Antioxidant</td>
<td>Bitter, astringent</td>
</tr>
<tr>
<td>Glucosinolates</td>
<td>Cruciferous vegetables</td>
<td>Anti-cancer</td>
<td>Bitter</td>
</tr>
<tr>
<td>Catechins</td>
<td>Tea</td>
<td>Antioxidant</td>
<td>Bitter, astringent</td>
</tr>
<tr>
<td>Phytoestrogens</td>
<td>Soy, berries</td>
<td>Anti-cancer</td>
<td>Bitter</td>
</tr>
<tr>
<td>Anthocyanins</td>
<td>Red and blue fruits</td>
<td>Antioxidant</td>
<td>Bitter</td>
</tr>
</tbody>
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1.3 Taste Physiology and PROP Taster Status

1.3.1 PROP Taster Status

The sensitivity to 6-n-propylthiouracil (PROP) is a genetically inherited trait. PROP is a thiourea compound similar to the glucosinolates and their breakdown products, isothiocyanates, found in broccoli, cabbage, and other cruciferous vegetables, as well as phenylthiocarbamide (PTC) [22]. It is believed that the nitrogen-sulfur bond leads to the bitter taste of these compounds. Approximately 70% of the United States population is sensitive to PROP (tasters) and 30% are taste blind to PROP (non-tasters), however, ratios of tasters to non-tasters (NT) differ in other parts of world [24]. The taster population can be further divided by sensitivity, those who are extremely sensitive have been labeled super tasters (ST), while those with moderate sensitivity are referred to as medium tasters (MT). Approximately 30% of PROP tasters in the U.S. are super tasters, and, although gender differences will not be addressed in this research, it should be noted that a higher percentage of men are non-taster than women [25]. The gene TAS2R38 has been shown to relate strongly to the taster phenotype and the ability to taste both PTC and
PROP [26]. Three single nucleotide polymorphisms at positions P49A, A262V, and V296I, are responsible for the variation in the gene, where non-tasters have an AVI/AVI (recessive) haplotype and tasters have at least one dominant allele, PAV [24, 27]. Bufe, et al. showed bitterness intensity ratings of PTC and PROP differed by group, such that individuals with the homozygous recessive, AVI/AVI haplotype had higher thresholds for PTC than the other two groups [27]. Additionally, this research showed PAV/PAV individuals had higher suprathresholds for PTC and PROP than the heterozygotes.

1.3.2 Food Preferences by PROP Taster Group – Bitterness

The three PROP taster groups have been shown to vary in their food preferences, as well as their bitterness intensity ratings of different bitter compounds beyond PROP and PTC, although the research has shown this to varied degrees, as will be discussed in the next paragraphs. Differences have been shown in the intake and/or preference of bitter fruits, such as citrus, and vegetables, such as broccoli and cauliflower, through a number of different data collection methods, including diet recalls, food frequency questionnaires, and food preference questionnaires. Additionally, studies have been performed in both children and adults, in which differences have been found in both subject populations.

Studies in adults have looked at food preferences by PROP or PTC taster group using questionnaires and/or sensory tests. In a study by Drewnowski, et al., women with greater sensitivity to PROP had lower acceptance for cruciferous vegetables, based on a food preference questionnaire [28]. In a study in adults, ST gave higher bitterness ratings to naringin solutions than both MT and NT (using a nine-point intensity scale) [29].
Additionally, this study showed increased PROP sensitivity was associated with lower liking ratings of grapefruit juice through the use of a food preference questionnaire. In another study, in which the subjects rated bitterness intensity (in this case of glucosinolate and non-glucosinolate-containing foods), PAV/PAV individuals rated the glucosinolate-containing foods as significantly more bitter than the AVI/AVI individuals [30]. This study did not quantify intake of these foods by genotype, however. This same study showed no association between orange juice liking and PROP taster status, most likely due to the lack of naringin, or other bitter compounds, in the beverage. In addition, Akella, et al. showed PROP sensitivity was correlated with an increased bitterness perception, as well as dislike for Japanese green tea, using nine-point category scales [31]. This same study showed PROP tasters had an increased preference (using a food preference questionnaire) for sweetened soy products over unsweetened soy products, perhaps due to the reduction in bitterness (soy isoflavones) caused by the sugar.

Various studies in children have been performed, also looking at food preferences. In a study of children ages three to four, PROP-sensitive children had lower acceptance (using a food preference test) of raw broccoli and American cheese [32]. A second study by Turbull, et al., showed a similar pattern for raw spinach (through the use of a verbal questionnaire), such that taster children disliked it more than NT children [33]. A study by Bell and Tepper showed NT children liked raw broccoli more than taster children, using a five-point facial hedonic scale (a food preference test) [34].

Differences have also been shown among taster groups during studies looking at food intake. Jerzsa-Latta et al. showed PTC NT used cooked turnip and raw watercress more than PTC tasters did, based on the results of a food frequency questionnaire [35].
study by Turnbull, et al., mentioned above, also looked at the order in which children selected foods to eat. This research showed NT were more likely to select cheese earlier in an order-of-choice ranking test than taster children [36]. Alternatively, milk was selected later by NT than ST. A fourth study in children, by Bell and Tepper, showed NT consumed more vegetables in a free-choice eating session, reflected in the increased intake of bitter vegetables; olives, cucumber, and broccoli [34].

While the studies above have shown a correlation between PROP taster status and the acceptance and frequency of consumption of various foods and food compounds, a number of studies have shown no relationship. A study by Niewind in an elderly population showed taste preferences and vegetable use (reported using a FFQ) were unaffected by PTC taster status. This study did show, however, that PTC tasters rated the flavor intensity of cooked cabbage higher than NT using quantitative descriptive analysis [37]. In the study mentioned earlier by Jerzsa-Latta, no differences between PTC taster groups were seen for the other eleven vegetables tested, including broccoli, Brussel sprouts, and cabbage, rated using a Food Frequency Questionnaire [35]. Another study by Yackinous, et al., showed no effect of PROP taster status on the intake of bitter fruits, vegetables, or beverages (with the exception of NT eating more green salad than ST; measured using a FFQ) based on the results of a FFQ [38]. Mattes and Labov showed there were no differences in dietary intake of goitrogens (cabbage, carrots, celery, rutabaga, and strawberries) by PTC taster status using a FFQ [39]. A study by Drewnowski, et al., showed no influence of PROP taster status on either food preferences, measured with a nine-point hedonic scale, or food intake, measured using a FFQ, in female subjects [40]. Relationships between taster status and bitter salts,
isohumulones from beer, and red wine, have also been shown to be non-existent by some researchers [41-43].

1.3.3 Food Preferences by PROP Taster Group – Interpretation

One explanation for these conflicting results is the influence of food adventurousness on food choice and intake, as shown by Ullrich, et al [44]. This study included the variation due to the willingness of the participants to try new foods. ST, when food adventurous, did not show the expected lowered preference for bitter and pungent foods, suggesting that food adventurousness could greatly mask the anticipated taster group preferences for various foods. In addition, ethnic background, socioeconomic status, environmental influence, and other factors at all as children and through adulthood, play a role in influencing food choices and, ultimately, likes and dislikes, further masking the influence of taster status [45]. Furthermore, the influences of emotional factors, such as dietary restraint, disinhibition, and their interaction (which will be discussed later), have been shown to influence food choice and affect weight status, especially in adult women, which is of particular relevance in this study population [46, 47]. In addition, PROP taster status was assigned using different methods across studies and food intakes and preferences were recorded using assorted methods, from FFQs to diet recalls. These disparate research methods may have profound influences on the study outcomes. While studies have clearly shown PROP tasters rate the bitterness of various foods as higher than PROP non-tasters, the influence this has over food choice and actual intake remains unclear. Even though foods are perceived as more bitter, there are clearly additional factors that influence the intake of these foods, beyond preference for non-bitter foods.
1.3.4 Food Preferences by PROP Taster Group – Anatomical Differences and Texture of Fats

1.3.4.1 Anatomical Differences

The surface of the tongue is covered with taste papillae, which hold and orient the taste buds. These papillae are distributed and oriented on three types of taste papillae, organized into regions on the tongue. The fungiform papillae (which respond to sweet and salty) are located mainly on the sides and tip of the tongue. The circumvallate papillae, which respond to bitter compounds, are located on the back of the tongue, near the throat. The third, less abundant type of papillae, the foliate papillae, are located on the sides of the tongue in folds. The taste buds are arranged on the papillae to maximize the area exposed to taste compounds dissolved in the saliva through chewing [48]. In the case of sweet and bitter tasting compounds, the dissolved compounds come in contact with the microvilli on the taste cells, found inside each taste bud. Once the sweet or bitter compounds enter the taste cell and binds with the taste receptor, a G-protein coupled receptor reaction occurs. This reaction involves the G-protein (gustducin) beginning a chain of events, resulting in the release of a neurotransmitter (indicating a sweet or bitter tastant has been ingested) which sends a message to the cerebral cortex via the nerves that innervate the tongue [49, 50].

There are 25 human bitter taste receptors that make up the class of TAS2R taste receptors [25]. The ability to taste bitterness in food is an important evolutionary development in terms of food intake, as bitter tastes are often indicators of poisonous substances in foods or less than optimum nutritional benefit (under-ripeness). Though these bitter taste receptors bind a variety of bitter compounds, the receptor of interest in
the current study is TAS2R38, as this receptor binds PTC and PROP. It is hypothesized that the ability to perceive bitterness from PTC and PROP is necessary to avoid dietary goitrogens (anti-thyroid compounds) from cruciferous vegetables, especially within populations where dietary iodine is low. Thus, the ability to taste the specific bitterness from these iodine uptake inhibitors serves as a warning to reduce consumption [51].

Aside from differences in bitter taste perception, PROP taster status has also been linked to a higher density of fungiform taste papillae. Bartoshuk, et al. showed differences in papillae density by both taster status (ST had a greater density of papillae than NT) and gender (women had a higher papillae density than men) [52]. In the Delwiche, et al. study, increased papillae density was shown to correlate with increased perception of the bitterness of PROP solutions, but not to the bitterness of quinine solutions [53]. In a study by Tepper and Nurse in 1996, ST had the highest fungiform papillae density, followed by MT, with NT having the lowest density [54]. Differences were also shown between men and women, as in the Bartoshuk study, in that women had significantly higher papillae densities than men. These findings were further confirmed in a 2002 study, which also showed ST had a significantly higher fungiform papillae density, as well [55]. The higher density of fungiform papillae may be the cause of increased trigeminal sensations in ST and MT, as the taste papillae are innervated with nerves which send information on the cooling, burning, chemical heat, etc., to the brain [56]. The trigeminal nerves also transmit information about the texture of food, which may be related to the ability of PROP tasters to better determine the amount of fat in food and the preference NT have for higher fat foods.
These physiological differences in the trigeminal system may also relate to other taste preference differences between NT and tasters with regards to oral irritants. PROP tasters have been shown to be more sensitive to the chemical burn from capsaicin, as shown by Karrer, et al. in 1991 [57]. This was further confirmed in 2000 in a study looking at capsaicin and ethanol mouth burn in PROP tasters and NT, where tasters were shown to perceive more oral burn from these compounds [58]. Ullrich, et al. showed PROP tasters had a lower preference for pungent spices, such as horseradish and ginger [44]. In a study looking at the trigeminal sensations attributed to red wine, NT gave significantly lower ratings to the astringency and acidity than tasters [59].

1.3.4.2 Texture of Fats

In addition to differences among taster groups with regards to bitter tasting foods and compounds, taster groups show differences in fat perception and other trigeminal stimuli, like astringency and oral irritation. This was shown by Tepper, et al., where ST were able to discriminate between high and low-fat salad dressings, while NT could not [54]. Kirkmeyer, et al. showed that while ST and NT perceived creaminess in dairy products in a similar manner, the two groups used different words to describe the creaminess and different cues to determine the creaminess intensity [60]. These findings differed from the results from a study by Yackinous, et al, however, in which there were no taster differences in fattiness ratings for potato chips, vanilla pudding, or chocolate drinks [61].

A difference in fattiness rating by taster status was found, in this study, using mashed potatoes as a stimulus, however the researchers has little confidence this indicated a true PROP taster difference in fattiness perception. The Yackinous study did show, however,
that ST were more sensitive to general stimulation of the tongue. Drewnowski, et al, also failed to show differences in fat perception in the mixtures of sugar and fat in women [62]. The lack of difference may be explained by the use of food mixtures in both studies, where other food cues and tastes may have interfered with the subjects’ ability to distinguish fat content. While there are conflicting data regarding fat perception and taster status, studies of other trigeminal sensations clearly show ST and NT perceive foods differently, based on many textural and oral irritation characteristics, not just flavor [57, 63].

These differences in trigeminal sensation perception may also relate to a higher (or lower) preference for fat. In the study mentioned earlier, Tepper showed that NT could not distinguish between low and high-fat salad dressings, but they preferred the high-fat dressing [64]. A study in children in 2001 reported a similar preference for higher fat by NT, showing ST girls had a lower preference for full fat milk than NT. One study by Hayes and Duffy relating sweet-fat mixtures to PROP bitterness ratings found that those individuals who found PROP to be most bitter also perceived the most creaminess from heavy cream, regardless of the level of sucrose added, although papillae density did not show as strong a relationship to creaminess perception [65]. The authors attributed this to creaminess perception not being solely related to tactile information from the trigeminal nerve, but also due to olfactory or retronasal cues. A second study by the same authors compared ratings for the bitterness of both PROP and quinine to hedonic responses to sweet-fat mixtures. Among subjects rating PROP more bitter than quinine, creaminess of the sweet-fat mixtures was rated as more intense than those that perceived less bitterness from PROP [66]. Previous research from that lab had shown subjects with
the opposite pattern (high quinine/low PROP) consumed more sweet foods and added sugars, perhaps due to taste damage due to chronic otitis media [67]. In this same group of subjects, high PROP/low quinine, the “moderate” sweet and fat samples were liked the best, meaning those samples with a mid-level of fat and sugar (3.3% and 10%, respectively) were optimal. In contrast, those subjects who rated PROP as less bitter than quinine like the samples with the highest levels of fat and sugar the best (36% and 20%, respectively). In this same study population, women with a high density of taste papillae preferred lower fat mixtures, while those with a lower density of papillae were not influenced by the level of fat in the mixtures in reporting their liking. A study by Drewnowski, et al., however, failed to show taster group differences in hedonic ratings of varying concentrations of sweet-fat mixtures [62]. Obviously more research needs to be performed in this area to resolve these conflicting findings, however, there seems to be a different eating pattern between tasters and NT for fat, which may be relevant with regard to the current obesity concerns in the U.S [68].

1.4 Dietary Restraint

While many factors influence eating behaviors, including availability, cost, taste preferences, and environment, cognitive factors can greatly influence eating patterns and have, as such, received a good deal of research attention. Two of the main outside cues influencing dietary intake are dietary restraint and disinhibition. Dietary restraint is the intent to restrict food intake in order to lose weight or for other health reasons [69, 70]. Disinhibition is eating in response to emotional cues, rather than because one is hungry [71-75]. These eating behaviors can be measured by questionnaire; the ones most
commonly used are the Dutch Eating Behavior Questionnaire (DEBQ) and the Three-Factor Eating Questionnaire. It should be noted, however, that research indicates these questionnaires do not measure actual food restriction, but rather the strength of the subjects’ intent to restrict [76]. Dietary restraint has been shown to influence diet reporting, as well, in that women and men with high restraint scores were more likely to underreport food intake [77]. These two eating behaviors can clearly influence food choices and the amount of food consumed, as well as other nutrient intakes and must be taken into account when researching eating behaviors. These scores must also be accounted for when using self-reported dietary intake data, as emotional eating behaviors influence not just intake, but also reported intake.

1.5 PROP Taster Status and Antioxidants in Blood Plasma

A similar study to the one proposed here was conducted in 2004 using undergraduate women (ages 18-21) as subjects. The study showed differences between NT and tasters, such that NT had significantly higher levels of \( \alpha \)-tocopherol in their blood plasma than tasters [78]. This finding was interpreted to mean that NT were eating more discretionary fats, such as salad dressings and oils, and more dark green, leafy vegetables. These findings confirmed research by Tepper and Nurse, who showed that NT have an increased preference for high fat foods, as well other research showing decreased acceptance and preference of dark green, leafy vegetables by PROP tasters [64]. No other differences in the major antioxidants (Vitamin A, C, lycopene, and uric acid) were found. One explanation for finding no difference, was the lack of variation in the diet of the women, due to the fact their food choices were limited to mostly what was available...
at the University dining halls, rather than having the option to shop for and select foods on their own. Thus, the current study seeks to relate specific dietary intake data to plasma levels of α-tocopherol.

1.6 Vitamin E

1.6.1 Vitamin E Function

There are eight naturally occurring forms of Vitamin E (α-, β-, γ-, and δ-tocopherol and (α-, β-, γ-, and δ-tocotrienol), all with similar antioxidant properties, however only the α-tocopherol form is biologically active [79]. The structures of Vitamin E can be seen in Figure 1 [80]. Of all the eight stereoisomeric forms (stemming from the chirality of the 2, 4’, and 8’ positions) of α-tocopherol, only RRR-α-tocopherol and the three RR isomers are maintained in the plasma and are the only forms of α-tocopherol considered to be “active” forms of the compound [81]. The RRR form is the only isomer found naturally in foods, while the 2R stereoisomers are synthesized and added to Vitamin E fortified foods and dietary supplements. The synthesized forms of α-tocopherol are about half as active as natural α-tocopherol in the body [12].

Upon entering the intestine post-ingestion, Vitamin E is taken up by chylomicrons in the lumen and is then distributed throughout the body, including the liver (Figure 2) [12]. Once in the liver, RRR and 2R forms bind to a transfer protein (α-TTP), which is then able to transfer the α-tocopherol between liposomes and membranes [82]. Excess Vitamin E is excreted in bile or in urine (at very high intakes) as α-2,5,7,8-tetramethyl-2(2’-carboxyethyl)-6-hydroxychroman (α-CEHC) [83, 84]. Only the RRR and 2R forms of α-tocopherol are biologically active, as the other forms of tocopherol are unable to
bind or bind with very low efficiency to $\alpha$-TTP. While the RRR form of $\alpha$-tocopherol binds to $\alpha$-TTP with 100% efficiency, the other forms of Vitamin E bind with various, much lower frequency; for example $\beta$-tocopherol at 38% and $\gamma$-tocopherol at 9% [85].

Hosomi, et al., further showed that the methyl group at position five and the hydroxyl group located on the chromanol ring are the most important groups, necessary for $\alpha$-tocopherol to bind to the transfer protein. While it is known that $\alpha$-TTP is necessary for the secretion of tocopherol from the liver cells, it is currently unknown how this occurs [86]. It is known that $\alpha$-tocopherol becomes associated with very low density lipoproteins (VLDLs) once secreted from the liver, and the metabolism and transport of cholesterol appear to be linked to $\alpha$-TTP transport, but it is unclear how this association occurs and how $\alpha$-TTP is involved [79, 87]. Once Vitamin E is oxidized in the cell, it can be reduced by various reducing agents, one of the main ones being Vitamin C in order to be reused in the body [88, 89]. The oxidized forms are excreted from the body if they are not regenerated. Vitamin E intake is often reported in the form of $\alpha$-tocopherol equivalents, which calculates the activity of the other forms of Vitamin E based on the activity of $\alpha$-tocopherol equaling one. The equation to calculate $\alpha$-tocopherol equivalents is:

$$\text{mg } \alpha\text{-tocopherol equivalents} = \text{mg total } \alpha\text{-tocopherol} + (0.45 \times \text{mg } \beta\text{-tocopherol}) + (0.1 \times \text{mg } \gamma\text{-tocopherol}) + (0.01 \times \text{mg } \delta\text{-tocopherol})$$

The known function of Vitamin E is that of a lipid-soluble antioxidant, scavenging and preventing the propagation of free radicals in the lipid-based parts of the cell, including the cell membrane (phospholipids) and blood plasma (lipoproteins) [90]. This antioxidant function stabilized cell membranes as well as prevents the oxidation of other
important biological compounds, such as hormones and enzymes [5]. In addition to its function as an antioxidant, Vitamin E inhibits protein kinase C activity, known to play a role in cell proliferation, thereby inhibiting smooth muscle cell proliferation [91]. This inhibition may have an important role in preventing many forms of heart disease, including atherosclerosis [92]. Furthermore, Vitamin E has been shown to prevent platelet aggregation, again an important part of cardiovascular disease prevention [93]. Vitamin E has also been shown to be vital to fetal development in rats, as fetal rats show Vitamin E deficiency when the mothers are fed diets low in Vitamin E [94, 95]. Vitamin E is vital to neurological function as well [96].

1.6.2 Vitamin E and Disease

Many studies have used supplementation with Vitamin E to try to relate \(\alpha\)-tocopherol with a decreased risk for heart disease or death from cardiovascular-related incidents, due to the research showing \(\alpha\)-tocopherol may have a protective effect against cardiovascular disease (CVD) and prevents platelet adhesion and aggregation [97, 98]. The CHAOS study in England showed \(\alpha\)-tocopherol levels in serum significantly increased in the group supplemented with Vitamin E, while there was no increase in the control group given a placebo. The supplemented group showed a 47% reduction in death due to CVD and a 77% reduction in non-fatal heart attacks [99]. The SPACE study in Israel yielded similar results, in that the supplemented group showed a decreased risk for various heart-related illnesses and a significant reduction in heart attack rates [100]. However, the HOPE study showed no significant difference in death from CVD, stroke or heart attack, between supplemented and placebo groups [101]. A study measuring
artery wall thickness as an indicator of future heart disease showed no difference in thickness after Vitamin E supplementation when compared to a placebo group [102]. A study looking at cancer and heart disease prevention in women using Vitamin E supplementation also showed no significant difference in risk reduction or heart attack between placebo and supplemented groups [103]. The authors of a paper reviewing the research on $\alpha$-tocopherol supplementation and CVD believe conflicting data may be due to the mixtures of $\alpha$-tocopherol used in the supplemented groups, noting that trials using only RRR-$\alpha$-tocopherol yielded positive results for reduced risk of CVD [104]. They also state that perhaps there is a threshold dose of Vitamin E, above which is needed to be effective and when subjects are supplemented at a lower level, there is no effect.

A few studies have looked at dietary intake of Vitamin E (from food, dietary supplements, or both) and how it relates to risk for CVD and other diseases, with mixed results. Two studies by Kushi, et al., tried to relate antioxidant vitamins to the risk for heart disease and stroke in women [105, 106]. In both studies, the relative risk for death from each disease was significantly lowest among those subjects in the highest quintile of Vitamin E intake (measured by a FFQ) from food only ($p = 0.008$ for heart disease and $p = 0.006$ for stroke). When Vitamin E supplement use was examined separately and in combination with Vitamin E from food, this association was not statistically significant. It should be noted that the researchers did not collect information on length of Vitamin E supplement use, only whether or not supplements were used, which may have affected the relative risk outcomes. An earlier study in women by Stampfer, et al., showed a significantly reduced risk for coronary disease among those taking dietary Vitamin E supplements (relative risk = 0.57 versus those not taking supplements) or multivitamins.
containing Vitamin E (relative risk = 0.78), but only among women who had taken these supplements for two years or more [107]. There was no reduced risk for subjects whose sole source of Vitamin E was diet, not supplements. A third study, quantifying fruit, vegetable, and overall antioxidant intake (FFQ) with mortality risk from all-cause, cancer, and CVD, showed no association between Vitamin E intake from food alone or from food and supplements, although increased fruit and vegetable intake overall was associated with reduced mortality risk and cancer mortality risk [108]. A study looking at plasma \( \alpha \)-tocopherol and mortality from ischemic heart disease (IHD) showed increased plasma \( \alpha \)-tocopherol was associated with reduced risk of death from IHD (\( r^2 = 0.63 \) [109]. It should be noted, however, that this study looked only at plasma \( \alpha \)-tocopherol and no information about dietary intake was collected. Among a group of male smokers studied in Finland, Vitamin E (and other antioxidant vitamins) intake did not alter the risk for coronary death [110].

While Vitamin E acts as an important lipid antioxidant and has been shown to reduce the risk for CVD, its relationship to several diseases and their prevention has been questionable. In a study using meta-analysis to review 68 trials (n=232,606) looking at antioxidant supplementation and all-cause mortality, Vitamin E supplementation was shown to possibly increase mortality [111]. This study also showed beta carotene and Vitamin A supplementation may increase mortality. Research looking at the influence of Vitamin E supplementation on cancer has also been negative. One study comparing Vitamin E supplemented groups to a control group showed no decrease in the incidence of total cancer or cancer deaths between groups [103]. A number of studies showed no relationship between Vitamin E supplementation and reduced cancer risk [112-114].
1.6.3 Dietary Intake of Vitamin E

1.6.3.1 Recommended Intake and Sources

The RDA for Vitamin E for adult women (ages 19-50 years) is 15 mg/day and the EAR is 12 mg/day. These values were set based on studies measuring the plasma $\alpha$-tocopherol concentration that limited hydrogen peroxide-induced hemolysis to 12% or less [12]. The upper limit of Vitamin E intake for adults is 1000 mg/day, based on several animal studies which showed hemorrhagic effects at very high intakes (500 mg/kg/day), as well as a study in humans in which the risk of death from hemorrhagic stroke increased by 50 percent in male smokers consuming 50 mg/day RRR-$\alpha$-tocopherol over six years [12]. Additional studies have shown a wide range of adverse effects from excessive $\alpha$-tocopherol supplementation, including fatigue, emotional disturbances, inflammation of veins (thrombophlebitis), breast soreness, cretinurea, changes in serum lipids and lipoproteins, gastrointestinal disturbances, and thyroid effects, however these studies were uncontrolled and not replicated, and clinical trials to assess these effects are necessary [12]. The RDA for Vitamin E was raised to 15 mg/day in the 2000 DRI, from 7 mg/day for women, based on research indicating that blood serum concentrations of 30-33 $\mu$mol/L were necessary for optimal disease-risk reduction [115]. Further research showed an intake of 12 mg/day of Vitamin E maintained serum $\alpha$-tocopherol between 25.8-28 $\mu$mol/L, as such, the EAR was set at 12 mg/day and the RDA was raised two standard deviations from that EAR to 15 mg/day [116].

Several studies have argued the RDA is impossible to consume based on the current U.S. population’s dietary patterns [117]. Indeed, current findings suggest that few women in the U.S. population reach this recommended intake on a daily basis, with a
mean intake of 6.1 - 7 mg/day from diet alone (not dietary supplements), well below the recommended intake and research has shown that the population as a whole is limited in their Vitamin E intake [118-120]. One study using linear modeling to create a diet for maximal \( \alpha \)-tocopherol intake, showed the best diet for \( \alpha \)-tocopherol intake was 11 servings of fruit and vegetables, plus 0.6 servings of nuts, per day [117]. This is clearly not an attainable goal, based on previous diet research from the NHANES III study, which showed vegetables contributed only 7.3% of total energy intake (fifth on the list of food groups), while fruit was not in the top ten food groups contributing to intake [121].

The main dietary sources of Vitamin E (contributing more than 1 mg/serving) are dark green vegetables (kale, cabbage, collard greens), nuts (including peanut butter), oils (from cooking, salad dressing, etc.), and pizza, which often contains vegetable oils [117]. In addition, foods such as nuts, meat, fruit, and processed foods fortified with Vitamin E (the RR forms of \( \alpha \)-tocopherol), such as breakfast cereals and prepackaged entrees, are major contributors to Vitamin E intake [120].

**1.6.3.2 Vitamin E Dietary Intake and Blood Concentration Correlations**

Despite this low intake, studies have shown a range in the level of \( \alpha \)-tocopherol in blood plasma to be between 9.7 and 34.4 \( \mu \)M (mean 21.6 ± 0.2 \( \mu \)M) for women ages 19-30 years and between 13.7 and 52.8 \( \mu \)M (mean 24.9 ± 0.2 \( \mu \)M) for women ages 31 – 50 years [12]. Many studies have aimed to relate dietary intake of Vitamin E to blood \( \alpha \)-tocopherol levels with mixed results. In a study comparing weighed food records (WFR) and FFQ reported antioxidant intake to serum levels of the same antioxidants, Vitamin E serum levels showed poor correlations with both the FFQ and WFR values of Vitamin E.
intake from food alone. FFQs, in which participants indicate how often they eat a variety of foods and the approximate amount eaten, are used to determine long-term food intake patterns, while WFR, in which participants weigh all food before eating and record the amount of food eaten, are used to look at short-term, “snapshots” of dietary intake. Only when intake from supplements was added to the analysis (mean FFQ intake = 37.69 ± 119.84 mg/day; mean WFR intake = 51.44 ± 91.92 mg/day), did the serum levels (mean = 34.8 ± 11.4 μmol/L) correlate with FFQ Vitamin E intake, but this relationship was not shown with the WFR [122]. The authors attributed this to the fact that the records measured α-tocopherol-equivalents (i.e., including β-tocopherol, γ-tocopherol, and δ-tocopherol, as well) while the serum measurements were only for α-tocopherol and also to the poor diet-serum relationship for Vitamin E. Previous research had shown that increased Vitamin E intake through supplements did raise serum α-tocopherol levels, however the intakes reported in these studies are not possible to achieve through diet alone [123]. A second study in 2001 also showed no correlation between high α-tocopherol intake and plasma α-tocopherol measures. In this study, subjects were instructed to eat low-antioxidant diets for two weeks, which was then followed by a two week period of either antioxidant supplementation or dietary increase of antioxidant-containing foods. While there was an increase in plasma ascorbic acid, α- and β-carotene and lutein plus zeaxanthin following both diet increases and supplementation, α-tocopherol levels in the plasma remained unchanged (mean plasma concentrations before and after supplementation = 28.8 μmol/L) and [124]. Data from the NHANES III study also showed no association between increased dietary intake of α-tocopherol (mean = 8.8
± 0.3 mg/day) and serum levels (992 ± 9 μg/dL), despite showing positive associations for Vitamin C and β-carotene [125].

Other studies have, however, showed correlations between Vitamin E intake from food and blood concentrations of α-tocopherol, using a FFQ to quantify intake. A study in African-American adults showed a strong positive correlation ($r^2 = 0.50$, 0.058, and 0.51; $p < 0.001$) between serum α-tocopherol (mean non-supplemented = 25.10 ± 0.80; mean supplemented = 39.75 ± 1.37) and dietary intake (recorded via 24-hour recalls (mean intake = 73.2 ± 8.3 mg/day (supplemented); mean intake = 6.58 ± 0.19 mg/day (non-supplemented)), a short FFQ (mean intake = 66.7 ± 9.58 mg/day (supplemented); mean intake = 6.86 ± 0.14 mg/day (non-supplemented)), and a long FFQ (mean intake = 82.4 ± 7.29 mg/day (supplemented); mean intake = 6.94 ± 0.13 mg/day (non-supplemented))) of α-tocopherol [126]. However, when the researchers controlled for supplement intake, no associations were found except among those subjects in the highest quartile of intake. A study looking at dietary intake of α-tocopherol from a FFQ and the relationship to plasma levels of α-tocopherol showed a significant difference among α-tocopherol intake tertiles, such that those in the highest tertile for intake also had the highest plasma concentrations of α-tocopherol [127]. This difference was seen both in all subjects ($p = 0.003$; average total intake = 9.9 mg/day, average plasma concentration = 25.8 μmol/L) and in subjects that did not take dietary Vitamin E supplements, as well ($p = 0.008$; average total intake = 5.4 mg/day, average plasma concentration = 22.0 μmol/L). This research further broke down the sources of α-tocopherol to each groups’ diet, showing Hispanics were most likely to get α-tocopherol predominantly from oils, followed by milk and milk products, fruit, beans, and fish. Alternatively, the non-
Hispanic population consumed \( \alpha \)-tocopherol mostly from ready-to-eat breakfast cereals, followed by oils/salad dressings and fruit. Those subjects in the cereal and fruit group were more likely to not have high \( \alpha \)-tocopherol levels in their plasma, which they attributed to the fortified cereal intake. In addition, subjects consuming milk were likely to not have low \( \alpha \)-tocopherol levels in plasma, either, perhaps due to increased bioavailability due to its dispersal in milk, as the fat in milk and butter has been shown to increase the absorption of Vitamin E by the body [128, 129]. While studies have produced mixed results when correlating blood plasma and serum levels of \( \alpha \)-tocopherol with dietary intake, it has been possible to show an association in some studies. No studies have been performed in younger, non-supplemented women, thus this study aims to correlate blood concentrations and dietary intake of \( \alpha \)-tocopherol.

1.6.3.3 Vitamin E Dietary Intake and \( \alpha \)-CEHC Excretion

Recently, studies have researched the excretion of a novel \( \alpha \)-tocopherol metabolite, \( \alpha \)-CEHC in urine, as a potential biomarker for adequate \( \alpha \)-tocopherol intake. These studies have shown \( \alpha \)-CEHC to be a product of \( \omega \)- and \( \beta \)-oxidation of the phytol tail, leaving the chromanyl ring intact, indicating this metabolite has not been used for its free radical-scavenging purpose [83]. One study in seven healthy, male volunteers showed no increase in \( \alpha \)-CEHC excretion at supplementation levels below 50 mg/day (RRR-\( \alpha \)-tocopherol), although plasma increase did occur [84]. Excretion of \( \alpha \)-CEHC began between supplementation levels of 50 – 150 mg/day, and the authors calculated the value to be around 90 mg/day, and increased with increasing supplementation, without leveling off. Plasma \( \alpha \)-tocopherol increased the most between supplementation levels of 150 mg
– 350 mg/day, and supplementation at higher levels did not significantly increase plasma concentrations. Excretion of α-CEHC began when plasma levels reached a range of 30 – 50 μmol/L. This research was continued five years later, comparing these same healthy volunteers to three ataxia with isolated Vitamin E deficiency (AVED) patients, who have a defect in α-TTP [130]. The AVED patients were shown to excrete α-CEHC, even at very low plasma concentrations, and had very low plasma concentrations of α-tocopherol unless they were supplemented at levels between 1000-2500 mg/day [83]. This suggested to the authors that saturation of α-TTP in the liver, not plasma α-tocopherol concentration, determines when α-CECH is formed and excreted in the urine. As such, α-CEHC excretion could indicate adequate Vitamin E intake, as α-TTP would be saturated and plasma levels would not be able to be increased.

1.7 Summary

Two key dietary patterns have been shown among PROP taster groups; (1) PROP tasters prefer non-bitter foods, such as dark green, leafy vegetables, and may consume less of these types of foods, while this relationship is not seen among NT, and (2) NT consume more discretionary fat and prefer higher fat foods than ST. These two food groups (fats and leafy vegetables) are the main sources of α-tocopherol to the U.S. diet, thus it follows that NT may consume more α-tocopherol and, therefore, have higher levels circulating in their blood. This could have health implications, as α-tocopherol is a necessary compound for membrane stability and a key antioxidant shown to be important in CVD prevention. This research aims to link these two previously shown eating patterns to specific dietary intake and plasma α-tocopherol concentrations.
CHAPTER 2: OBJECTIVES

The objective of this study was to confirm and expand the research first performed by Tepper, et al. from 2004, specifically to examine the relationship between dietary intake and plasma levels of α-tocopherol, and to relate previously shown dietary patterns of PROP taster groups to the levels of α-tocopherol in blood plasma in healthy, young women [78]. This research pursued three objectives in order to measure and relate α-tocopherol to these dietary variables:

Objective 1: To determine whether PROP taster status influences dietary intake of foods high in α-tocopherol in women.

_Hypothesis:_ PROP non-tasters will have higher intakes of foods rich in α-tocopherol, such as dark green, leafy vegetables and added fats and oils.

Objective 2: To determine the α-tocopherol levels in blood plasma in women and relate levels to PROP taster status.

_Hypothesis:_ Non-tasters will have higher levels of α-tocopherol in their blood plasma, due to increased intake of α-tocopherol-containing foods.

Objective 3: To relate previously shown dietary patterns to the levels of α-tocopherol in blood plasma and determine the relationship to PROP taster status.

_Hypothesis:_ Previously reported dietary patterns by taster status will be seen in this population and will correlate with plasma α-tocopherol levels.
CHAPTER 3: METHODS

3.1 Subjects

Female subjects were recruited via email, newspaper ads, and in-person screenings, conducted both at Rutgers University and the surrounding area. Subjects were pre-screened before acceptance into the study to ensure they were not vegetarian, did not have any illnesses, and were not taking medications that would affect taste. In addition, subjects were screened to ensure they were within the weight and age requirements of the study. The study aimed to focus on young women within normal weight ranges for the population. Subjects were also screened to ensure they were not taking any dietary supplements, as the study focused on only antioxidant intake from foods. Subjects were also given the PROP test at the time of the pre-screening. Once accepted into the study, subjects gave their informed consent based on protocols approved by the Rutgers University Institutional Review Board for human subjects. Upon completion of the study, the subjects were compensated with $40 by check. A copy of the consent form and screening questionnaires can be found in Appendix I and II.

3.2 Classification of PROP Taster Status

Subjects were classified as non-taster (NT), medium taster (MT) or super-taster (ST) according to the paper disk method, first developed by Zhao, et al [131]. Subjects used a labeled magnitude scale (LMS) to rate the intensity of two paper disks, one impregnated with sodium chloride and one with PROP. The LMS used is a semi-logarithmic, 100 mm line scale, anchored along its length with the descriptors “barely
detectable” at 2 mm, “weak” at 7 mm, “moderate” at 17 mm “strong” at 36 mm, “very strong” at 53 mm, and “strongest imaginable” at 100 mm. The NaCl disk is impregnated with a 1.0 mol/L NaCl solution, and is used as a standard, as NaCl ratings do not vary as a function of PROP taster status [131]. Relative to PROP, NT give higher intensity ratings to NaCl, MT give about equal ratings, while ST give lower ratings. Then subjects rate the second disk impregnated with 50 mmol/L PROP and rate using a second LMS scale. Subjects are instructed to rinse with spring water before and after tasting each paper disk. Subjects are then classified as NT, MT, or ST based on their PROP ratings, such that ratings less than 17 mm are NT, ratings between 18 and 68 mm are MT, while those rating PROP above 69 mm are ST. The LMS ballot can be found in Appendix I.

3.3 Dietary Intake

Three, 24-hour recalls were taken from each subject, from one weekend day and two weekdays. Diet recalls were used, as opposed to a food frequency questionnaire, in order to obtain a quantitative measure of food intake. While a FFQ provides a good picture of long-term intake, it is difficult to quantify the exact amount of food consumed over time. Dietary recalls, on the other hand, provide specific information about the foods consumed, as well as the exact amount (based on the subject’s recollection) eaten. The three recalls were averaged together in order to obtain an estimate of intake over several days. If the subject stated their intake was not normal for any reason, the recall was rescheduled for a different day in order to obtain an accurate record of food intake over the three days.
The Nutrient Data System for Research (NDS-R), Nutrition Coordinating Center, University of Minnesota, Minneapolis, MN, multiple-pass approach was used to collect diet information. The multiple-pass approach separated the recall into three stages. In the first stage, the subject listed everything eaten during the previous twenty-four hours and the time at which each food was eaten, ignoring portion sizes and specific food information. During the second stage, each food was considered individually, including portion size, cooking method, and specific brand names, if applicable. During this stage, a “Food Amounts Booklet” was used to help the subject accurately describe the size and/or amount of food eaten during each meal. During the third stage, the interviewer reported the list back to the subject to ensure all information was recorded correctly and no foods were left out. While many data output options were available, only the ones important to the current study were used, including total calories, macronutrients and vitamins, and food group intakes. The first recall was done in-person, and the next two were as well, where possible. If time constraints did not allow the second two recalls to be done in-person, phone recalls were conducted at pre-arranged times.

3.4 Food Groups and Nutrients

The food intake output from NDS-R was combined into groups of interest for the study, based on the USDA food groups. While NDS-R automatically generates a list of 160 different food groups with serving sizes based on standard USDA reference amounts, these groups were combined to provide a better overview of overall food group intake patterns. These 160 groups were reduced to 30 groups, including citrus fruit and juice, non-citrus fruit and juice, dark green vegetables, other vegetables, and added fats/oils. A
complete list of foods can be seen in Table 2. Among foods that contributed to α-tocopherol intake, 13 groups were formed to analyze their contribution to overall α-tocopherol intake, regardless of portion size. Food groups were based on the USDA groups, however an “other” group was created to encompass all foods that contributed a minimal amount of α-tocopherol to the diet. The complete list of these food groups and the individual foods that make up each group can be seen in Table 3.

Table 2: List and description of food groups and (standard serving sizes) used for analysis of nutrient intake.

<table>
<thead>
<tr>
<th>USDA Main Groups</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Citrus Fruit</td>
<td>Citrus fruit and citrus juice (½ cup, 1 M piece, 4 fluid oz.)</td>
</tr>
<tr>
<td>Fruit</td>
<td>All non-citrus fruit and fruit juice (½ cup, 1 M piece, 4 fluid oz.)</td>
</tr>
<tr>
<td>Dark Green Vegetables</td>
<td>Dark green, leafy vegetables, broccoli, asparagus, artichokes (½ cup chopped, 1 cup leafy)</td>
</tr>
<tr>
<td>Yellow Vegetables</td>
<td>Sweet potatoes, pumpkin, yellow squash (½ cup)</td>
</tr>
<tr>
<td>Tomatoes</td>
<td>Tomatoes and tomato-based products (½ cup)</td>
</tr>
<tr>
<td>Starchy Vegetables</td>
<td>Potatoes (including fried), peas, corn (½ cup)</td>
</tr>
<tr>
<td>Whole Grains</td>
<td>Whole grain pasta, rice, breads, crackers (½ cup, 28 g bread slice, 1 oz crackers)</td>
</tr>
<tr>
<td>Grains</td>
<td>Refined or some whole grain pasta, rice, breads, crackers, muffins (½ cup, 28 g bread slice, 1 oz crackers)</td>
</tr>
<tr>
<td>Unsweetened Ready-to-Eat Cereals</td>
<td>Unsweetened Ready-to-Eat Cereals (1 oz)</td>
</tr>
<tr>
<td>Sweetened Ready-to-Eat Cereals</td>
<td>Sweetened Ready-to-Eat Cereals (1 oz)</td>
</tr>
<tr>
<td>Grain - Desserts</td>
<td>Cakes, cookies, pies, cobblers, doughnuts, pastries (30 g cookie, 125 g cake/pastry/pie)</td>
</tr>
<tr>
<td>Snack Bars</td>
<td>Granola bars, cereal bars (40 g bar)</td>
</tr>
<tr>
<td>Salty Snacks</td>
<td>Popcorn, snack chips (1 oz)</td>
</tr>
<tr>
<td>Red Meat</td>
<td>Regular and lean beef, veal, lamb, pork, game, cold cuts (1 oz)</td>
</tr>
<tr>
<td>Poultry</td>
<td>Poultry, lean poultry, fried chicken (1 oz)</td>
</tr>
<tr>
<td>Seafood</td>
<td>Fish, shellfish, fried fish and shellfish (1 oz)</td>
</tr>
<tr>
<td>Unsweetened Dairy</td>
<td>Milk, unsweetened yogurt, cheese (1 cup milk/yogurt, ½ fluid oz.)</td>
</tr>
</tbody>
</table>
Table 3: List and description of food groups and used for analysis of \( \alpha \)-tocopherol intake.

<table>
<thead>
<tr>
<th>USDA Main Groups</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Dark Green Vegetables</td>
<td>Spinach, broccoli, green beans, cabbage, kale, green beans, artichokes, lettuce, other dark, leafy vegetables</td>
</tr>
<tr>
<td>Other Vegetables</td>
<td>Onions, peppers, yellow vegetables, all other non-starchy and non-dark, leafy vegetables, tomatoes</td>
</tr>
<tr>
<td>Nuts</td>
<td>Nuts, nut butters, sesame seeds</td>
</tr>
<tr>
<td>Fruit</td>
<td>Fruit and fruit juices, citrus and citrus juices</td>
</tr>
<tr>
<td>Grains</td>
<td>Breads, rice, pasta, tortillas</td>
</tr>
<tr>
<td>Ready-to-Eat Cereals</td>
<td>All ready-to-eat breakfast cereals, oatmeal</td>
</tr>
<tr>
<td>Oils</td>
<td>Cooking oils, margarine, butter, salad dressings, olives, avocado, French fries</td>
</tr>
</tbody>
</table>
Dairy: Milk, cheese, yogurt, sweet dairy, dairy-based beverages (latte, cappuccino), sour cream, cream-based soups

Meats: All animal products, eggs, tofu, soy milk

Meal Replacements: Energy bars, breakfast drinks and bars,

Mixed Dishes: Pizza, stews, frozen dinners, soups, sandwiches, stir-fry, sushi

All Snacks and Desserts: Cakes, cookies, pies, crackers, muffins, granola bars, cereal bars, popcorn, pretzels, tortilla chips, potato chips

Other: Spices, coffee, starchy vegetables, candy (chocolate and non-chocolate)

The nutrient file output from NDS-R was used to determine overall calorie intake, percent fat, carbohydrate, and protein intake, and total α-tocopherol intake in mg per 24-hour period. Total α-tocopherol includes α-tocopherol from plant sources (RRR form), as well as the two-R forms, synthesized in industry and added to Vitamin E fortified foods.

3.5 Dietary Restraint

The subjects completed the Dutch Eating Behavior Questionnaire (DEBQ), included in Appendix I, in order to determine dietary restraint. The DEBQ is a 33-question questionnaire that measures restraint, emotional eating (divided into clearly labeled emotions and diffuse emotions) and external eating. The questionnaire uses a 5-point scale where 1 = never, 2 = rarely, 3 = sometimes, 4 = often and 5 = very often. The subjects are then given an individual score for each eating behavior, based on the ratings given. Subjects were divided into two groups; “restrained eaters” and “non-restrained eaters,” based on a median split of the restraint scores.
3.6 Blood Plasma Measures

Subjects were scheduled to give a blood sample in the morning, after an overnight fast. Twenty ml blood samples were extracted by a trained phlebotomist from an antecubital vein into vacutainers EDTA for plasma analysis. Blood samples were kept on ice for no longer than one half hour, then centrifuged for 15 minutes at 3200 rpm. The plasma was separated from the samples and transferred to freezer tubes and stored at -70C until α-tocopherol analysis could be performed. Samples were analyzed for α-tocopherol based on the methods described in Stedman, et al., described briefly, as follows [132]. A portion of each sample was mixed with an equal volume of ethanol, which also contained butylated hydroxyl anisole and an internal standard of γ-tocopherol. This mixture was vortexed, hexane was added, then vortexed again. The phases were separated through centrifugation and the organic layer was transferred to a second tube. The aqueous layer was re-extracted with hexane and the organic layer was added to the second tube. The contents were then concentrated under nitrogen and re-suspended in ethanol. Twenty μl was injected onto a HPLC column for Vitamin E analysis. The concentrations were quantified by coulometric electrochemical detection using a CoulArray detector (ESA, Chelmsford, MA).
3.7 Study Design

**Pre-screening**
- General Screening Questionnaire
- PROP Taste Test
- Body Weight and Height Measurement

**Study Day 1**
- Consent Forms
- Cheek swab for genetic analysis
- Diet recall 1
- Saliva sample
- DEBQ

**Study Day 2**
- Diet recall 2 (in-person or via phone)

**Study Day 3**
- Diet recall 3 (in-person or via phone)

**Study Day 4**
- Overnight fast and blood sample collection

3.8 Statistical Analysis

Statistical analysis was performed using Statistical Analysis Software (SAS) versions 9.1 and 9.2 (SAS Institute, Cary, NC). PROP and NaCl ratings were analyzed using analysis of variance (ANOVA) for differences among PROP taster groups. Dietary intake, nutrient intake, and blood plasma α-tocopherol levels were also analyzed using ANOVA, using PROP taster status (NT, MT, and ST) and dietary restraint (restrained or non-restrained), and their interaction as factors. Analysis of covariance (ANCOVA) was also used to remove the influence of energy intake and body weight on both dietary and nutrient intake and plasma α-tocopherol levels. Correlations among dietary intake and nutrient intake were examined to determine the foods contributing to α-tocopherol intake. Correlations were also performed to associate dietary intake and nutrient intake of α-tocopherol to the plasma levels of α-tocopherol. Logistic regression analysis with forward, stepwise selection, was used to predict the odds of having “higher” intakes of α-
tocopherol, as well as “higher” plasma concentrations of α-tocopherol. Multiple linear regression was used to analyze the factors that predicted “higher” or “lower” concentrations of α-tocopherol in blood plasma.
CHAPTER 4: RESULTS

4.1 PROP Taster Status

Ninety-three women between the ages of 21 and 44 participated in the study. Subjects were classified as NT, MT, and ST according to the paper disk method [131]. Thirty were NT (32%), 33 were MT (36%), and 30 were ST (32%). Care was taken during the pre-screening process to obtain equal numbers of NT, MT, and ST, in the subject population. ST had significantly higher NaCl ratings than MT, while NT were not different from either (p < 0.05, F = 3.24, df = 2). ST had the highest PROP ratings, followed by MT, with NT having the lowest ratings (p < 0.0001 F = 351.17, df = 2). These differences can be seen in Figure 3.

4.2 Demographics

The subjects were primarily Caucasian, with Asian (both East (Chinese, Japanese, etc.) and South (Indian, etc.)) and other (predominantly African-American and Hispanic) making up roughly one-half the study population. All subjects had completed or were currently completing a college degree and a large percent had completed a post-graduate degree. The subjects ranged in age from 21 to 44 years, with an average age of 25.8 ± 0.5 years. The body mass index (BMI) of the subjects ranged from normal (18 kg/m^2) to overweight (30 kg/m^2), with an average BMI of 23.8 ± 0.4 kg/m^2. See Table 4 for overall demographic information.
Table 4: Demographics of study participants.

<table>
<thead>
<tr>
<th>Demographic</th>
<th>Percent</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Ethnicity</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caucasian</td>
<td>49%</td>
<td>46</td>
</tr>
<tr>
<td>Asian</td>
<td>25%</td>
<td>23</td>
</tr>
<tr>
<td>Other</td>
<td>26%</td>
<td>24</td>
</tr>
<tr>
<td><strong>Education</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Some College</td>
<td>36%</td>
<td>33</td>
</tr>
<tr>
<td>College Graduate</td>
<td>34%</td>
<td>32</td>
</tr>
<tr>
<td>Post-Graduate Degree</td>
<td>30%</td>
<td>28</td>
</tr>
<tr>
<td><strong>Taster Group</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NT</td>
<td>32%</td>
<td>30</td>
</tr>
<tr>
<td>MT</td>
<td>36%</td>
<td>33</td>
</tr>
<tr>
<td>ST</td>
<td>32%</td>
<td>30</td>
</tr>
</tbody>
</table>

### 4.3 Dietary Restraint

Eating behaviors were measured using the DEBQ during the first session of the study. Dietary restraint scores ranged from 1.40 – 4.20, with a median of 2.65. Disinhibition scores ranged from 1.92 – 5.33, with a median score of 2.88. Disinhibition was not deemed to be important for the analysis of intake data and was not used in any statistical analysis. There were no differences in dietary restraint or disinhibition scores across PROP taster group. See Table 5 for dietary restraint and disinhibition scores.

### 4.4 Dietary Intake

#### 4.4.1 Total Energy and Nutrients

To account for any underreporting of food intake, subjects reporting less than their calculated Basal Metabolic Rate (BMR), as calculated using the Harris-Benedict Equation, were removed from the analysis. As a result, one subject was removed from the analysis for a final n = 93. Of the 93 subjects, 88 subjects completed all three diet recalls, three subjects completed two recalls, and two subjects completed one recall. The
diet recalls were averaged together to obtain an overall dietary intake picture for each subject. There were no significant differences in caloric intake or percent macronutrients by PROP taster status alone, when the interaction of PROP taster status and dietary restraint was considered, or when body weight was used as a controlling variable (ANCOVA). NT consumed an average of 1820 kcal/day, MT: 1920 kcal/day, and ST: 1846 kcal/day. The caloric intakes for all subjects ranged from 1170 – 2926 kcal/day and the mean overall intake was 1846 kcal/day. There were also no differences in macronutrient intake among PROP taster groups, when the interaction of PROP taster status and dietary restraint was considered, or when body weight was used as a controlling variable (ANCOVA). Overall nutrient intakes (30.0% fat, 52.3% carbohydrate, and 15.5% protein) were within the recommended ranges for this age group. Energy intake, macronutrient intake, BMI, and dietary restraint scores by PROP taster group can be seen in Table 5. Energy intake, macronutrient intake, BMI, and dietary restraint scores by PROP taster group can be seen in Table 5.

There were no differences in vitamin intake by PROP taster group (Table 5), however, due to the reported influence of dietary restraint on food intake, the interaction of dietary restraint and PROP taster status was examined. BMI, energy intake, dietary restraint score, and nutrient intakes by PROP taster group and dietary restraint group can be seen in Table 6. Among non-restrained eaters, NT consumed significantly more \( \alpha \) -tocopherol than either non-restrained MT or ST (p<0.05, F=3.50, df = 2). There were no differences among the restrained eaters. These differences can be seen in Figure 4.

Intakes of Vitamin A and Vitamin C were within recommended ranges, 700 retinol activity equivalents/day and 75 mg/day, respectively, while intakes of Vitamin E
were below recommended ranges (15 mg/day) [12]. Nutrient intakes by PROP taster group and dietary restraint group can be seen in Table 6.

Table 5: Weight status, dietary restraint, energy intake, and nutrient intake by PROP taster status. Mean ± SEM.

<table>
<thead>
<tr>
<th></th>
<th>Non-taster (n=30)</th>
<th>Medium Taster (n=33)</th>
<th>Super Taster (n=30)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>BMI (kg/m²)</strong></td>
<td>24.9 ± 0.8</td>
<td>23.5 ± 0.6</td>
<td>23.0 ± 0.6</td>
</tr>
<tr>
<td><strong>Energy Intake (kcal/day)</strong></td>
<td>1820.3 ± 78.5</td>
<td>1920.4 ± 72.8</td>
<td>1846.2 ± 42.1</td>
</tr>
<tr>
<td><strong>Dietary Restraint</strong></td>
<td>2.7 ± 0.1</td>
<td>2.6 ± 0.1</td>
<td>2.5 ± 0.1</td>
</tr>
<tr>
<td><strong>Disinhibition</strong></td>
<td>3.1 ± 0.1</td>
<td>3.1 ± 0.1</td>
<td>3.0 ± 0.1</td>
</tr>
<tr>
<td><strong>β-carotene (RAE/day)</strong></td>
<td>838.5 ± 83.6</td>
<td>719.3 ± 75.3</td>
<td>773.7 ± 96.1</td>
</tr>
<tr>
<td><strong>α-tocopherol (mg/day)</strong></td>
<td>9.5 ± 1.6</td>
<td>8.3 ± 1.0</td>
<td>8.1 ± 0.7</td>
</tr>
<tr>
<td><strong>Vitamin C (mg/day)</strong></td>
<td>109.0 ± 14.1</td>
<td>89.6 ± 9.0</td>
<td>106.1 ± 12.9</td>
</tr>
<tr>
<td><strong>% Fat</strong></td>
<td>28.4 ± 1.0</td>
<td>31.3 ± 0.8</td>
<td>30.3 ± 1.1</td>
</tr>
<tr>
<td><strong>% Carbohydrate</strong></td>
<td>52.6 ± 1.4</td>
<td>50.9 ± 1.0</td>
<td>53.5 ± 1.3</td>
</tr>
<tr>
<td><strong>% Protein</strong></td>
<td>16.2 ± 0.7</td>
<td>15.8 ± 0.7</td>
<td>14.4 ± 0.7</td>
</tr>
</tbody>
</table>

¹RAE = Retinol Activity Equivalents, where 1 RAE = 12 µg β-carotene, 24 µg α-carotene, or 24 µg β-cryptoxanthin.

Table 6: Weight status, dietary restraint, energy intake, and nutrient intake by PROP taster status and dietary restraint group. Mean ± SEM.

<table>
<thead>
<tr>
<th></th>
<th>Non-taster (n=30)</th>
<th>Medium Taster (n=33)</th>
<th>Super Taster (n=30)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>BMI</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Restrainted</td>
<td>24.5 ± 0.9</td>
<td>24.2 ± 0.7</td>
<td>23.7 ± 0.8</td>
</tr>
<tr>
<td>Non-Restrained</td>
<td>25.4 ± 1.4</td>
<td>22.7 ± 1.0</td>
<td>22.4 ± 0.9</td>
</tr>
<tr>
<td><strong>Energy Intake (kCals)</strong></td>
<td>1795.0 ± 110.4</td>
<td>1851.5 ± 90.3</td>
<td>1873.3 ± 97.4</td>
</tr>
<tr>
<td>Restrainted</td>
<td>1892.1 ± 95.8</td>
<td>1993.6 ± 123.7</td>
<td>1727.4 ± 91.7</td>
</tr>
<tr>
<td>Non-Restrained</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Dietary Restraint (DEBQ score)</strong></td>
<td>3.2 ± 0.1</td>
<td>3.0 ± 0.1</td>
<td>3.1 ± 0.1</td>
</tr>
<tr>
<td>Restrainted</td>
<td>2.2 ± 0.1</td>
<td>2.1 ± 0.1</td>
<td>2.1 ± 0.1</td>
</tr>
<tr>
<td><strong>β-Carotene RAE (mcg)</strong></td>
<td>916.5 ±130.9</td>
<td>758.5 ± 134.3</td>
<td>907.2 ± 177.6</td>
</tr>
<tr>
<td>Restrainted</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
In order to determine the food groups and other factors contributing to total \( \alpha \) -tocopherol intake, correlations were performed. The strongest correlations were between \( \alpha \) -tocopherol intake and dark green vegetable, nuts, yellow vegetable, non-citrus fruit and juice, and wholegrain intake. There were also modest, yet still significant correlations between \( \alpha \) -tocopherol intake and energy, citrus fruit and juice, and oil intake. These data can be seen in Table 7.

Table 7: Dietary \( \alpha \)-tocopherol intake correlations with food group and demographic variables.

<table>
<thead>
<tr>
<th>Intake Group</th>
<th>Pearson Correlation Coefficient</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy Intake (kcals)</td>
<td>0.27</td>
<td>0.01</td>
</tr>
<tr>
<td>Dark Green Vegetables</td>
<td>0.35</td>
<td>0.001</td>
</tr>
<tr>
<td>Yellow Vegetables</td>
<td>0.33</td>
<td>0.001</td>
</tr>
<tr>
<td>Citrus Fruit and Juice</td>
<td>0.22</td>
<td>0.04</td>
</tr>
<tr>
<td>Non-citrus Fruit and Juice</td>
<td>0.41</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Oil</td>
<td>0.20</td>
<td>0.05</td>
</tr>
<tr>
<td>Nuts</td>
<td>0.65</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Whole Grain</td>
<td>0.32</td>
<td>0.002</td>
</tr>
</tbody>
</table>
4.4.2 Food Groups

4.4.2.1 ANOVA

Food group intake was analyzed by PROP taster group to determine differences in dietary patterns by group. Differences were observed for the “reduced fat/non-fat dairy” and “sweet beverages” groups, such that MT consumed more reduced and non-fat dairy products than ST (p < 0.05, F = 3.59, df = 2) and ST drank more sweet beverages than NT (p < 0.05, F = 3.83, df = 2). A non-significant trend was observed for the “condiments” group, such that MT consumed more condiments than either other taster group (p=0.07, F=2.79, df = 2). No other differences were found among food groups. Analysis of covariance (ANCOVA) was performed to correct for the effect of energy intake and body weight on food intake, however this did not affect the results by PROP taster group. Overall food group intake by taster status and overall can be seen in Table 8.

A second analysis was performed to determine which foods contributed to total α-tocopherol intake by percentage and foods that did not contribute were removed. These foods’ contributions to α-tocopherol intake were summed to obtain the total amount of α-tocopherol consumed across all subjects. Individual foods that contributed to α-tocopherol intake were then grouped based on USDA food groups to obtain 13 groups. The contribution of each group to total α-tocopherol intake was calculated as a percentage. Mixed dishes, such as pizza, stews, and stir fry, along with nuts, snacks and desserts, meats, and fruit provided more than half of the total α-tocopherol intake across all subjects, followed by oils, vegetables, and fortified, ready-to-eat cereals. These 13 food groups were then used for subsequent analysis. This data can be seen in Table 9.
Table 8: Average dietary intake of food groups (servings/day) by PROP taster status.

<table>
<thead>
<tr>
<th>Food Group</th>
<th>Non-tasters (n=30)</th>
<th>Medium Tasters (n=33)</th>
<th>Super Tasters (n=30)</th>
<th>Overall Intake (n=93)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Citrus Fruit</td>
<td>0.5 ± 0.2</td>
<td>0.5 ± 0.1</td>
<td>0.6 ± 0.2</td>
<td>0.5 ± 0.1</td>
</tr>
<tr>
<td>Fruit</td>
<td>1.6 ± 0.3</td>
<td>1.3 ± 0.3</td>
<td>1.3 ± 0.3</td>
<td>1.4 ± 0.2</td>
</tr>
<tr>
<td>Dark Green Vegetables</td>
<td>2.1 ± 0.3</td>
<td>1.8 ± 0.2</td>
<td>1.7 ± 0.2</td>
<td>1.8 ± 0.2</td>
</tr>
<tr>
<td>Yellow Vegetables</td>
<td>0.4 ± 0.1</td>
<td>0.2 ± 0.1</td>
<td>0.4 ± 0.1</td>
<td>0.3 ± 0.1</td>
</tr>
<tr>
<td>Tomatoes</td>
<td>0.7 ± 0.1</td>
<td>0.6 ± 0.1</td>
<td>0.5 ± 0.1</td>
<td>0.6 ± 0.1</td>
</tr>
<tr>
<td>Starchy Vegetables</td>
<td>0.4 ± 0.1</td>
<td>0.3 ± 0.1</td>
<td>0.4 ± 0.1</td>
<td>0.4 ± 0.1</td>
</tr>
<tr>
<td>Whole Grains</td>
<td>1.2 ± 0.2</td>
<td>0.7 ± 0.2</td>
<td>0.8 ± 0.3</td>
<td>0.9 ± 0.1</td>
</tr>
<tr>
<td>Grains</td>
<td>3.9 ± 0.4</td>
<td>4.8 ± 0.4</td>
<td>3.8 ± 0.4</td>
<td>4.2 ± 0.2</td>
</tr>
<tr>
<td>Unsweetened RTE Cereals</td>
<td>0.2 ± 0.1</td>
<td>0.4 ± 0.1</td>
<td>0.3 ± 0.1</td>
<td>0.3 ± 0.1</td>
</tr>
<tr>
<td>Sweetened RTE Cereals</td>
<td>0.3 ± 0.1</td>
<td>0.6 ± 0.1</td>
<td>0.3 ± 0.1</td>
<td>0.3 ± 0.1</td>
</tr>
<tr>
<td>Desserts</td>
<td>0.8 ± 0.2</td>
<td>0.6 ± 0.1</td>
<td>0.7 ± 0.2</td>
<td>0.7 ± 0.1</td>
</tr>
<tr>
<td>Snack Bars</td>
<td>0.1 ± 0.1</td>
<td>0.1 ± 0.02</td>
<td>0.1 ± 0.1</td>
<td>0.1 ± 0.02</td>
</tr>
<tr>
<td>Salty Snacks</td>
<td>0.3 ± 0.1</td>
<td>0.5 ± 0.1</td>
<td>0.4 ± 0.1</td>
<td>0.4 ± 0.1</td>
</tr>
<tr>
<td>Red Meat</td>
<td>1.1 ± 0.2</td>
<td>1.3 ± 0.2</td>
<td>1.3 ± 0.3</td>
<td>1.2 ± 0.1</td>
</tr>
<tr>
<td>Poultry</td>
<td>1.6 ± 0.2</td>
<td>1.5 ± 0.3</td>
<td>1.5 ± 0.3</td>
<td>1.5 ± 0.2</td>
</tr>
<tr>
<td>Seafood</td>
<td>0.8 ± 0.2</td>
<td>0.8 ± 0.2</td>
<td>0.5 ± 0.2</td>
<td>0.7 ± 0.1</td>
</tr>
<tr>
<td>Unsweetened Dairy</td>
<td>1.7 ± 0.2</td>
<td>2.1 ± 0.2</td>
<td>1.5 ± 0.2</td>
<td>1.8 ± 0.1</td>
</tr>
<tr>
<td>Products</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sweetened Dairy Products</td>
<td>0.3 ± 0.1</td>
<td>0.4 ± 0.1</td>
<td>0.3 ± 0.1</td>
<td>0.3 ± 0.1</td>
</tr>
<tr>
<td>Full Fat Dairy Products</td>
<td>0.7 ± 0.1</td>
<td>0.8 ± 0.1</td>
<td>0.9 ± 0.2</td>
<td>0.8 ± 0.1</td>
</tr>
<tr>
<td>Reduced/Non-Fat Dairy</td>
<td>1.3 ± 0.2&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>1.7 ± 0.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.9 ± 0.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.3 ± 0.1</td>
</tr>
<tr>
<td>Products</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Full Fat Added Fats</td>
<td>2.8 ± 0.4</td>
<td>3.3 ± 0.4</td>
<td>2.6 ± 0.3</td>
<td>2.9 ± 0.2</td>
</tr>
<tr>
<td>Reduced Fat Added Fats</td>
<td>0.2 ± 0.1</td>
<td>0.2 ± 0.1</td>
<td>0.2 ± 0.1</td>
<td>0.2 ± 0.1</td>
</tr>
<tr>
<td>Added Sugar</td>
<td>2.0 ± 0.4</td>
<td>1.67 ± 0.3</td>
<td>2.2 ± 0.7</td>
<td>1.7 ± 0.2</td>
</tr>
<tr>
<td>Candy</td>
<td>0.3 ± 0.1</td>
<td>0.3 ± 0.1</td>
<td>0.3 ± 0.1</td>
<td>0.3 ± 0.1</td>
</tr>
</tbody>
</table>
Sweet Beverages & 0.7 ± 0.2\textsuperscript{a} & 1.3 ± 0.2\textsuperscript{a,b} & 2.2 ± 0.7\textsuperscript{b} & 1.4 ± 0.2 \\
Tea/Coffee & 1.7 ± 0.3 & 1.0 ± 0.2 & 1.1 ± 0.2 & 1.3 ± 0.1 \\
Water & 4.3 ± 0.5 & 3.3 ± 0.5 & 3.7 ± 0.5 & 3.7 ± 0.3 \\
Alcohol & 0.6 ± 0.2 & 0.4 ± 0.1 & 0.3 ± 0.1 & 0.4 ± 0.1 \\
Non-Meat Protein & 1.5 ± 0.3 & 1.3 ± 0.35 & 1.6 ± 0.3 & 1.5 ± 0.2 \\
Condiments & 1.0 ± 0.4 & 2.2 ± 0.6 & 0.9 ± 0.3 & 1.4 ± 0.3 \\

\textsuperscript{1}Mean ± SEM. Differences among taster groups are noted by different letters.

Table 9: Food groups percent contribution to total \( \alpha \)-tocopherol intake.

<table>
<thead>
<tr>
<th>Food Group</th>
<th>Percent Contribution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mixed Dishes</td>
<td>12.0%</td>
</tr>
<tr>
<td>Nuts</td>
<td>11.7%</td>
</tr>
<tr>
<td>Snacks/Dessert</td>
<td>11.6%</td>
</tr>
<tr>
<td>Meat</td>
<td>11.0%</td>
</tr>
<tr>
<td>Fruit</td>
<td>10.2%</td>
</tr>
<tr>
<td>RTE Cereals</td>
<td>9.6%</td>
</tr>
<tr>
<td>Vegetables</td>
<td>7.9%</td>
</tr>
<tr>
<td>Oils</td>
<td>7.3%</td>
</tr>
<tr>
<td>Dark Green Vegetables</td>
<td>4.9%</td>
</tr>
<tr>
<td>Grains</td>
<td>4.4%</td>
</tr>
<tr>
<td>Meal Replacement Drinks/Bars</td>
<td>4.1%</td>
</tr>
<tr>
<td>Dairy</td>
<td>2.8%</td>
</tr>
<tr>
<td>Other</td>
<td>2.0%</td>
</tr>
</tbody>
</table>

The influence of “higher” or “lower” plasma \( \alpha \)-tocopherol and the interaction of “higher” or “lower” plasma \( \alpha \)-tocopherol and PROP taster group on food group intake was also examined, due to potential differences in food intake between the two groups (“higher” or “lower” plasma \( \alpha \)-tocopherol). A median split of \( \alpha \)-tocopherol concentrations in blood plasma was taken and subjects were divided into “higher” and “lower” groups. The median value was 18.65 \( \mu \text{M} \). To simplify the results, for this analysis the 30 food groups were combined into fourteen and food groups with low intake
were removed from the analysis. The groups were combined as follows: all citrus and non-citrus fruit into a fruit group; all vegetables (dark green, yellow, tomato, and starchy) into a vegetables group; sweetened and unsweetened cereal into a RTE cereal group; whole grain and refined grain into a grain group; unsweetened and sweetened dairy into an all dairy group; salty snacks, snack bars, and desserts into a snack group; red meat, poultry, seafood, and non-meat proteins into a meat group; and candy and added sweets into a sweet group. Also included in this analysis were sweetened beverages, water, tea/coffee, alcoholic beverages, condiments, and added fats. The percent of macronutrients, overall energy intake per day, and total \( \alpha \)-tocopherol consumed in mg/day were examined, as well. Significant differences were found between the “higher” and “lower” \( \alpha \)-tocopherol in plasma group, such that subjects with “higher” levels of \( \alpha \)-tocopherol in their plasma consumed more servings of fruit per day (\( p=0.05, F=4.07, \text{df} = 1 \)). A non-significant trend was also seen between the two food groups for vegetable intake, such that those subjects with “higher” \( \alpha \)-tocopherol concentrations in their plasma tended to consume more servings of vegetables per day (\( p=0.08, F=3.09, \text{df} = 1 \)). These results can be seen in Table 10.

Differences were observed when the interaction of “higher” or “lower” plasma \( \alpha \)-tocopherol and PROP taster group was examined for alcoholic beverage intake (\( p=0.02, F=4.17, \text{df} = 2 \)) and percent protein intake (\( p=0.03, F=3.53, \text{df} = 2 \)). For alcoholic beverage intake, MT with “higher” \( \alpha \)-tocopherol concentrations consumed more than MT with “lower” \( \alpha \)-tocopherol concentrations. With regards to percent protein intake, “lower” \( \alpha \)-tocopherol ST consumed less protein than all groups, except for “higher” \( \alpha \)-tocopherol MT. All other groups were not different from one another. A non-significant trend was observed for fruit intake when this interaction
was examined, such that NT and ST with “higher” levels of α-tocopherol consumed more servings of fruit than NT and ST with “lower” α-tocopherol, respectively. These results can be seen in Table 11 (“lower” α-tocopherol NT (n=16), MT (n=11), ST (n=13); “higher” α-tocopherol NT (n=8), MT (n=19), ST (n=12)).

Table 10: Average dietary intake of food groups by PROP taster status. Mean ± SEM.

<table>
<thead>
<tr>
<th>Food Group</th>
<th>“Lower” α-tocopherol in plasma (n=40)</th>
<th>“Higher” α-tocopherol in plasma (n=39)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vegetables</td>
<td>2.9 ± 0.3</td>
<td>3.5 ± 0.4</td>
</tr>
<tr>
<td>Fruit</td>
<td>1.6 ± 0.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.3 ± 0.3&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Grains</td>
<td>5.2 ± 0.3</td>
<td>4.8 ± 0.3</td>
</tr>
<tr>
<td>RTE Cereal</td>
<td>0.6 ± 0.1</td>
<td>0.6 ± 0.1</td>
</tr>
<tr>
<td>Snacks/Desserts</td>
<td>1.0 ± 0.1</td>
<td>1.4 ± 0.2</td>
</tr>
<tr>
<td>Meat</td>
<td>4.6 ± 0.3</td>
<td>5.2 ± 0.4</td>
</tr>
<tr>
<td>Dairy</td>
<td>2.0 ± 0.2</td>
<td>2.3 ± 0.2</td>
</tr>
<tr>
<td>Sweets</td>
<td>1.9 ± 0.3</td>
<td>2.1 ± 0.3</td>
</tr>
<tr>
<td>Sweet Beverages</td>
<td>1.5 ± 0.4</td>
<td>1.2 ± 0.3</td>
</tr>
<tr>
<td>Tea/Coffee</td>
<td>1.2 ± 0.2</td>
<td>1.4 ± 0.2</td>
</tr>
<tr>
<td>Water</td>
<td>3.7 ± 0.4</td>
<td>3.8 ± 0.4</td>
</tr>
<tr>
<td>Alcoholic Beverages</td>
<td>0.4 ± 0.1</td>
<td>0.5 ± 0.1</td>
</tr>
<tr>
<td>Condiments</td>
<td>1.0 ± 0.2</td>
<td>1.9 ± 0.5</td>
</tr>
<tr>
<td>Added Fats</td>
<td>1.9 ± 0.2</td>
<td>2.6 ± 0.3</td>
</tr>
<tr>
<td>Energy Intake (kcals)</td>
<td>1819.8 ± 55.5</td>
<td>1882.8 ± 65.1</td>
</tr>
<tr>
<td>Percent Fat</td>
<td>29.2 ± 0.6</td>
<td>31.2 ± 1.0</td>
</tr>
<tr>
<td>Percent Protein</td>
<td>15.3 ± 0.6</td>
<td>15.7 ± 0.6</td>
</tr>
<tr>
<td>Percent Carbohydrate</td>
<td>53.3 ± 0.9</td>
<td>50.9 ± 1.0</td>
</tr>
<tr>
<td>Total α-tocopherol (mg)</td>
<td>8.3 ± 0.9</td>
<td>9.0 ± 1.1</td>
</tr>
</tbody>
</table>

<sup>1</sup>Differences among taster groups are noted by different letters.
Table 11: Average dietary intake of food groups by PROP taster status and “higher” or “lower” plasma concentrations of α-tocopherol. Mean ± SEM.

<table>
<thead>
<tr>
<th>Food Group</th>
<th>Non-taster</th>
<th>Medium Taster</th>
<th>Super Taster</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Vegetables</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>“lower” α-tocopherol</td>
<td>3.1 ± 0.4</td>
<td>2.6 ± 0.3</td>
<td>2.7 ± 0.5</td>
</tr>
<tr>
<td>“higher” α-tocopherol</td>
<td>4.2 ± 0.9</td>
<td>3.2 ± 0.6</td>
<td>3.5 ± 0.5</td>
</tr>
<tr>
<td><strong>Fruit</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>“lower” α-tocopherol</td>
<td>2.2 ± 0.3</td>
<td>1.1 ± 0.3</td>
<td>1.3 ± 0.3</td>
</tr>
<tr>
<td>“higher” α-tocopherol</td>
<td>1.8 ± 0.6</td>
<td>2.3 ± 0.5</td>
<td>2.6 ± 0.6</td>
</tr>
<tr>
<td><strong>Grains</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>“lower” α-tocopherol</td>
<td>5.2 ± 0.3</td>
<td>6.3 ± 0.7</td>
<td>4.5 ± 0.4</td>
</tr>
<tr>
<td>“higher” α-tocopherol</td>
<td>4.9 ± 0.8</td>
<td>4.9 ± 0.4</td>
<td>4.6 ± 0.6</td>
</tr>
<tr>
<td><strong>RTE Cereal</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>“lower” α-tocopherol</td>
<td>0.6 ± 0.1</td>
<td>0.7 ± 0.2</td>
<td>0.6 ± 0.2</td>
</tr>
<tr>
<td>“higher” α-tocopherol</td>
<td>0.6 ± 0.2</td>
<td>0.6 ± 0.1</td>
<td>0.6 ± 0.3</td>
</tr>
<tr>
<td><strong>Snacks/Dessert</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>“lower” α-tocopherol</td>
<td>0.9 ± 0.2</td>
<td>1.1 ± 0.2</td>
<td>1.2 ± 0.3</td>
</tr>
<tr>
<td>“higher” α-tocopherol</td>
<td>1.9 ± 0.5</td>
<td>1.3 ± 0.3</td>
<td>1.1 ± 0.3</td>
</tr>
<tr>
<td><strong>Meat</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>“lower” α-tocopherol</td>
<td>4.9 ± 0.5</td>
<td>4.6 ± 0.6</td>
<td>4.3 ± 0.6</td>
</tr>
<tr>
<td>“higher” α-tocopherol</td>
<td>5.1 ± 0.8</td>
<td>5.1 ± 0.6</td>
<td>5.4 ± 0.8</td>
</tr>
<tr>
<td><strong>Dairy</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>“lower” α-tocopherol</td>
<td>2.0 ± 0.3</td>
<td>2.6 ± 0.4</td>
<td>1.5 ± 0.2</td>
</tr>
<tr>
<td>“higher” α-tocopherol</td>
<td>2.3 ± 0.4</td>
<td>2.3 ± 0.3</td>
<td>2.2 ± 0.4</td>
</tr>
<tr>
<td><strong>Sweets</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>“lower” α-tocopherol</td>
<td>2.5 ± 0.5</td>
<td>1.3 ± 0.4</td>
<td>1.7 ± 0.5</td>
</tr>
<tr>
<td>“higher” α-tocopherol</td>
<td>1.6 ± 0.6</td>
<td>2.4 ± 0.5</td>
<td>1.9 ± 0.7</td>
</tr>
<tr>
<td><strong>Sweet Beverages</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>“lower” α-tocopherol</td>
<td>0.5 ± 0.2</td>
<td>1.5 ± 0.4</td>
<td>2.7 ± 1.0</td>
</tr>
<tr>
<td>“higher” α-tocopherol</td>
<td>1.0 ± 0.4</td>
<td>1.1 ± 0.3</td>
<td>1.6 ± 0.7</td>
</tr>
<tr>
<td><strong>Tea/Coffee</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>“lower” α-tocopherol</td>
<td>1.8 ± 0.4</td>
<td>0.6 ± 0.2</td>
<td>1.0 ± 0.3</td>
</tr>
<tr>
<td>“higher” α-tocopherol</td>
<td>1.4 ± 0.4</td>
<td>1.4 ± 0.3</td>
<td>1.3 ± 0.4</td>
</tr>
<tr>
<td><strong>Water</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>“lower” α-tocopherol</td>
<td>4.4 ± 0.7</td>
<td>2.8 ± 0.7</td>
<td>3.5 ± 0.7</td>
</tr>
<tr>
<td>“higher” α-tocopherol</td>
<td>4.2 ± 0.9</td>
<td>3.6 ± 0.6</td>
<td>4.0 ± 0.8</td>
</tr>
<tr>
<td><strong>Alcoholic Beverages</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>“lower” α-tocopherol</td>
<td>0.7 ± 0.2a</td>
<td>0.1 ± 0.1b</td>
<td>0.4 ± 0.1ab</td>
</tr>
<tr>
<td>“higher” α-tocopherol</td>
<td>0.3 ± 0.3</td>
<td>0.7 ± 0.2</td>
<td>0.2 ± 0.1</td>
</tr>
<tr>
<td><strong>Condiments</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>“lower” α-tocopherol</td>
<td>0.7 ± 0.3</td>
<td>1.5 ± 0.5</td>
<td>1.0 ± 0.4</td>
</tr>
</tbody>
</table>
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“higher” α-tocopherol 2.0 ± 1.3 2.7 ± 0.9 0.7 ± 0.4

**Added Fat**

“lower” α-tocopherol 1.9 ± 0.3 2.0 ± 0.4 1.9 ± 0.4
“higher” α-tocopherol 2.0 ± 0.9 2.7 ± 0.5 2.7 ± 0.5

**Energy Intake (kCals/day)**

“lower” α-tocopherol 1787.6 ± 90.4 1875.0 ± 123.7 1816.2 ± 84.5
“higher” α-tocopherol 1910.1 ± 163.7 1953.9 ± 89.8 1752.1 ± 113.8

**% Fat**

“lower” α-tocopherol 27.2 ± 1.0 30.8 ± 1.0 30.3 ± 1.2
“higher” α-tocopherol 31.7 ± 2.6 31.6 ± 1.2 30.2 ± 2.3

**% Protein**

“lower” α-tocopherol 16.1 ± 0.8a 17.0 ± 1.1a 13.1 ± 0.8b
“higher” α-tocopherol 16.5 ± 1.7 15.0 ± 0.8 16.4 ± 1.1

**% Carbohydrate**

“lower” α-tocopherol 53.4 ± 1.6 51.8 ± 1.3 54.4 ± 1.8
“higher” α-tocopherol 50.4 ± 2.6 50.2 ± 1.4 52.1 ± 1.9

**Total α-tocopherol (mg/day)**

“lower” α-tocopherol 9.7 ± 2.0 6.6 ± 0.5 8.0 ± 1.0
“higher” α-tocopherol 9.1 ± 3.2 9.5 ± 1.7 8.2 ± 0.9

1 Differences within α-tocopherol group, among taster groups are noted by different letters.

### 4.4.2.2 Logistic Regression

Logistic regression is used to predict the odds of an event occurring (or not occurring). Logistic regression was used in the current study to predict a “high” total α-tocopherol intake using forward stepwise selections. The data were divided by median split into “higher” and “lower” α-tocopherol intake level; the median value was 6.99 mg/day. The best-fit model for total α-tocopherol intake had an 84.2% concordance rate for predicting “higher” α-tocopherol intake (Wald Chi-square p<0.10). The intake of Vitamin C and energy intake were found to be positive predictors of “higher” α-tocopherol intake (Table 12). The variables dairy, RTE cereal, fruit, vegetable, dessert,
snack bar, and added fat intake, as well as age, BMI, PROP rating, dietary restraint, and the interaction of taster status and dietary restraint did not contribute to the model. For the purposes of regression analysis, intake of sweetened and unsweetened cereals was combined into one “RTE cereal” group and unsweetened and sweetened dairy intake were combined into an “all dairy” group.

Table 12: Logistic Regression model for predicting “higher” Total α-tocopherol Intake.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Point Estimate</th>
<th>95% Wald Confidence Limits</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin C Intake</td>
<td>1.023</td>
<td>1.011-1.034</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Energy Intake</td>
<td>1.001</td>
<td>1.000-1.003</td>
<td>0.0340</td>
</tr>
</tbody>
</table>

4.5 Plasma α-tocopherol Concentrations

4.5.1 ANOVA

Blood samples from 80 subjects were obtained and two samples were discarded due to errors in collection and processing, as such, α-tocopherol concentrations of 78 samples were used for data analysis. Plasma concentrations of α-tocopherol ranged from 9.67 μM to 34.41 μM. The concentrations were within normal ranges for the subject population and there were no differences among PROP taster groups with regards to plasma α-tocopherol concentration (Table 13). There were also no differences among groups when the effect of the interaction of PROP taster status and dietary restraint was explored. As such, a median split of α-tocopherol plasma concentration was then taken, and subjects were grouped into two groups based on “higher” or “lower” blood plasma concentrations. The interaction of the “higher” or “lower” group and PROP taster status
was explored. A significant difference was found among the “higher” α-tocopherol group, such that NT had higher levels of α-tocopherol than both MT and ST (p<0.0001, F=22.04). These values are shown in Figure 5.

Table 13: Mean plasma α-tocopherol concentrations by PROP Taster Group.

<table>
<thead>
<tr>
<th></th>
<th>Non-taster (n = 30)</th>
<th>Medium Taster (n = 33)</th>
<th>Super Taster (n = 30)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma α-tocopherol</td>
<td>19.1 ± 1.3</td>
<td>19.1 ± 0.9</td>
<td>18.5 ± 1.0</td>
</tr>
</tbody>
</table>

Correlations were used to determine which demographic variables and food groups related to α-tocopherol concentrations in blood plasma. α-tocopherol in plasma was only correlated with age and modestly, although not significantly, correlated with non-citrus fruit intake. This data can be seen in Table 14.

Table 14: Blood plasma α-tocopherol correlations with food groups and demographic variables.

<table>
<thead>
<tr>
<th>Intake Group</th>
<th>Pearson Correlation Coefficient</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>0.26</td>
<td>0.02</td>
</tr>
<tr>
<td>Fruit Intake</td>
<td>0.20</td>
<td>0.08</td>
</tr>
</tbody>
</table>

4.5.2 Logistic Regression

In the current study, logistic regression using forward, stepwise selections, was used to predict the event “higher” α-tocopherol plasma measures. Data was divided by median split into “higher” and “lower” α-tocopherol plasma levels (n=39 for each
group); the median value was 18.65 μM. The best-fit model for α-tocopherol in blood plasma had a 75.2% concordance rate for predicting “higher” α-tocopherol plasma levels (Wald Chi-square p<0.10). The intake of fruit and the subjects’ age, were found to be significant, positive predictors of “higher” α-tocopherol levels in plasma (Table 15). Additionally, salty snack intake and PROP rating were non-significant, though trending, positive predictors of “higher” α-tocopherol levels in plasma. The variables Vitamin C, total energy, dairy, RTE cereal, vegetable, dessert, snack bar, and added fat intake, as well as, BMI, dietary restraint, and the interaction of taster status and dietary restraint did not contribute to the model.

**Table 15: Logistic Regression model for predicting α-tocopherol plasma levels.**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Point Estimate</th>
<th>95% Wald Confidence Limits</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fruit Intake</td>
<td>1.588</td>
<td>1.095-2.302</td>
<td>0.0148</td>
</tr>
<tr>
<td>Salty Snack Intake</td>
<td>1.981</td>
<td>0.947-4.141</td>
<td>0.0694</td>
</tr>
<tr>
<td>Age</td>
<td>1.132</td>
<td>1.022-1.253</td>
<td>0.0175</td>
</tr>
<tr>
<td>PROP Rating</td>
<td>1.015</td>
<td>0.998-1.032</td>
<td>0.0768</td>
</tr>
</tbody>
</table>

**4.5.3 Multiple linear regression results - α-tocopherol plasma concentrations**

Due to the interaction of both PROP taster status and “higher” or “lower” α-tocopherol plasma concentrations, a sub-analysis was done using multiple linear regression. This regression was done to determine which variables predicted plasma levels of α-tocopherol, in those individuals with higher and lower values. The best-fit model for “higher” concentrations using backward, stepwise selections accounted for 30.7% of the variance (p < 0.05). The factors included in the model were Vitamin C, RTE cereal, fruit, snack bar, salty snack, dairy, vegetable, dessert, total α-tocopherol, and
added fat intake, as well as age, PROP rating, energy intake, dietary restraint, BMI, and the interaction of dietary restraint and PROP taster status. One factor included in the final model positively influenced “higher” \( \alpha \)-tocopherol plasma concentrations; total dairy intake. The factors that negatively influenced “higher” plasma concentrations were total energy intake and PROP rating. None of the other factors contributed to the model at \( p < 0.05 \). This data can be seen in Table 16.

Table 16: Multiple linear regression variables for predicting \( \alpha \)-tocopherol plasma concentrations in those individuals with high plasma levels. Variables that were not significant in predicting “higher” \( \alpha \)-tocopherol concentrations at \( p=0.05 \) were removed from the model.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Parameter Estimate</th>
<th>Standard Error</th>
<th>SS</th>
<th>F-Value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>30.86</td>
<td>3.25</td>
<td>1194.98</td>
<td>90.16</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Dairy Intake</td>
<td>1.37</td>
<td>0.48</td>
<td>106.11</td>
<td>8.01</td>
<td>0.01</td>
</tr>
<tr>
<td>PROP Rating</td>
<td>-0.06</td>
<td>0.02</td>
<td>108.00</td>
<td>8.15</td>
<td>0.01</td>
</tr>
<tr>
<td>Energy Intake</td>
<td>-0.004</td>
<td>0.002</td>
<td>81.11</td>
<td>6.12</td>
<td>0.02</td>
</tr>
</tbody>
</table>

The best-fit model for predicting “lower” plasma \( \alpha \)-tocopherol concentrations using backward, stepwise selections accounted for 20.3% of the variance (\( p < 0.05 \)). The factors included in the model were Vitamin C, RTE cereal, fruit, snack bar, salty snack, dairy, vegetable, dessert, total \( \alpha \)-tocopherol, and added fat intake, as well as age, PROP rating, energy intake, dietary restraint, BMI, and the interaction of dietary restraint and PROP taster status. The only factor included in the model was dessert intake, which negatively influenced “lower” plasma \( \alpha \)-tocopherol concentrations. None of the other factors contributed to the model at \( p < 0.05 \). This data can be seen in Table 17.
Table 17: Multiple linear regression variables for predicting “lower” α-tocopherol plasma concentrations. Variables that were not significant in predicting “higher” α-tocopherol concentrations at p=0.05 were removed from the model.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Parameter Estimate</th>
<th>Standard Error</th>
<th>SS</th>
<th>F-Value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>15.82</td>
<td>0.53</td>
<td>5569.53</td>
<td>882.02</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Dessert Intake</td>
<td>-1.80</td>
<td>0.59</td>
<td>57.84</td>
<td>9.16</td>
<td>0.01</td>
</tr>
</tbody>
</table>
CHAPTER 5: DISCUSSION

5.1 Dietary Intake – Nutrient Totals

5.1.1 Overall Intake and Intake by Dietary Restraint

Overall, the subjects’ nutrient consumption was within the recommended ranges and comparable to other reported nutrient intakes from studies following similar populations. According to the Dietary Reference Intakes, women between the ages of 21–45, energy intakes of 1800–2200 kcals/day are recommended (depending on physical activity), while macronutrient intakes should 20-35% fat, 45-65% carbohydrate, and 10-35% protein [12]. The subjects from this population were within these ranges, with an average of 1846 kcals, 30% fat, 52% carbohydrate, and 16% protein consumed. In terms of vitamin and the other nutrient intakes measured in the current study, the subjects were within the recommended, and on par with reported, ranges, with the exception of Vitamin E intake. As much of the α-tocopherol in the U.S. diet comes from added fats, such as cooking oils, salad dressings, potato chips, and other discretionary fat sources, foods that would most likely be consumed less when attempting to restrict one’s diet, the influence of dietary restraint on α-tocopherol intake was investigated. While it is true that those subjects classified as “higher” in dietary restraint may not be actually restricting their intake, only intending to restrict, the potential influence of this dietary eating behavior was strong and warranted investigation. Upon examining dietary restraint, no differences were seen in overall intake of total α-tocopherol between non-restrained and restrained eaters.
5.1.2 Nutrient Intake by PROP Taster Status and Dietary Restraint

As previously shown by Tepper, et al., two key dietary patterns have been shown to exist in NT, higher consumption of and preference for discretionary fats and higher acceptance of dark green, bitter vegetables, thus the influence of PROP and dietary restraint interaction were examined with regards to nutrient intakes (Table 5 and Table 6) [25]. When divided by PROP taster status, each taster group also consumed the expected number of calories and percent macronutrients for this population. Again, a similar pattern was seen with regards to vitamin intake, in that all intakes were within the recommended ranges, except for that of Vitamin E, where all groups were below the recommended 15 mg/day guideline.

Since each dietary pattern contributes to overall \( \alpha \)-tocopherol intake, and fat intake may be related to dietary restraint and bitter vegetable intake may be related to PROP taster status, the interaction of dietary restraint and PROP taster group was examined. As shown earlier (Figure 3), non-restrained NT had the highest intakes of \( \alpha \)-tocopherol among NON-non-restrained eaters, while there were no differences among restrained eaters. This can be explained as restrained eaters eat based on cognitive influences, rather than taste, and perhaps are better able to overcome innate preferences and genetics as a result of these cognitive factors in order to “eat healthy” and reduce weight status. Again, however, it should be noted that the DEBQ used to classify subjects as restrained or non-restrained eater can only classify subjects into those who INTEND to restrict intake and those who DO NOT intend to restrict. Among non-restrained eaters, taste is arguably the most important factor in deciding what to eat and
NT, not hindered by genetic predisposition against bitter foods and the increased preference for high-fat foods, will consume more of these foods.

5.2 Dietary Intake – Food Groups

The food group intakes in this population were close to or within the expected and recommended ranges for women in this age group. The USDA recommends women in the age range of the participants in the current study eat four to four and one-half cups of fruits and vegetables per day; in this population two servings (one serving = ½ cup) of fruit and approximately four servings (one serving = ½ cup) of vegetables were eaten per day [133]. The recommended intake of grains is six one-ounce equivalents, the intake in this population was approximately six servings/day. For meat, the recommendation is five one-ounce equivalents and for dairy, three cups per day. In this population, an average of five servings of meat were eaten per day (including one and one half servings each of red meat and poultry, one half serving of seafood, and one serving of non-meat protein) and just under three servings of dairy per day. Six teaspoons of oils per day is recommended by the USDA and this population consumed approximately three servings (teaspoons) per day.

When divided by PROP taster status, the food group intakes by NT, MT, and ST were within or close to the USDA recommendations (Table 8). There were no differences in intake by PROP taster group, with the exception of reduced fat/non-fat (RF/NF) dairy and sweet beverage intake. MT ate the most RF/NF dairy, while ST ate the least and NT were not different from either group. These differences are difficult to explain based on PROP taster status, previous studies have shown PROP tasters are better discriminators
of fat and that NT tend to prefer full-fat products, however this does not explain the consumption of RF/NF dairy products seen here [64]. Based on those findings, ST should eat more RF/NF foods (like dairy products) than NT and vice versa for full fat products, however, in this population, there were no differences in full fat dairy consumption. It should be noted that the RF/NF dairy group includes both sweetened and unsweetened dairy products, which may be confounding the findings and PROP taster status has been show to relate to the preference for sweet fat foods. The intakes of these foods were relatively low, however, making it difficult to parse out differences among PROP taster groups. Alternatively, these differences may be due to factors other than PROP taster status, perhaps cognitive factors or general environmental variables.

Differences in sweet preference among taster groups have been shown previously by many researchers, such that ST perceive sweet more intensely than NT and that ST are more likely to dislike sweet foods [134, 135]. Other studies have found no associations between PROP taster status and sweet preference and intake [136]. Differences in sweet beverage intake in this population, however, seem to go against previously reported associations. ST consumed more sweet beverages than NT, while MT were not different from either group. While ST may perceive sweetness as more intense, it is fair to say this does not always influence intake of sweet foods. In the current study, the sweetened beverages group includes beverages sweetened with sugar and also non-nutritive sweetener. Research has shown ST perceive more bitterness from non-nutritive sweeteners, although a minimal in influence on intake was observed [137]. Thus, despite the bitterness associated with these types of drinks, intake does not seem to have been affected by PROP taster status.
There were no differences in fruit, vegetable, or added fat intake, in contrary to the hypothesized differences. As stated earlier, while clear differences in preference for bitter fruits, vegetables, and full fat products have been observed, the actual influence these preferences have on intake are unclear. There were also no differences in the intake of any food groups between dietary restraint group or when looking at the interaction between dietary restraint and PROP taster group. The subjects who participated in this study were all young, healthy women, and, anecdotally, the majority were very interested in nutrition and healthy eating. It is entirely possible the desire to “eat healthy” had a more profound influence on their food choices than we would otherwise expect. Another possibility is that the dietary intake information collected either did not accurately depict the subjects’ intake over the three day time period, or that the intake across the three days was not indicative of their usual eating habits. Dietary underreporting is quite commonplace when collecting diet information, especially among women and overweight subjects and could substantially affect the reported intakes of high-fat foods, which are especially important to α-tocopherol intake [138, 139].

5.3 Dietary Intake – Food Group and α-tocopherol Relationship

Correlations were conducted to determine which foods contributed the most to total α-tocopherol, in order to better focus food group analysis on the foods most relevant to intake. The expected foods contributed to overall intake: dark green vegetables, yellow vegetables, citrus fruit, non-citrus fruit, oils, and whole grains, as well as energy intake (Table 7). It was expected that foods fortified with Vitamin E, such as granola bars and meal replacement drinks and bars, as well as salty snacks, like potato chips,
would correlate with total $\alpha$-tocopherol intake, however this was not the case. This may be due to the overall low to moderate intakes of these foods in this population, one quarter and one half serving per day, respectively.

Furthermore, a breakdown of the percent contribution of each food group to the total $\alpha$-tocopherol intake of this population illustrated the importance of determining all foods that correlated with $\alpha$-tocopherol intake (Table 9). In addition to the foods expected to contribute to total $\alpha$-tocopherol (dark green vegetables, nuts, vegetable oils), a large variety of other foods contributed, as well. More than half the total $\alpha$-tocopherol intake was from mixed dishes, desserts and snacks, meat, fruit, and RTE cereals. This very much parallels a study comparing Hispanic and Caucasian eating habits and sources of $\alpha$-tocopherol. In that study, RTE cereal, sweet, baked desserts, oils and fruit contributed the most $\alpha$-tocopherol to the diet among the Caucasian population [127].

Within the current study population, a variety of mixed dishes were consumed, and were often prepared with oil, or were commercially prepared and fortified with $\alpha$-tocopherol, thus it makes sense these types of foods would significantly contribute to intake. Meats were often prepared with oil, contributing to total $\alpha$-tocopherol intake, despite a low level of $\alpha$-tocopherol inherent to the meat. Many of the RTE cereals consumed quite frequently by this population (one serving/day) were highly fortified with Vitamin E. Fruits such as berries are quite high in $\alpha$-tocopherol and all non-citrus fruits were consumed by this population frequently (two servings/day). Nuts, although consumption was low, are quite high in $\alpha$-tocopherol, making their contribution much higher, despite low intake.
Vegetables and added fats contributed a modest amount to the total α-tocopherol intake in this population, despite frequent consumption (three servings of each per day) and correlation with α-tocopherol intake. Dark green vegetable consumption, however, was low in this population, so while overall vegetable consumption was high, consumption of those rich in α-tocopherol was not. In addition, grain intake contributed very little to total α-tocopherol intake, but was highly correlated with α-tocopherol. This could be due to overall low whole grain intake (one serving per day) as compared to refined and some whole grain intake (four servings/day). Whole grains are the highest in α-tocopherol, so while correlated with α-tocopherol, the low intake prevented them from contributing much to overall intake. The intake of fortified, energy bars and meal replacement drinks was quite low in this population, in fact very few subjects even consumed these types of foods. However these fortified foods contributed about 4% of the total α-tocopherol consumed by this study group. The low consumption explains the lack of correlation, however it is clear that among subjects consuming these types of fortified foods, α-tocopherol intake would be quite high. The intake of these types of α-tocopherol-fortified foods may be confounding the results, however, as care was taken to exclude subjects who were taking dietary supplements from the study so only α-tocopherol intake from whole foods would be considered. While none of the participating subjects were taking Vitamin E supplements, a few were supplementing themselves with Vitamin E through the use of these types of fortified foods.

In attempting to predict α-tocopherol intake from demographic information and nutrient and food group intakes, only Vitamin C intake and energy intake predicted overall α-tocopherol intake (Table 12). Higher energy intakes could influence α-
tocopherol intake as added fats contribute more calorically to overall energy intake, while also contributing to α-tocopherol intake. In addition, the more calories consumed would certainly increase the amount of all macronutrients and vitamins consumed. Vitamin C intake could potentially predict α-tocopherol intake, as fruit contributed a fair amount of α-tocopherol to the diet and is also quite high in Vitamin C [140]. In addition, leafy vegetables high in Vitamin E are quite high in Vitamin C, as well, so combined with fruit intake, these two groups could potentially contribute both essential nutrients. Other foods, such as RTE cereals, snacks, and juices fortified with Vitamin E may also be fortified with, or naturally high in Vitamin C, as well.

5.4 Plasma Concentrations of α-tocopherol

The plasma concentrations of α-tocopherol in this population were within reported ranges of other studies in similar populations, although the subjects were on the lower end of the reported range [12]. Despite low intake of α-tocopherol, plasma levels were well within normal ranges. Additionally, there was no correlation between α-tocopherol concentration in plasma and total α-tocopherol intake. While studies have shown increased α-tocopherol intake (supplementation with 15, 75, and 150 mg) increased plasma α-tocopherol in the short-term, intakes in the current population were quite low [82]. At the low, non-supplemented intakes (average intake = 8.6 ± 0.7 mg) in the current population, it is unlikely that differences would be seen, because changes in plasma levels would be likely be minor. Currently, very little is known about Vitamin E biokinetics in humans, so it is difficult to describe a regulation range for Vitamin E in blood plasma, or at what level of intake blood plasma levels begin to correlate with intake.
From the results of the supplementation studies described earlier, it is clear that plasma values are sensitive to intake at levels much higher than those achieved in this study. It is also possible that the dietary information collected did not accurately reflect the long-term dietary patterns of this population. While care was taken to obtain three recalls, reflective of usual eating patterns, it is difficult to assume all subjects ate normally while knowing their intakes were to be reported the next day. The underreporting associated with 24-hour dietary recalls has been well documented, and may have influenced this study [138, 139]. While the information collected was most likely precise with regards to what was eaten, the amounts consumed may not have been accurately represented either due to cognitive underreporting or lack of knowledge of portion size. Furthermore, the information collected may have been accurate for the week reported, but not truly represent the eating behaviors of the subject long term. A food frequency questionnaire would have been better for determining long term eating patterns, however quantitative information about specific nutrients is difficult to obtain from these types of questionnaires. To accurately relate diet and plasma concentrations of \( \alpha \)-tocopherol, it was necessary to collect quantitative dietary information and, currently, diet recalls are the best way to collect quantitative dietary intake information.

Differences among subjects with “higher” and “lower” levels of \( \alpha \)-tocopherol in plasma were examined to determine whether subjects with “higher” levels had different dietary patterns and behaviors than those with “lower” levels. These two groups apply solely to this population of subjects, as all plasma levels were within normal ranges and a median split was taken to divide the subjects into two groups. Among subjects with “higher” levels of \( \alpha \)-tocopherol in their blood, NT had significantly higher levels than
both MT and ST (Figure 4). There were no differences among subjects with “lower” levels of \( \alpha \)-tocopherol in their plasma. The factors which positively contributed to “higher” \( \alpha \)-tocopherol concentrations in blood plasma were fruit and salty snacks intake, as well as age and PROP rating (Table 15). “Higher” concentrations due to fruit and salty snack intake is intuitive, as fruit and fruit juices are either high naturally in \( \alpha \)-tocopherol, or are fortified with Vitamin E, in the case of fortified citrus juice. Salty snacks include potato chips and other food fried in vegetable oils, which are quite high in \( \alpha \)-tocopherol and have been shown to contribute significantly to total \( \alpha \)-tocopherol intake [126]. In addition many of these salty snacks may be further fortified by industry to increase the nutritional content of these snacks. Intake of these foods over time could influence plasma levels of \( \alpha \)-tocopherol. The influence of age is not as clear, perhaps as the subjects became older, there was more focus on healthy eating, therefore an increase in fruit and dark green vegetable intake. These older subjects also may be more likely to consume foods fortified with Vitamin E. Additionally, there may be biological explanations as to why higher levels of \( \alpha \)-tocopherol are present in older women, although it should be noted that the median age of this population was 27 years, so the influence of age may be able to be more clearly defined in a population with a much more broad range of ages. The influence of age on Vitamin E intake should be further explored to determine differences in food group intake and any biological influences that contribute to \( \alpha \)-tocopherol blood plasma concentrations.

Among subjects with “higher” levels of \( \alpha \)-tocopherol in their blood plasma, dairy intake, energy intake, and PROP rating significantly contributed to higher concentrations (Table 16). Dairy intake was positively correlated, indicating the increased dairy intake
raised plasma α-tocopherol concentrations. This confirms the findings of Hayes, et al., in which subjects with high dairy intake also had high α-tocopherol plasma concentrations, despite relatively low intake of α-tocopherol [128]. This may be due to the increase in bioavailability of Vitamin E consumed with full fat or reduced fat dairy products, rather than skim milk or water [129]. PROP rating was negatively associated with increased α-tocopherol blood concentration, indicating lower sensitivity to PROP equates to higher α-tocopherol levels in blood plasma. This was thought to be due to the food preference and dietary intake patterns discussed earlier, in which PROP NT prefer high fat foods and may consume more of these foods, as well as PROP tasters having reduced preference for bitter, green vegetables and possibly consuming less of them. However, the intake of high fat foods and dark green vegetables did not influence plasma α-tocopherol concentrations. Energy intake was very modestly, negatively associated with increased α-tocopherol plasma concentrations, indicating the less energy consumed, the higher α-tocopherol concentration would be. This could be due to fruit and vegetable intake among the subjects with “higher” plasma concentrations, as these foods are high in α-tocopherol, however relatively low in calories. Among those with “higher” concentrations, increased intake of these foods would raise plasma α-tocopherol without increasing overall energy intake.

Dessert intake was the only predictor of α-tocopherol plasma concentrations among subjects with “lower” levels (Table 17). Dessert intake was negatively associated with higher α-tocopherol concentrations in this “lower” group. This is somewhat counterintuitive, as sweet, baked desserts are often made with oil, and therefore should contribute to α-tocopherol intake, and plasma concentrations, in this population. This
also contradicts the findings in the Gao study, in that dessert intake among Caucasians contributed to overall \( \alpha \)-tocopherol intake [127]. It is possible the desserts eaten by this group did not contain significant amounts of Vitamin E or that those who ate more desserts were also eating less of the foods that contributed to Vitamin E intake. Either of these theories would explain lower intake and, in turn, lower plasma concentrations.

Taken together, it seems apparent that there are different dietary patterns that contribute to “higher” or “lower” \( \alpha \)-tocopherol blood plasma concentrations in this population. Among subjects with “higher” intake, dairy intake and PROP rating seem to have the most influence, this confirms two theories: (1) PROP NT have different eating patterns than PROP tasters, which contribute to increased antioxidant status in plasma, with regards to Vitamin E, and (2) Vitamin E bioavailability is dependent on full fat or reduced fat dairy intake. Those subjects with “higher” concentrations of \( \alpha \)-tocopherol appear to eat foods that help the body absorb Vitamin E more efficiently, therefore it might imply they would have higher levels circulating in plasma. Dairy products are not naturally good sources of Vitamin E, therefore the association with higher plasma levels of \( \alpha \)-tocopherol, may be attributed to the ability of these types of foods to increase absorption of \( \alpha \)-tocopherol. Differences among PROP taster groups are also more apparent among the “higher” concentration subjects due to increased intake of Vitamin E-containing foods. Among those subjects with “lower” levels, PROP taster group has no effect, and only RTE cereal and dessert intake show any influence on plasma levels of \( \alpha \)-tocopherol. Levels among this “lower” group may be regulated solely by the liver, thus no differences among groups are seen. Plasma levels of \( \alpha \)-tocopherol will likely not
show large differences unless intake of \( \alpha \)-tocopherol is quite high, either due to increased Vitamin E-containing food intake or dietary supplementation.

5.5 Summary/Conclusions

No differences in \( \alpha \)-tocopherol intake were seen, except when looking at the influence of dietary restraint and PROP taster status. This is most likely due to the cognitive influence of intention to restrict on fat intake. Among non-restrained eaters, there seems to be an influence of PROP taster status on \( \alpha \)-tocopherol intake, such that NT had higher intakes of \( \alpha \)-tocopherol than both MT and ST, however more subjects are needed to clearly define differences in eating patterns among PROP taster groups. Perhaps in the future, care can be taken to eliminate restrained eaters from the subject pool, thereby eliminating the influence of dietary restraint. No differences in food group intake were seen with regards to PROP taster status, with the exception of reduced and non-fat dairy intake and sweet beverage intake. The reasons for these differences are unclear, although differences in sweet preference have been show among PROP taster groups, this preference has not been shown to affect intake, which has been confirmed here.

There appears to be some influence of dairy intake on \( \alpha \)-tocopherol plasma concentrations in this population, as has been shown previously [127]. Additionally, this study showed RTE cereal and salty snacks, such as potato chips and other fried snacks contribute significant amounts of \( \alpha \)-tocopherol to the diet. While differences in non-citrus fruit intake also had an influence on overall \( \alpha \)-tocopherol intake, the fortified and high-fat foods are, perhaps, lesser known sources of dietary \( \alpha \)-tocopherol. While,
certainly, the intake of \( \alpha \)-tocopherol-containing foods such as fruit and dark, green vegetables, should be encouraged, the moderate consumption of other foods may provide a beneficial amount of \( \alpha \)-tocopherol in the standard U.S. diet.

In general, this study could not relate dietary intake of \( \alpha \)-tocopherol to concentration in blood plasma, most likely due to low intakes of \( \alpha \)-tocopherol. Intakes among this population were low, when compared to the recommended intakes and people consuming dietary Vitamin E supplements, however they were on par with that of the U.S. population as a whole. Despite the low intake of \( \alpha \)-tocopherol, the population had \( \alpha \)-tocopherol blood plasma concentrations within other reported ranges. Other studies (both small and population) have also reported this lack of association between \( \alpha \)-tocopherol intake from food alone and increased blood concentrations, however many of these same studies have showed a strong correlation between intake and plasma \( \alpha \)-tocopherol when including subjects who use Vitamin E supplements. The study by Gao, et al., did show associations between dietary intake and plasma concentrations of \( \alpha \)-tocopherol, both in supplemented and non-supplemented elderly subjects [127]. However, this was not shown through correlation, however, but rather \( \alpha \)-tocopherol plasma concentrations by intake tertile groups and no such association among intake tertile groups was seen in the current population. The subjects in the Gao study had similar intakes from food of \( \alpha \)-tocopherol, but had higher plasma concentrations than the current study population, which may have contributed to the differences seen by tertile group. The reasons for the differences in plasma concentration between that and the current study populations are unclear at the time, perhaps the difference in age between the two groups has some influence, however. Both in a small study by McNaughton, et al. and two larger studies
by Talegawkar and Ascherio, associations between blood \( \alpha \)-tocopherol and intake (from a FFQ) were found only among subjects taking Vitamin E supplements \([122, 126, 141]\). Additionally, a small study by Record, et al. showed no changes in plasma \( \alpha \)-tocopherol when subjects moved from a low antioxidant diet to a high antioxidant diet for two weeks, or after consuming a high antioxidant supplement for two weeks \([124]\). Similarly, a larger study in men showed no associations between intake of \( \alpha \)-tocopherol measured by FFQ or weighed record to plasma \( \alpha \)-tocopherol concentrations \([142]\). Many of these studies also corrected the \( \alpha \)-tocopherol plasma concentrations for either cholesterol or triglycerides or both, in order to obtain a more accurate value, as \( \alpha \)-tocopherol concentrations in blood have been shown to be affected by these biological constituents.

In this study, diet recalls were used to obtain a quantitative measurement of \( \alpha \)-tocopherol intake, however most other studies have used food frequency questionnaires, alone or with diet records, as they give a more accurate picture of long-term intake. Unfortunately, the intakes calculated via the FFQ showed little to no correlation with plasma \( \alpha \)-tocopherol, thus it seems unlikely differences would be seen in the current study, using only diet recalls (short-term intake) and excluding people who did not take any Vitamin E supplements. Additionally, the current study only reflects the intake of 93 subjects and the blood plasma concentrations of 78 subjects, making it difficult to relate blood \( \alpha \)-tocopherol concentrations with the intake of specific foods. A larger, population-based study, better able to look at long term dietary intake would most likely be better able to show a relationship between diet and blood plasma measures.

As mentioned earlier, a previous study performed in 2002-2004 showed differences in \( \alpha \)-tocopherol concentration in blood plasma by PROP taster status, such that NT had
significantly higher levels of \(\alpha\)-tocopherol than either MT or ST [78]. The current study was not able to replicate these results, except within the subset of the study population with “higher” levels of plasma \(\alpha\)-tocopherol. The reasons for this disconnect are unclear at the present time. Based on previous research on Vitamin E and its availability in the human body, it seems unlikely differences in blood plasma levels would be seen, with the exception of individuals with very high intakes of \(\alpha\)-tocopherol. The current study results seem to agree with this research, as differences among PROP taster groups were only seen among those subjects in the top 50% of \(\alpha\)-tocopherol blood concentrations. The differences from the earlier study cannot be explained in this manner, perhaps these results were independent to that subject population (as were the current study results), not a reflection of women in that age range as a whole. Additionally, the a small percentage of the subjects in the current study population consumed foods highly fortified with \(\alpha\)-tocopherol, despite care being taken to eliminate subjects from the study population taking dietary supplements. The earlier study in 2002-4 may not have been affected by these fortified foods, as (anecdotally) the prevalence and use of these types of foods, especially in the form of vitamin-fortified beverages, has increased over the past five years. While there seems to be some influence of PROP taster status on plasma \(\alpha\)-tocopherol, how this relates to other factors that influence both dietary intake and biological antioxidant status remains to be seen.

Overall, \(\alpha\)-tocopherol may not have been the best biomarker of fruit, dark, green vegetable, and added fat intake to measure in plasma, as intakes of this Vitamin are low in the U.S. population and differences in intake are not measurable in plasma except at very high intake amounts. Future research should seek to quantify other biomarkers of
fruit and dark green vegetable intake in plasma, such as α-CEHC or π-GST (see Appendix III) which may provide a more accurate picture of the intake-plasma antioxidant relationship. In addition, the use of food frequency questionnaires in tandem with diet recalls may be able to better portray the long-term eating behaviors of the subjects. The quantification of biomarkers indicative of fruit and vegetable consumption may be quite important in determining risk for a variety of diseases. Certainly decreased consumption of these foods due to genetic or other influences could increase the risk for these diseases and are important considerations to be addressed in diet research. Despite the lack of association between plasma and dietary α-tocopherol and the relatively low intakes of α-tocopherol among this population, the plasma levels were all within normal ranges. This indicates that the current recommendations may be unnecessarily high and should perhaps be reconsidered based on the dietary sources of α-tocopherol available to the U.S. population.
BIBLIOGRAPHY


The only form 100% biologically active in humans is \( \alpha \)-tocopherol, due to the positions of the methyl groups on the chromanyl ring and the configuration of the methyl groups on the tail. Only the RRR (position of methyl groups at 2, 4', and 8') form of \( \alpha \)-tocopherol is found naturally in foods.
Figure 2: Vitamin E Metabolism – Vitamin E is absorbed by chylomicrons in the intestine and transported to the liver. In the liver, $\alpha$-TTP preferentially binds RRR-$\alpha$-tocopherol and incorporates it into VLDLs. From there, $\alpha$-tocopherol is transferred to LDL and HDLs and brought to tissues. Excess Vitamin E is excreted in bile.
Figure 3: NaCl and PROP Ratings by PROP Taster Group – mean (±SE) ratings for NaCl and PROP by PROP taster group (NT n = 30, MT n = 33, ST n = 30) using the paper disk method and the LMS. There was a significant main effect of taster group for both NaCl (p < 0.05) and PROP ratings (p < 0.0001) using ANOVA (p < 0.05) and Duncan’s post-hoc test (p < 0.05). Means with different letters are statistically different at p < 0.05.
Figure 4: α-Tocopherol Intake by PROP Taster Group and Dietary Restraint Group – mean (±SE) intake of total α-tocopherol by PROP taster group and restraint group. The number of subjects in each group can be seen in the figure. There was a significant taster group x restraint group interaction by ANOVA (p < 0.05) and pairwise comparison (p < 0.05). Means with different letters are statistically different at p < 0.05.
Figure 5: α-Tocopherol Blood Plasma Concentrations by PROP Taster Group and α-Tocopherol Plasma Group mean (±SE) plasma α-tocopherol by PROP taster group and plasma group. The number of subjects in each group can be seen in the figure. There was a significant taster group x plasma group interaction by ANOVA (p < 0.05) and pairwise comparison (p < 0.05). Means with different letters are statistically different at p < 0.05.
APPENDIX I
Consent Form
CONSENT FORM
BITTER TASTE AND MARKERS OF DIETARY BEHAVIOR - Food Habits Study

Principal Investigator: Beverly J. Tepper, Ph.D.
Sensory Evaluation Laboratory (Room 211)
Department of Food Science, Rutgers University
65 Dudley Road, New Brunswick, NJ 08901
732) 932-9611 Ext. 221 email: tepper@aesop.rutgers.edu

PURPOSE: Dietary phytochemicals may act as anti-oxidants to protect against the development of chronic diseases such as cancer. This study will examine the relationship between taste responses, dietary behaviors and blood markers of anti-oxidants status.

PROCEDURES: As a subject in this study, I will be asked to participate in 2, 30 min sessions. During one of the sessions, I will taste and evaluate laboratory solutions (dried onto filter papers) and will provide saliva samples by placing dental rolls into my mouth for several minutes. My weight and height will be measured and I will complete a questionnaire about my food attitudes. In a separate session, a small blood sample (~1-1/2 tablespoons) will be taken from my arm by a needle. I will be asked to fast overnight prior to the blood test. I should not eat or drink anything except plain water from 11:00 pm until after I have had my blood drawn the following morning. The blood sample will be analyzed for anti-oxidants. I will also complete 3 diet interviews. Two interviews will be scheduled during the test sessions and a third interview will either be conducted in person or by telephone.

RISKS/BENEFITS: The activities I will be participating in pose minimal risks to my health. However, I might experience some discomfort or swelling at the site of the needle insertion or some mild dizziness before or during the blood draw. Although I will receive no direct benefits from participating in this study, this research will benefit society by providing a better understanding of the relationship between diet and disease risk.

COMPENSATION: At the completion of the study I will receive a single payment of $40. If I withdraw from the study prior to its completion, my payment will be pro-rated for each session completed.

MY RIGHTS AS A RESEARCH SUBJECT/CONFIDENTIALITY: My participation in this study is completely voluntary and I have the right to withdraw at any time without explanation or penalty. The information collected in this experiment will be kept strictly confidential, my identity protected by a code number, and all data kept in a locked filing cabinet or on a pass-word protected computer. Only research staff involved in this study will have access to these files.

AGREEMENT: I have read the above description. All my questions have been answered to my satisfaction and I agree voluntarily to participate. I understand that I have the right to leave the experiment at any time without penalty. I also understand that Rutgers University has made no general provision for financial compensation or medical treatment for any physical injury resulting from this research. If I have questions about this research, I can contact the Principal Investigator at the number listed above or the Rutgers University Institutional Review Board for the Protection of Human Subjects, Office of Research and Sponsored Programs, 3 Rutgers Plaza, New Brunswick, NJ 08901-8559. Tel: 732-932-0150 ext. 2104 or Email: humansubjects@orsp.rutgers.edu
Name of participant (print) ____________________________ Date ____________________________

Signature of Participant ____________________________ Signature of Investigator ____________________________

I have received a copy of this statement for my records_______
(initials)

This informed consent form was approved by the Rutgers Institutional Review Board for the Protection of Human Subjects on ______; approval of this form expires on ______.
APPENDIX II
Questionnaires
Demographic and Health Information

Instructions
Please answer these questions about you to the best of your knowledge and make sure you answer every question. Thank you for your time.

GENERAL INFORMATION ABOUT YOU

Please provide the following information:

1. Name: ____________________________
2. Date of birth: 
   month ___ day ___ year ___
3. Age: __________
4. Gender: 
   1 male  2 female
5. Contact Telephone Number: ________________________________
6. Email Address: ________________________________
7. Home Address: ________________________________
8. Occupation: ________________________________
9. Were you born in the United States? 
   1 Yes  2 No
   If “No,” Please write in the country in which you were born: ________________________________
10. To which of the following races do you consider yourself to belong? You may choose all that apply.
   1 Black or African-American  2 White  3 American Indian or Alaska native
   4 Asian or Pacific islander  5 Hispanic or Latino
   Other (please specify): ________________________________
11. In addition, which of the following groups describes your ethnicity? You may choose all that apply.

- [ ] 1. African (please specify):
- [ ] 10. Chinese
- [ ] 11. Korean
- [ ] 2. West Indian / Caribbean (please specify):
- [ ] 12. Filipino
- [ ] 13. Vietnamese
- [ ] 3. Mexican / Mexican-American/ Chicano
- [ ] 14. Other Asian (please specify):
- [ ] 15. Native Hawaiian
- [ ] 16. Guamanian or Chamorro
- [ ] 4. Other Latino/Hispanic (please specify):
- [ ] 17. Samoan
- [ ] 18. Tongan
- [ ] 5. Asian Indian
- [ ] 19. Other (please specify):
- [ ] 6. Japanese
- [ ] 20. None of the above

B. HEALTH INFORMATION

12. Do you have a history of or are currently being treated for any of the following medical conditions? (Please check all that apply.)

- [ ] 1. Diabetes (Type I or Type II)
- [ ] 2. Heart problems
- [ ] 3. Blood problems (haemophilia)
- [ ] 4. Kidney problems
- [ ] 5. Hypertension
- [ ] 6. Stroke
- [ ] 7. PKU (phenylketonuria)
- [ ] 8. Otitis Media (chronic ear infection, especially as a young child)
- [ ] 9. Severe hayfever or allergies
- [ ] 10. Asthma
- [ ] 11. Cancer
- [ ] 12. Sinusitis

Continued on next page
13. “Have you had a cold/flu or ear infection in the past 2 weeks?” (Please check one.)

☐ YES  ☐ NO

If yes, please describe:

14. What, if any, prescription medications are you currently taking (including birth control) and how often?

15. Have you been to the dentist in the past 2 weeks? (Please check one.)

☐ YES  ☐ NO

16. Have you had hay fever/nasal allergies in the past two weeks? (Please check one.)

☐ YES  ☐ NO

17. Do you dislike or avoid eating certain foods? (Please check one.)

☐ YES  ☐ NO

If yes, please describe

18. Do you have any food allergies? (Please check one.)

☐ YES  ☐ NO

If yes, please describe

19. Are you currently dieting to lose weight? (Please check one.)

☐ YES  ☐ NO
20. How many times have you been on a diet to lose weight over the past six months? ______

21. Have you unintentionally gained or lost more than five pounds in the past six months? (Please check one.)

☐ a. YES  ☐ b. NO

22. What is your current height?

☐ FT.  ☐ IN.  | OR  ☐ M.

23. What is your current weight?

☐ LBS.  OR  ☐ KG

24. What is the highest weight you have ever been?

☐ LBS.  OR  ☐ KG

25. What is the lowest weight you have ever been?

☐ LBS.  OR  ☐ KG

26. Do you currently smoke? (Please check one.)

☐ a. YES  ☐ b. NO

If yes, please specify cigarettes, cigar, or pipe

_________________________

27. If you smoke, how many:
   cigarettes per day? _________
   cigars per day? _____________
   pipes per day? _______________
28. Have you smoked in the past?

☐ 1 YES  ☐ 2 NO

If yes, how many years ago did you quit?

_______________________________________

29. Have you taken multi-vitamins or Vitamin A, C, or E supplements in the past month?

☐ 1 YES  ☐ 2 NO

30. On average, how many hours of sleep do you get per night?____________________

31. How often do you try unfamiliar foods?

☐ 1 Never  ☐ 2 Rarely  ☐ 3 Sometimes  ☐ 4 Often  ☐ 5 Very Often
C. FAMILY INFORMATION

Please answer the following questions about your family.

32. What is the highest education level you have finished? (Please “X” only one answer)

- [ ] 6th grade or less
- [ ] 8th grade or less
- [ ] Attended some High School
- [ ] High School Graduate or GED
- [ ] Technical School
- [ ] Some College
- [ ] College Graduate
- [ ] Post Graduate Study

33. What was the approximate total income, before taxes, of your household last year? Please include wages, salaries, social security, interest, child support, public assistance, unemployment compensation, rent from property and all other income. (Please “X” only one answer)

- [ ] Less than $5,000
- [ ] $5,000 - $9,999
- [ ] $10,000 - $19,999
- [ ] $20,000 - $29,999
- [ ] $30,000 - $39,999
- [ ] $40,000 - $49,999
- [ ] $50,000 - $59,999
- [ ] $60,000 - $69,999
- [ ] $70,000 - $79,999
- [ ] $80,000 - $89,999
- [ ] $90,000 - $99,999
- [ ] Over $100,000

Thank you. You are done with this form. Please return this form to the test administrator.
Adult Eating Behavior Questionnaire

Please read each question and than decide whether each item is true in relation to you, using the following rating scale: never; rarely; sometimes; often; very often. Check the box that corresponds to your rating. Please respond to all items, making sure that you check the box for the rating that is true about you. If you make a mistake or need to change an answer, change the check to a cross and then check the correct box. (CHECK ONLY ONE BOX FOR EACH.)

<table>
<thead>
<tr>
<th></th>
<th>Question</th>
<th>Never</th>
<th>Rarely</th>
<th>Sometimes</th>
<th>Often</th>
<th>Very Often</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>Do you have the desire to eat when you are irritated?</td>
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<td>2</td>
<td>If food tastes good to you, do you eat more than usual?</td>
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<td>3</td>
<td>Do you have a desire to eat when you have nothing to do?</td>
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<td>4</td>
<td>If you have put on weight, do you eat less than you usually do?</td>
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<td>5</td>
<td>Do you have a desire to eat when you are depressed or discouraged?</td>
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<td>6</td>
<td>If food smells and looks good, do you eat more than usual?</td>
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<td>7</td>
<td>How often do you refuse food or drink offered because you are concerned about your weight?</td>
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<td>8</td>
<td>Do you have a desire to eat when you are feeling lonely?</td>
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<td>9</td>
<td>If you see or smell something delicious, do you have a desire to eat it?</td>
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<td>10</td>
<td>Do you have a desire to eat when somebody lets you down?</td>
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<td>11</td>
<td>Do you try to eat less at mealtimes than you would like to eat?</td>
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<td>12</td>
<td>If you have something delicious to eat, do you eat it straight away?</td>
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<td>Question</td>
<td>Never</td>
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<td>13. Do you have a desire to eat when you are angry?</td>
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<td>14. Do you watch exactly what you eat?</td>
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<td>15. If you walk past the baker do you have the desire to buy something delicious?</td>
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<td>16. Do you have a desire to eat when you are approaching something unpleasant to happen?</td>
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<td>17. Do you deliberately eat foods that are slimming?</td>
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<td>18. If you see others eating, do you also have the desire to eat?</td>
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<td>19. When you have eaten too much, do you eat less than usual the following days?</td>
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<td>20. Do you get the desire to eat when you are anxious, worried to tense?</td>
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<td>21. Do you find it hard to resist eating delicious foods?</td>
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<td>22. Do you deliberately eat less in order not to become heavier?</td>
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<td>23. Do you have a desire to eat when things are going against you or when things have gone wrong?</td>
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<td>24. If you walk past a snack bar or a café, do you have the desire to buy something delicious?</td>
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<td>25. Do you have the desire to eat when you are emotionally upset?</td>
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<td>26. How often do you try not to eat between meals because you are watching your weight?</td>
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<td>27. Do you eat more than usual, when you see others eating?</td>
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<td>28. Do you have a desire to eat when you are bored or restless?</td>
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<tr>
<td>Question</td>
<td>Never</td>
<td>Rarely</td>
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<td>29. How often in the evening do you try not to eat because you are watching your weight?</td>
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<td>30. Do you have a desire to eat when you’re frightened?</td>
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<td>31. Do you take into account your weight with what you eat?</td>
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<td>32. Do you have a desire to eat when you are disappointed?</td>
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<td>33. When you are preparing a meal are you inclined to eat something?</td>
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<td>34. How often do you try unfamiliar foods?</td>
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</table>
Paper Disc Samples

Instructions:

You will receive two paper discs to taste. Rinse your mouth thoroughly with water before you begin. Place the disc that matches the number below on the tip of the tongue for 30 seconds or until it is wet. Rate the intensity of the taste of the paper disc by drawing a mark on the scale for your answer. You can draw your mark on any place on the scale. For the next sample, go to the next page.

First sample #: 151

Strongest Imaginable

Very Strong

Strong

Moderate

Weak

Barely Detectable
Please rinse with water and wait for 45 seconds before you begin.

Second sample #: 627
Appendix III

$\pi$-Glutathione-s-Transferase Analysis
1. **Background**

   Glutathione-s-transferases (GSTs) are induced by isothiocyanate compounds (the breakdown products of glucosinolates) and these compounds also serve as substrates for GSTs [143]. These glucosinolate and isothiocyanate compounds are found in the *Brassica* family have been linked to reduced cancer risk [144]. Increased intake of *Brassica* vegetables, therefore, may increase the concentrations of GST in human blood plasma. Previous research has indicated differences in preferences, and perhaps intake, among PROP taster groups with regards to glucosinolate-containing vegetables, so differences in GST concentrations in blood plasma might be observed among taster groups [25]. If differences are seen, GSTs in plasma could be an indicator of long-term bitter vegetable intake.

   This pilot study aimed to see if differences in $\pi$-GST concentrations existed between PROP NT and ST. The hypothesis was that NT would have higher intakes of *Brassica* vegetables and, therefore, higher levels of $\pi$-GST in their plasma than PROP ST. If such differences exist, perhaps $\pi$-GST could be used as a biomarker for glucosinolate-containing vegetable intake based on taste genetics.

2. **Method**

   **2.1 Blood Sample Collection**

   Thirty-four blood samples were collected from healthy, female subjects by a trained phlebotomist after an overnight fast in a vacutainer containing EDTA. The samples were centrifuged within one-half hour of collection for fifteen minutes at 3200 rpm. The plasma was transferred to freezer tubes and kept frozen at -70C until the analysis for $\pi$-
GST could be performed. Seventeen NT plasma samples and seventeen ST plasma samples were analyzed for $\pi$-GST.

2.2 $\pi$-GST Analysis

Plasma samples were analyzed for $\pi$-GST using the method provided with the Biotrin $\pi$-GST EIA kit (Biotrin International, Ltd., Dublin, Ireland) as follows. A wash solution was made by diluting (1/20) a 20x phosphate buffered saline/Tween 20 with deionized water. Calibrators were prepared using the provided calibrator stock solution and the sample dilutent (protein solution with added stabilizers) at the following concentrations: 100, 50, 25, 12.5, 6.25, 3.12, and 0 $\mu$g/L. Plasma samples were thawed on ice and immediately upon thawing, 50 $\mu$L of sample was diluted with 200 $\mu$L sample dilutent. The calibrators, positive control ($\pi$-GST in protein with added stabilizers), and samples were added to a Microassay plate, the plate was covered and incubated at room temperature for 60 minutes while being uniformly shaken. The cover was then removed and the wells were washed four times with the prepared wash solution (250-350 $\mu$L/well). 100 $\mu$L conjugate (anti-$\pi$-GST IgG conjugated to horseradish peroxidase) was added to each well and the plate was covered and incubated as before. After incubation, the wells were washed as before. 100 $\mu$L substrate (stabilized liquid TMB solution) was added to each well using a multi-channel pipet and the plate was incubated for exactly fifteen minutes. After fifteen minutes, 100 $\mu$L stop solution (0.5M sulphuric acid) was added and the plate was immediately read at 450 nm, using 630 nm as a reference. Of the thirty-four samples analyzed, 27 were analyzed in duplicate, while the remaining seven samples were analyzed once.
2.3 Food Group Analysis

Dark green vegetable intake information was collected using three, 24-hour diet recalls, using NDS-R software (NCC, Minneapolis, MN). An average of the three recalls was taken for each subject and this value was used for dietary intake of dark, green vegetables. The vegetables included in this group are based on the USDA food groups and include: broccoli, collard greens, romaine lettuce, spinach, etc. The mean intakes by NT and ST group can be seen in Table 1.

3. Results

A calibration curve was plotted and the best-fit line used the equation: $y = 1.1199x^3 + 6.7902x^2 + 24.061x - 0.2985$ ($r^2 = 0.9996$). $\pi$-GST concentrations were calculated based on this equation and the range of concentrations was 10.8 – 165 μg/L. The mean values of dark, green vegetable intake and $\pi$-GST by taster group can be seen in Table 1. There were no differences between taster groups for either dark, green vegetable intake or $\pi$-GST plasma concentration. All statistical analysis was performed using Statistical Analysis Software (SAS, Cary, NC).

Table 1: Mean dark, green vegetable intake (servings/day) and $\pi$-GST concentrations (μg/L) by PROP taster group. Mean ± SE.

<table>
<thead>
<tr>
<th></th>
<th>NT (n=17)</th>
<th>ST (n=17)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dark Green Vegetable</td>
<td></td>
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</tr>
<tr>
<td>Intake (servings/day)</td>
<td>1.7 ± 0.3</td>
<td>1.8 ± 0.3</td>
</tr>
<tr>
<td>$\pi$-GST in Plasma</td>
<td>32.9 ± 9.1</td>
<td>28.8 ± 6.1</td>
</tr>
</tbody>
</table>
A median (median = 1.5 servings/day) split of dark, green vegetable intake was taken and subjects were divided into “high” or “low” dark, green vegetable intake groups as well as NT and ST groups. When the data were divided this way, there appeared to be a trend for the NT in the “high” dark, green vegetable intake group to have the highest GST concentrations in plasma, although this difference was not significant (NT “low” intake n = 10, NT “high” intake n = 7, ST “low” intake n = 6, ST “high” intake n = 11). These data can be seen in Figure 1.

Figure 1: Mean (±SE) π-GST concentrations by dark, green vegetable intake group and PROP taster group. There were no significant differences among groups by ANOVA.

4. Discussion

There were no significant differences between taster groups in either π-GST plasma concentrations or dark, green vegetable intake, however the interaction between these
two variables showed a trend in the expected direction, that NT would have the highest levels of $\pi$-GST in plasma. This trend was seen only among those subjects with “high” intakes of dark, green vegetables, indicating that a certain level of cruciferous vegetable intake may need to be reached in order to elucidate differences in blood plasma levels of $\pi$-GST.

The dark, green vegetable food group did contain non-glucosinolate-containing vegetables, so future analysis may want to focus solely on those types of vegetables, eliminating those not of interest. Additionally, subjects with high intakes of these types of Brassica vegetables would be most relevant to the study; therefore targeted recruiting may be necessary in order to better obtain subjects of interest. $\pi$-GST levels seem to vary by taster status and may indicate consumption of foods based on genetic influences. This enzyme may be able to be used as a biomarker for dark, green vegetable intake, and, therefore, dietary patterns and health status based on genetic taste differences.