Genetic Determinants of Plasma $\alpha$-tocopherol

by

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A thesis submitted in conformity with the requirements for the degree of Master of Science
Department of Nutritional Sciences
University of Toronto

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Abstract

\( \alpha \)-tocopherol is the most abundant form of vitamin E in human plasma and tissues. Inter-individual differences in plasma \( \alpha \)-tocopherol concentration or its response to dietary \( \alpha \)-tocopherol may be due, in part, to polymorphisms in vitamin E metabolism genes (\( \alpha \)-tocopherol transfer protein (\( \alpha \)-TTP), tocopherol associated protein (TAP) and CYP4F2). The thesis objectives were to determine whether common polymorphisms in the \( \alpha \)-TTP (rs6994076 A>T), TAP (rs2072157 C>T and Arg11Lys) and CYP4F2 (Val433Met) genes influence plasma \( \alpha \)-tocopherol concentration or modify the association between dietary and plasma \( \alpha \)-tocopherol. Subjects (n=1248), 20 to 29 years from the Toronto Nutrigenomics and Health study completed a food frequency questionnaire. Fasting blood samples were used for genotyping and to measure plasma \( \alpha \)-tocopherol concentration. The \( \alpha \)-TTP and TAP Arg11Lys polymorphisms significantly altered plasma \( \alpha \)-tocopherol. The \( \alpha \)-TTP polymorphism only influenced plasma \( \alpha \)-tocopherol in individuals not using supplements. None of the polymorphisms examined modified the plasma \( \alpha \)-tocopherol response to dietary \( \alpha \)-tocopherol.
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<tr>
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<td>α-tocopherol transfer protein</td>
</tr>
<tr>
<td>A</td>
<td>Adenine</td>
</tr>
<tr>
<td>A/A</td>
<td>Homozygote for the adenine allele</td>
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<td>A/T</td>
<td>Heterozygote for the adenine and thymine alleles</td>
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<tr>
<td>A&gt;T</td>
<td>Adenine to thymine nucleic acid substitution</td>
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<tr>
<td>ABCA1</td>
<td>ATP-binding cassette A1 transporter</td>
</tr>
<tr>
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<td>Applied Biosystems</td>
</tr>
<tr>
<td>ACD</td>
<td>Acid-trisodium citrate dextrose</td>
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<td>Apolipoprotein</td>
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<td>Arg158Cys</td>
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<tr>
<td>ATBC</td>
<td>α-tocopherol β-carotene cancer prevention clinical trial</td>
</tr>
<tr>
<td>AVED</td>
<td>Ataxia with vitamin E deficiency</td>
</tr>
<tr>
<td>BHT</td>
<td>Butylated hydroxytoluene</td>
</tr>
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<td>BMI</td>
<td>Body mass index</td>
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<td>Cytosine</td>
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<tr>
<td>DRI</td>
<td>Dietary Reference Intake</td>
</tr>
<tr>
<td>EAR</td>
<td>Estimated Average Requirement</td>
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<td>FFQ</td>
<td>Food frequency questionnaire</td>
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<td>Food preference checklist</td>
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<td>Guanine</td>
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<td>HDL</td>
<td>High density lipoprotein</td>
</tr>
<tr>
<td>HPLC</td>
<td>High performance liquid chromatography</td>
</tr>
<tr>
<td>HWE</td>
<td>Hardy-Weinberg equilibrium</td>
</tr>
<tr>
<td>IDL</td>
<td>Intermediate density lipoprotein</td>
</tr>
<tr>
<td>IU</td>
<td>International unit</td>
</tr>
<tr>
<td>LDL</td>
<td>Low density lipoprotein</td>
</tr>
<tr>
<td>LPL</td>
<td>Lipoprotein lipase</td>
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<tr>
<td>Lys</td>
<td>Lysine</td>
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<td>mdr2</td>
<td>Multidrug resistance 2</td>
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<tr>
<td>Acronym</td>
<td>Full Form</td>
</tr>
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<td>------------------------------------------------</td>
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<td>Metabolic equivalent of task</td>
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<td>Phosphoinositide 3-kinase</td>
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<tr>
<td>PKC</td>
<td>Protein kinase C</td>
</tr>
<tr>
<td>PLTP</td>
<td>Phospholipid transfer protein</td>
</tr>
<tr>
<td>PP2A</td>
<td>Protein phosphatase 2A</td>
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<tr>
<td>PUFA</td>
<td>Polyunsaturated fatty acid</td>
</tr>
<tr>
<td>PXR</td>
<td>Pregnane X receptor</td>
</tr>
<tr>
<td>RDA</td>
<td>Recommended Dietary Allowance</td>
</tr>
<tr>
<td>SAS</td>
<td>Statistical Analysis Systems</td>
</tr>
<tr>
<td>SNP</td>
<td>Single nucleotide polymorphism</td>
</tr>
<tr>
<td>SPF</td>
<td>Supernatant protein factor</td>
</tr>
<tr>
<td>SR-BI</td>
<td>Scavenger receptor class B type I</td>
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<tr>
<td>SRM</td>
<td>Standard reference material</td>
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<td>T</td>
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<td>TAP</td>
<td>Tocopherol associated protein</td>
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<td>Toronto Nutrigenomics and Health study</td>
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<td>Very low density lipoprotein</td>
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Chapter ONE:
Introduction
Vitamin E is a generic term used to describe eight lipid-soluble compounds of related structure [1]. These compounds include the four tocopherols (α-, β-, γ- and δ-tocopherol) and the four tocotrienols (α-, β-, γ- and δ-tocotrienol) [1]. Vitamin E is a major lipophilic antioxidant in cellular membranes which acts by terminating the propagation phase of lipid autooxidation [2]. In addition, vitamin E also has several non-antioxidant functions including gene regulation and the inhibition of smooth muscle cell proliferation, monocyte-endothelial adhesion, monocyte reactive oxygen species and cytokine release and platelet adhesion and aggregation [3-6]. While γ-tocopherol is the most common form of vitamin E in the diet, α-tocopherol is the most biologically active and the predominant vitamin E form in the plasma and tissues of humans and animals as a result of its selective retention by the body [7, 8]. This difference in the retention between the eight forms of vitamin E occurs post-absorption and stems largely from the action of the liver proteins, α-tocopherol transfer protein (α-TTP) and CYP4F2 [8-11].

Plasma α-tocopherol concentration is often used as a biomarker for short-term vitamin E nutritional status [12]. Since plasma α-tocopherol concentration is a function of α-tocopherol absorption and metabolism, it may be a better indicator of vitamin E exposure for epidemiological studies [13, 14]. In addition to dietary α-tocopherol, several other factors can influence plasma α-tocopherol concentration [15]. These factors, however, do not fully explain all the variability in plasma α-tocopherol concentration [16-21]. Furthermore, there is a large variation in the plasma α-tocopherol response to dietary α-tocopherol between individuals [22, 23]. Therefore, further investigation is required in order to determine other
variables that can alter plasma α-tocopherol concentration or its response to dietary α-tocopherol.

Genetics may be able, in part, to explain some of this unaccounted variability in plasma α-tocopherol concentration and its response to dietary α-tocopherol. Candidate genes include those that are involved in vitamin E metabolism since plasma α-tocopherol concentration is a function of α-tocopherol absorption, cellular uptake and efflux, utilization, catabolism and excretion. Variation in genes involved in any of these processes could potentially lead to differences in plasma α-tocopherol concentrations between individuals. Therefore, the present thesis examined whether polymorphisms in three candidate vitamin E metabolism genes influence plasma α-tocopherol concentration or modify its response to dietary α-tocopherol. The three genes studied were the α-tocopherol transfer protein (facilitates the selective secretion of α-tocopherol into very low density lipoproteins resulting in the selective retention of α-tocopherol by the body compared with other vitamin E forms), tocopherol associated protein (cellular α-tocopherol binding protein) and CYP4F2 (α-tocopherol catabolism). Knowledge of the determinants of plasma α-tocopherol concentration is important in order to better assess vitamin E nutritional status.
Chapter TWO:
Literature Review
2.1 Vitamin E chemistry

The term vitamin E encompasses eight lipid-soluble compounds of related structure which include α-, β-, γ- and δ-tocopherol and α-, β-, γ- and δ-tocotrienol [1]. Each of these compounds contains a chromanol ring structure with a hydroxyl group attached to carbon 6 and a varying number of methyl groups (Figure 1) [1]. The number and position of these methyl groups on the chromanol ring determines whether the compound is given the α-, β-, γ- or δ designation. The α form has three methyl groups at carbon positions 5, 7 and 8. The β and γ forms both have two methyl groups at carbons 5 and 8 and carbons 7 and 8, respectively. The δ form only has one methyl group which is attached to carbon 8 [1]. The chromanol ring is attached to a 16 carbon atom long side chain at carbon position 2 [1]. The tocoferols contain a saturated phytol side chain, while the tocotrienols contain an unsaturated isoprenoid side chain, consisting of three double bonds at position 3’, 7’ and 11’ [1].

The addition of the phytol chain to the chromanol ring results in three chiral centres for the tocopherols at positions 2, 4’ and 8’. Of the eight possible tocopherol stereoisomers, the RRR (also named d-tocopherol) configuration is naturally occurring [1, 24, 25]. Only one chiral centre is present in the tocotrienols at position 2 [24]. Similarly to the tocopherols, the R configuration occurs naturally [24].

Although γ-tocopherol is the form of vitamin E most common in the diet, α-tocopherol is the most biologically active and the predominant form found in the plasma and tissues of both humans and animals due to the selective retention of α-tocopherol by the body.
(The mechanism for this selective retention is reviewed in section 2.4.1.) As a result, commercially available vitamin E supplements typically contain only α-tocopherol [1]. The supplement either contains natural α-tocopherol (RRR-α-tocopherol or d-α-tocopherol) or synthetic α-tocopherol (all-rac-α-tocopherol or dl-α-tocopherol) which is a mixture containing equal amounts of the eight stereoisomers of α-tocopherol [1, 25]. RRR-α-tocopherol is more bioavailable than all-rac-α-tocopherol as the four 2R stereoisomers of all-rac-α-tocopherol are preferentially retained by the body compared with the four 2S stereoisomers [26]. Regardless of the form, α-tocopherol in supplements is commonly in ester form, with either an acetate or succinate conjugated to the O atom at position 6 of the chromanol ring to form α-tocopheryl acetate or α-tocopheryl succinate, respectively [1, 27].

The addition of the acetate or succinate to α-tocopherol renders it more stable as it is less prone to oxidation [1, 27]. Vitamin E supplements may be sold in international units (IU), where 1 IU of vitamin E is equal to 1 mg of all-rac-α-tocopheryl acetate [1]. The conversion factors to convert from mg to IU vary depending on whether the supplement is in the natural or synthetic form and also whether it is in free or ester form. For the synthetic forms of α-tocopherol, the following conversion factors from the United States Pharmacopoeia are used [1]:

- 1 mg of all-rac-α-tocopherol is equal to 1.10 IU
- 1 mg of all-rac-α-tocopheryl acetate is equal to 1 IU
- 1 mg of all-rac-α-tocopheryl succinate is equal to 0.89 IU.

If the supplement contains natural α-tocopherol, the conversion factors are [1]:

- 1 mg of RRR-α-tocopherol is equal to 1.49 IU
- 1 mg of RRR-α-tocopheryl acetate is equal to 1.36 IU
1 mg of RRR-α-tocopheryl succinate is equal to 1.21 IU.

Although these are the established ratios, there is still some debate regarding whether these ratios hold true [28-30].

![Chemical structure of vitamin E](image)

**Figure 1.** The chemical structure of vitamin E. Vitamin E contains a chromanol ring structure with a hydroxyl group attached to carbon 6. The chromanol ring is attached to a 16 carbon atom long side chain at carbon position 2. a) If the side chain is a saturated phytol side chain, the compound is referred to as a tocopherol. b) If the side chain is an unsaturated isoprenoid side chain, consisting of three double bonds at position 3’, 7’ and 11’, the vitamin E compound is referred to as a tocotrienol. c) The number and position of methyl groups on the chromanol ring determines whether the compound (tocopherol or tocotrienol) is designated as α-, β-, γ- or δ.

2.2 Vitamin E function

Vitamin E was first discovered in 1922 as an anti-sterility factor essential for rat reproduction and required for a full term pregnancy in the rat [2, 24]. The term tocopherol reflects this function as it comes from the Greek words “tokos” and “phorein” meaning “to
bring forth childbirth” [2, 24]. Later, the antioxidant activity of vitamin E was discovered and vitamin E is now known as a major lipophilic antioxidant in cellular membranes [2]. In the antioxidant reaction, vitamin E donates the hydrogen atom from the hydroxyl group located at carbon 6 of the chromanol ring to the lipid peroxyl radical resulting in a stable, non-radical hydroperoxide lipid and a tocopheroxyl radical [1]. The reaction of the peroxyl radical with vitamin E occurs at a faster rate than the reaction of the peroxyl radical with another lipid [1]. This has the effect of removing the lipid radical before it reacts with another lipid, thereby terminating the propagation (chain reaction) phase of autooxidation and preventing subsequent free radical damage [1]. Although in the process vitamin E becomes a free radical itself, the tocopheroxyl radical is resonance stabilized and is thus a relatively stable radical [24]. This vitamin E radical has several fates. It may react with another tocopheroxyl radical to form a non-radical product [1]. Alternatively, the tocopheroxyl radical may be reduced back to its non-radical vitamin E form by vitamin C or ubiquinol [1, 31-34]. In addition, it is possible that the vitamin E radical may also act as a pro-oxidant by extracting a hydrogen atom from a lipid resulting in the formation of a lipid free radical however, the tocopheroxyl radical is more likely to react with another radical before it will extract a hydrogen atom from a lipid [1].

In addition to its antioxidant capabilities, non-antioxidant activities of vitamin E have been described. For example, vitamin E plays a role in gene regulation at the transcriptional, post-transcriptional and post-translational levels [35-47]. Examples of vitamin E-regulated genes include α-tropomyosin [35], collagenase [36, 37], α-tocopherol transfer protein [38-40], the scavenger receptors CD-36, SR-A and SR-BI [41-44], 5-lipoxygenase [45, 46] and cholesterol biosynthesis genes [47]. All forms of vitamin E are able to activate the pregnane X
receptor (PXR), which is a nuclear receptor that activates various enzymes involved in drug metabolism [48]. Inhibition of protein kinase C (PKC) by \( \alpha \)-tocopherol has also been shown [4, 49]. The mechanism involves an \( \alpha \)-tocopherol-induced activation of protein phosphatase 2A (PP2A) [50]. Upon activation, PP2A dephosphorylates PKC, thereby resulting in its inhibition [50]. This function is performed by \( \alpha \)-tocopherol, but not by \( \beta \)-tocopherol, a similar antioxidant, or other antioxidants, suggesting a non-antioxidant role of \( \alpha \)-tocopherol [49, 50]. In addition, vitamin E inhibits smooth muscle cell proliferation [4], monocyte-endothelial adhesion [5, 51, 52], monocyte reactive oxygen species and cytokine release [5, 46] and platelet adhesion and aggregation [6, 53]. Most of these effects are attributed to the gene regulation function of vitamin E. There is some debate, however, regarding whether gene regulation by vitamin E is a result of an antioxidant or non-antioxidant function [54, 55].

### 2.2.1 Vitamin E and health outcomes

The functions of vitamin E have prompted the hypothesis that vitamin E may have beneficial effects towards various health outcomes. Several health outcomes in relation to vitamin E have been studied, some of which include cardiovascular disease, cancer, cataract, Alzheimer's disease, Parkinson's disease, diabetes and immune function [56-61]. These studies, however, have yielded inconsistent findings. A beneficial or no effect of vitamin E on the health outcome has been reported, while a few studies have even found an adverse effect [56-62]. In addition, a meta-analysis suggested that high intakes of vitamin E exceeding 400 IU per day may increase the risk of all-cause mortality [63]. This meta-analysis has been criticized, however, for its selection of studies and statistical analysis [64-74].
There are several potential reasons that may explain the inconsistencies observed in these studies. Vitamin E may be a marker for a healthy diet or lifestyle or vitamin E in conjunction with other nutrients may work together to promote health and to combat chronic disease [56, 57, 62]. For example, vitamin E that has undergone antioxidant reactions requires other antioxidants such as vitamin C to regenerate it from its tocopheroxyl radical form back to its non-radical form following antioxidant reactions [1, 31-33]. If only vitamin E is administered in clinical trials, there may not be enough of the other nutrients that vitamin E interacts with in order for these interactions between vitamin E and other nutrients to occur. This may explain the differences in findings between observational and clinical trials. In addition, there is an inverse relationship between \( \alpha \)-tocopherol and \( \gamma \)-tocopherol, where high doses of \( \alpha \)-tocopherol lower levels of \( \gamma \)-tocopherol in the blood as a result of an increase in the metabolism and elimination of \( \gamma \)-tocopherol from the body following the ingestion of high doses of \( \alpha \)-tocopherol [75]. If adequate levels of \( \gamma \)-tocopherol are important in relation to the health outcome, as has been previously suggested, studies administering high doses of \( \alpha \)-tocopherol supplements may eliminate any benefits of \( \gamma \)-tocopherol [56, 76].

Other potential reasons for these inconsistencies include the dose of the vitamin E supplement used in clinical trials, the duration of supplementation and the form of the vitamin E in the supplement (\( RRR \)- or \( all-rac-\alpha \)-tocopherol) [56, 57, 62]. As discussed in section 2.4.1, whether vitamin E is taken with a meal or on an empty stomach can influence its bioavailability [77]. The fat content of the meal and the food matrix can also affect vitamin E bioavailability [78-81]. Therefore, whether the way in which the supplement was ingested
was standardized between subjects and between studies (for example, taking the supplement with milk following breakfast) may explain some of these inconsistencies [57, 62].

Differences in subject selection, geographical location and disease state (healthy, high-risk or diseased subjects) between studies may be another reason for the inconsistent findings [56, 62]. Prospective observational studies may involve initially healthy subjects whereas clinical trials often recruit subjects who are at high risk for developing the disease under investigation. If vitamin E has a preventative effect or if the timing of vitamin E exposure is important, it may be too late for high doses of vitamin E to have a beneficial effect on high-risk subjects. In addition, these high-risk subjects may be taking medications [62]. Since vitamin E activates the pregnane X receptor, which is a nuclear receptor that activates various drug metabolizing enzymes, it may be possible that any benefits of vitamin E supplementation may be masked by the harmful effects of the quick metabolism of subjects’ drugs [48, 62]. One study has tested whether vitamin E influences the metabolism of the drug midazolam [82]. Although vitamin E supplementation was found to have no effect on the metabolism of the medication, it may be possible that other medications are more vulnerable to vitamin E-induced drug metabolism. An additional explanation for the inconsistencies between studies is variation in the response to dietary vitamin E between individuals. This could lead to differences in the amount of vitamin E in the blood and tissues between subjects which could potentially influence the effect of vitamin E on the health outcome under study.
2.3 Dietary vitamin E

2.3.1 Canadian dietary recommendation for vitamin E

The Canadian recommendation for dietary vitamin E follows the 2000 Dietary Reference Intake (DRI) for vitamin E [83]. The DRI consists of an Estimated Average Requirement (EAR) and a Recommended Dietary Allowance (RDA) [83]. The EAR is defined as the daily intake of a nutrient that is estimated to meet the nutrient requirement in half the healthy individuals in a life stage or sex group [83]. The requirement is defined by an indicator of adequacy, which for vitamin E, is the vitamin E intake sufficient to achieve a plasma $\alpha$-tocopherol concentration (12 $\mu$mol/L) that is sufficient to prevent hydrogen peroxide-induced hemolysis (12% or less) [83]. These cut-points were established because at $\alpha$-tocopherol concentrations below 12 $\mu$mol/L, a large increase in hydrogen peroxide-induced hemolysis has been reported to occur [83, 84]. It was found that an intake of 12 mg of $\alpha$-tocopherol in experimentally depleted individuals resulted in a plasma $\alpha$-tocopherol concentration of 12 $\mu$mol/L [83, 85]. Thus, the EAR for vitamin E was set at 12 mg of $\alpha$-tocopherol for males and females aged 14 and up [83].

The RDA is calculated from the EAR and is defined as the average daily nutrient intake that is sufficient to meet the nutrient requirement of nearly all (97 to 98 percent) healthy individuals in a particular life stage and sex group [83]. The RDA for vitamin E for males and females aged 14 and up is 15 mg of $\alpha$-tocopherol for males and females [83]. There is no difference in the EAR or RDA for pregnant females [83]. The EAR and RDA for lactating females are 16 mg and 19 mg $\alpha$-tocopherol, respectively, to account for the amount of $\alpha$-tocopherol secreted in human milk during the first six months of lactation (4 mg) [83]. It
is important to note that the EAR and RDA apply only to the $\text{RRR-}\alpha$-tocopherol form of vitamin E from food as well as the $2R$ stereoisomers of synthetic $\alpha$-tocopherol ($\text{RRR-}$, $\text{RSR-}$, $\text{RRS-}$ and $\text{RSS-}\alpha$-tocopherol) that can be found in fortified food and supplements [83]. The other forms of vitamin E are not included in the current DRI because they are preferentially excreted and metabolized by the body, while $\alpha$-tocopherol is selectively retained [83] (as reviewed in section 2.4.1).

2.3.2 Sources and intake of vitamin E

Humans are unable to synthesize vitamin E and therefore, as an essential micronutrient, vitamin E must be obtained from the diet. Good sources of vitamin E include vegetable oils (corn, canola, olive, safflower, sunflower and wheat germ oils), nuts (particularly almonds), sunflower seeds, wheat germ, whole grains, green leafy vegetables and avocado [24, 83, 86-88]. Often, however, foods that are not particularly rich in vitamin E but are consumed frequently tend to be greater contributors to an individual’s vitamin E intake [88-90]. The major contributors to vitamin E intake can vary depending on the population. For example, Maras et al. found that sweet baked products, which do not contain high amounts of $\alpha$-tocopherol, provided more than 8% of total $\alpha$-tocopherol intake to the diets of Americans (ranked as the second main contributor of $\alpha$-tocopherol intake) because they are consumed frequently [88]. Conversely, in this same population, almonds and sunflower seeds, which are richer sources of $\alpha$-tocopherol, were not major contributors to $\alpha$-tocopherol intake because they were consumed less frequently [88]. In a Spanish population, fresh fruits and vegetables (particularly apples, pears, tomato and lettuce) contributed more
than 25% to vitamin E intake, whereas, nuts and seeds, which contain greater amounts of vitamin E, only contributed 8% [89].

Of the various forms of vitamin E, \( \gamma \)-tocopherol is the most common in the diet. It is estimated that individuals consume two to four times as much \( \gamma \)-tocopherol as \( \alpha \)-tocopherol from the diet [16, 91]. Despite this, \( \alpha \)-tocopherol is the most biologically active and the predominant form of vitamin E found in the blood and tissues of both humans and animals and there is ten times as much \( \alpha \)-tocopherol in the body as \( \gamma \)-tocopherol [7, 8, 92]. This difference arises because the body selectively retains \( \alpha \)-tocopherol, while the other vitamin E forms get preferentially eliminated from the body (as is reviewed in section 2.4.1) [83]. It is for this reason that the current RDA for vitamin E of 15 mg of \( \alpha \)-tocopherol for males and females aged 14 and up is expressed in milligrams of \( \alpha \)-tocopherol [83].

The average vitamin E intake from various populations tends to be below the RDA [88, 89, 93-97]. In a representative sample of Americans living in the United States, only 8.0% of men and 2.4% of women met the EAR of 12 mg of \( \alpha \)-tocopherol per day from food sources alone [88]. The men in this sample consumed only 56% of the EAR, while the women consumed 39% of the EAR [88]. In another representative sample of Americans, the average daily \( \alpha \)-tocopherol intake from the diet alone was 7.1 mg [94]. Although recent data from a representative Canadian population is lacking, one Canadian study reported average dietary vitamin E intake from foods ranged from 4 to 8 mg depending on the dietary assessment method used [95]. An average \( \alpha \)-tocopherol intake from food was reported to be 10.4 mg in a sample from Spain [89]. In a sample from Central-Northern Italy, \( \alpha \)-tocopherol intake from
food was 7.9 mg [96]. The majority of subjects from a South African sample (95%) had intakes below 12 mg (the Canadian EAR) [97].

Despite the reported low vitamin E intakes, clinical vitamin E deficiency is very rare and tends to only occur in individuals with certain genetic abnormalities affecting fat malabsorption, lipoprotein metabolism or maintenance of plasma $\alpha$-tocopherol concentrations [25, 83]. Although most people do not meet the RDA, an intake of 15 mg of $\alpha$-tocopherol is achievable, however, individuals must carefully choose $\alpha$-tocopherol-rich sources such as nuts and seeds [88, 93]. In spite of the low $\alpha$-tocopherol intakes described in studies, it may be possible that the actual $\alpha$-tocopherol intake is greater than that reported, but dietary assessment tools are not adequately capturing habitual $\alpha$-tocopherol intake [83]. Vitamin E is found in oils and individuals may be unable to recognize and quantify oil in their food [13, 98]. For example, individuals may not think to include added fats when they complete dietary assessments or it may be difficult for them to assess the amount of fats and oils added to food during the food preparation process [13, 83, 98]. Fats and oils can also be hidden in foods and if an individual is unaware of these hidden fats, they will not be able to accurately report their fat intake [98]. Bias such as under-reporting of fat intake, which can arise from an individual’s sensitivity to questions regarding their dietary fat intake, is very likely to occur for dietary fat reporting [83, 98]. In addition, the accuracy of assessing dietary $\alpha$-tocopherol intake is also reliant on the quality and completeness of food composition tables [99]. Food composition tables, which list the $\alpha$-tocopherol content of various foods, contain average values, however, there is a lot of variability in the amount of $\alpha$-tocopherol for a particular
food. Factors such as the product brand, harvesting, processing, storage and cooking can influence the \( \alpha \)-tocopherol content of food [18, 99-104].

2.4 Vitamin E metabolism

2.4.1 Absorption, transport and hepatic uptake and secretion of vitamin E

As a lipid-soluble vitamin, vitamin E absorption mirrors that of lipids (Figure 2) [105]. Once ingested, vitamin E becomes solubilized into mixed micelles in the intestinal lumen [106]. This process is dependent on the presence of bile acids in the intestinal lumen [107]. Before becoming solubilized by mixed micelles, vitamin E esters such as tocopheryl acetate and tocopheryl succinate are hydrolyzed by the bile acid-dependent pancreatic carboxyl ester hydrolase in the intestine [1, 106]. A previous study in which subjects were supplemented with 400 mg of \( \alpha \)-tocopheryl acetate found that the acetate ester was not detected in serum, indicating that vitamin E esters are likely hydrolyzed prior to absorption [108]. Following micelle formation, vitamin E is absorbed at the brush border membrane of the intestinal mucosa [92, 106]. It was originally believed that vitamin E absorption occurred via passive diffusion, however, in recent studies, it has been shown that the scavenger receptor class B type I (SR-BI) and Neimann-Pick C1-like 1 (NPC1L1) proteins are involved in the intestinal uptake of vitamin E [109-112]. SR-BI is found in several tissues and is the receptor responsible for cellular high density lipoprotein (HDL) uptake, while the NPC1L1 protein is involved in cholesterol absorption [113-117].

There does not appear to be a difference in the intestinal absorption between the free form of vitamin E and its acetate and succinate esters nor between the various forms (the 4
tocopherols and 4 tocotrienols) or stereoisomers (natural and synthetic) of vitamin E [27, 92, 118-121]. It is estimated that about 20 to 80% of dietary vitamin E is absorbed by the intestine [106]. The type of meal ingested with vitamin E can influence its absorption [77-81, 122]. The ingestion of plant sterols, for example may reduce vitamin E absorption [122]. The fat content of the meal can also influence vitamin E absorption since the presence of bile acids, which are required for micelle formation, is necessary for vitamin E absorption and dietary lipids stimulate bile acid secretion [77-79, 123, 124]. The amount of dietary fat required to maximize vitamin E absorption, however, is not yet known [79]. Roodenburg et al. reported no difference in plasma $\alpha$-tocopherol concentration following the consumption of 50 mg $RRR$-$\alpha$-tocopherol for seven days with either a low fat meal (3.1 g fat) or a high fat meal (34.1 g fat), suggesting a small amount of dietary fat is required for vitamin E absorption [125]. Bruno et al., however, found that $\alpha$-tocopherol absorption from apples fortified with $\alpha$-tocopheryl acetate (22 mg per serving) increased from 10% when consumed on their own (0% energy from fat breakfast) to 20% and 33% after consuming the fortified apple with a breakfast containing 6% and 21% of energy from fat, respectively [78]. In another study, 150 mg of labeled $RRR$-$\alpha$-tocopheryl acetate in a capsule consumed with a toast and butter breakfast (17.5 g fat) and a cereal with full-fat milk breakfast (17.5 g fat) resulted in significantly higher chylomicron and plasma labeled $\alpha$-tocopherol concentration compared with the consumption of the capsule with a cereal with semi-skimmed milk breakfast (2.7 g fat) [79]. The chylomicron and plasma labeled $\alpha$-tocopherol concentrations following consumption of the capsule together with a cereal with semi-skimmed milk breakfast (2.7 g fat) or water (0 g fat) were negligible and were not significantly different between these two groups [79]. Furthermore, ingesting vitamin E supplements with a meal compared with
ingesting the supplement on an empty stomach has been shown to increase vitamin E bioavailability [77].

**Figure 2.** Vitamin E metabolism. Various steps in the metabolism of vitamin E are shown in the figure. For details pertaining to these steps, please refer to the text. The size of the α- and γ- depict the differences in their abundance in the diet and in various compartments within the body (sizes are not to scale). The candidate proteins studied in this thesis are enclosed in rounded squares. Abbreviations: α-TTP, α-tocopherol transfer protein; ABCA1, ATP-binding cassette A1 transporter; CYP4F2, cytochrome P450, family 4, subfamily F, polypeptide 2; HDL, high density lipoprotein; IDL, intermediate density lipoprotein; LDL, low density lipoprotein; LPL, lipoprotein lipase; MDR3/ABCB4, multidrug resistance protein 3; NPC1L1, Neimann-Pick C1-like 1; SR-BI, scavenger receptor class B type I; TAP, tocopherol associated protein; VLDL, very low density lipoprotein.
The food matrix can also affect vitamin E bioavailability [79-81, 126]. For example, microdispersion of 100 mg all-rac-α-tocopheryl acetate in milk increased its bioavailability compared with the ingestion of the identical amount from a capsule containing all-rac-α-tocopheryl acetate [80]. In addition, vitamin E was more bioavailable when it was in a microdispersion in milk compared with a microdispersion in orange juice [80]. Absorption of vitamin E was also greater when it was in a fortified cereal compared with the same amount (400 IU all-rac-α-tocopheryl acetate) in a capsule when both were consumed with fat-free milk [81]. Additionally, when subjects were grouped according to their dietary intake pattern for α-tocopherol, those in the fruit and breakfast cereal and in the milk and milk products intake patterns had higher plasma α-tocopherol concentrations compared with the other dietary intake patterns even though these dietary patterns did not provide the highest α-tocopherol intake [126].

Once absorbed into the intestinal cells, there are two mechanisms through which vitamin E is secreted from the enterocyte [127, 128]. A small fraction of vitamin E may be secreted from the enterocytes with intestinal high density lipoproteins (HDL), a process which is mediated by the ATP-binding cassette A1 (ABCA1) transporter [127-129]. The major pathway, however, involves the secretion of vitamin E with chylomicrons, which involves the microsomal triglyceride transfer protein [127, 128]. Once vitamin E is packaged into chylomicrons along with the apolipoproteins (apo) apoB48, apoCII and apoE, it enters the lymphatic circulation and eventually the blood via the thoracic duct [105, 130]. While circulating in the blood, these chylomicrons are partially digested by the apoCII-activated, endothelium-bound lipoprotein lipase (LPL) [130]. In the process, triacylglycerols present in
the chylomicrons are hydrolyzed, releasing free fatty acids and monoacylglycerols [105]. These lipids as well as some vitamin E can then be transferred to extrahepatic tissues through an LPL-mediated process [105, 130-132]. Alternatively, the lipids and vitamin E can be transferred to HDL [105, 130]. Most of the vitamin E, however, remains in the resulting chylomicron remnants which then get taken up by the liver [105]. The apoE receptor is involved in chylomicron remnant uptake by the liver [130]. Recent studies have suggested that the SR-BI protein may also be involved in chylomicron remnant metabolism by acting as an initial recognition site for chylomicron remnants [133, 134].

In the liver, the $\alpha$-tocopherol transfer protein (\(\alpha\)-TTP) facilitates the selective secretion of $\alpha$-tocopherol into nascent very low density lipoproteins (VLDLs) [8, 9]. $\alpha$-TTP has a stronger affinity for $\alpha$-tocopherol than the other forms of vitamin E [135]. In addition, $\alpha$-TTP is also stereospecific for the $RRR$-$\alpha$-tocopherol stereoisomer [26, 120, 121]. Consequently, this strong preference for $\alpha$-tocopherol explains why $\alpha$-tocopherol is the most abundant form of vitamin E in the body even though the other vitamin E forms are equally absorbed [92, 118, 120, 121]. The mechanism of vitamin E incorporation into VLDLs by $\alpha$-TTP has not been completely elucidated. The mechanism seems to be independent of the Golgi pathway for VLDL assembly and secretion as the inhibition of this pathway and thus, VLDL secretion, did not alter $\alpha$-tocopherol secretion [9]. Therefore, $\alpha$-tocopherol secretion by $\alpha$-TTP is not coupled with VLDL assembly and secretion [9]. The way in which $\alpha$-TTP acquires $\alpha$-tocopherol seems to involve a direct interaction between $\alpha$-TTP and a membrane containing $\alpha$-tocopherol [136]. It appears that $\alpha$-TTP associates with a lipid bilayer containing $\alpha$-tocopherol, extracts $\alpha$-tocopherol from the bilayer and subsequently $\alpha$-
tocopherol is placed into the binding pocket of α-TTP [136]. The α-TTP then dissociates from the lipid bilayer membrane [136]. It is uncertain how α-TTP subsequently facilitates the secretion of α-tocopherol into VLDLs, however, recently, the ABCA1 transporter has been shown to be involved in this process by a yet undetermined mechanism [137, 138].

Circulating VLDLs can be further hydrolyzed by LPL into intermediate density lipoproteins (IDLs). About 50-60% of these IDLs are taken up by the liver through receptor-mediated endocytosis involving the apoE receptor [105, 130]. The remainder of the IDLs are once again hydrolyzed by LPL, forming low density lipoproteins (LDLs) [105]. Some vitamin E is transferred to HDL during the LPL-mediated hydrolysis [130]. Circulating lipoproteins can also exchange and acquire vitamin E by the action of the phospholipid transfer protein (PLTP) [139].

LDL and HDL are the major carriers of α-tocopherol in healthy adults [140]. Recently, it has been suggested that the protein afamin, which is a member of the albumin gene family and is located in plasma and extravascular fluids such as follicular and cerebrospinal fluids, is a vitamin E carrier as it was shown to bind α- and γ-tocopherol [141, 142]. It has been hypothesized that under the condition of lipoprotein system insufficiency, afamin assumes the role of vitamin E transport [141].

2.4.2 Cellular uptake of vitamin E

There are several mechanisms for the cellular uptake of vitamin E [143]. One mechanism involves facilitated uptake by lipid transfer proteins and lipases such as LPL [131,
132, 144, 145] and PLTP [139, 146]. Receptor-mediated lipoprotein endocytosis by the LDL-receptor is another mechanism for cellular vitamin E uptake [147, 148]. In addition to its role in intestinal uptake, SR-BI has also been suggested to be involved in the cellular uptake of vitamin E through the process of selective lipid uptake [149, 150]. Once it is taken up by cells, vitamin E may bind to the cytosolic tocopherol associated protein (TAP), which is present in many cell types and which may have a role in cellular vitamin E trafficking [151, 152].

2.4.3 Excretion and catabolism of vitamin E

2.4.3.1 Excretion and catabolism mechanism

The remaining forms of vitamin E not packaged by α-TTP into VLDLs, as well as excess α-tocopherol, are excreted in the bile or are catabolized and excreted in the urine (Figure 2) [105]. Multidrug resistance 2 (mdr2), which secretes phosphatidylcholine into the bile, has been shown to be involved in biliary α-tocopherol secretion in rats and mice [153]. The human equivalent, multidrug resistance 3 (MDR3/ABCB4) is, therefore, thought to secrete α-tocopherol into the bile in humans [154]. SR-BI may also be involved in biliary α-tocopherol secretion [150, 154].

Catabolism of vitamin E involves ω-hydroxylation followed by β-oxidation to produce carboxyethyl hydroxychroman (CEHC) metabolites, which have an intact chromanol ring and a truncated side chain [10, 11, 155]. The liver enzyme CYP4F2, a member of the cytochrome P450 (CYP P450) family, catalyzes the hydroxylation of the terminal methyl group of the side chain of vitamin E [10, 11]. Members of the CYP3A family, the major CYP
P450 isoform family in the liver, may also be involved in vitamin E catabolism [155, 156]. The resulting CEHC metabolites of the different vitamin E forms circulate in the blood before being excreted in the urine, mainly as conjugates of either glucuronide or sulphate [10, 157, 158].

2.4.3.2 Factors influencing catabolism and catabolism regulation

The various forms of vitamin E are not catabolized to the same extent [48, 159]. For example, \( \gamma \)-tocopherol is more readily catabolized than \( \alpha \)-tocopherol [48]. Differences in catabolism can be due to the structural features of the vitamin E forms [159]. The degree of saturation of the vitamin E side chain contributes to the differences in catabolism between the vitamin E forms [159]. Tocotrienols are metabolized to CEHCs to a greater extent than their corresponding tocopherols [159]. Vitamin E catabolism is also influenced by the position of the methyl groups on the chromanol ring, with a methyl group at the carbon 5 position resulting in lower catabolism [159].

Vitamin E is believed to regulate its own catabolism as it can up-regulate the pregnane X receptor (PXR), which is a nuclear receptor that activates numerous CYP enzymes [48]. The various forms of vitamin E have different affinities for the pregnane X receptor [48]. \( \alpha \)- and \( \gamma \)-tocotrienol appear to have the highest affinities for PXR, while \( \alpha \)-tocopherol displays a relatively weak affinity for PXR [48]. Despite this, the ability of a form of vitamin E to activate PXR is not related to the extent at which it is catabolized [48].

In addition to structural features and vitamin E-induced catabolism, vitamin E catabolism can be influenced by the ingestion of sesamin, which is present in sesame seeds
and sesame oil [10, 156]. Sesamin consumption has been shown to decrease vitamin E catabolism in both humans and animals, resulting in decreased excretion of the CEHC metabolites and increased vitamin E concentrations in the blood [160-162].

2.5 Plasma \(\alpha\)-tocopherol concentration

Plasma \(\alpha\)-tocopherol concentration is often used as a biomarker for short-term vitamin E nutritional status [12]. The responsiveness to dietary \(\alpha\)-tocopherol intake, the relative ease to obtain and process plasma \(\alpha\)-tocopherol as well as its suitability for large epidemiological studies contribute to the common use of plasma \(\alpha\)-tocopherol concentration as a biomarker for vitamin E nutritional status [15, 163]. Unlike dietary vitamin E, plasma \(\alpha\)-tocopherol concentration takes into account absorption and metabolism and may therefore be a better measure for vitamin E exposure for epidemiological studies [13, 14]. In addition to dietary \(\alpha\)-tocopherol, several factors can also influence plasma \(\alpha\)-tocopherol concentration and knowledge of these determinants is important in order to better assess vitamin E nutritional status [15].

2.5.1 Determinants of plasma \(\alpha\)-tocopherol concentration

2.5.1.1 Dietary \(\alpha\)-tocopherol

Dietary \(\alpha\)-tocopherol is the main determinant for plasma \(\alpha\)-tocopherol concentration. Several studies, however, have shown dietary \(\alpha\)-tocopherol to be weakly correlated to plasma \(\alpha\)-tocopherol concentration and there is a large inter-individual variation in the plasma response to dietary \(\alpha\)-tocopherol [22, 23, 164, 165]. This, therefore, suggests that other
variables are also contributing to the variability in plasma $\alpha$-tocopherol concentration and its response to dietary $\alpha$-tocopherol.

2.5.1.2 Type of meal ingested with $\alpha$-tocopherol, fat content of meal and food matrix

The type of meal ingested with $\alpha$-tocopherol, the fat content of the meal and the food matrix can influence $\alpha$-tocopherol absorption by the intestine [77-81]. The presence of lipids in the small intestine, for example, promotes bile secretion, which is required for $\alpha$-tocopherol absorption [123, 124]. Dietary lipids, therefore, enhance $\alpha$-tocopherol absorption and may consequently alter plasma $\alpha$-tocopherol concentration [77-79]. Identical amounts of dietary $\alpha$-tocopherol in different types of food have been shown to result in differences in $\alpha$-tocopherol bioavailability, indicating that the food matrix can influence $\alpha$-tocopherol absorption and therefore, plasma $\alpha$-tocopherol concentration [79-81]. However, even after the consumption of the identical meal, the plasma $\alpha$-tocopherol response to dietary $\alpha$-tocopherol varies widely between individuals [23]. Interestingly, intra-individual variation in response to the same vitamin E dose has been shown to be relatively stable over time [23]. This suggests that environmental, lifestyle and genetic factors may explain some of this variability in the plasma response to dietary $\alpha$-tocopherol that exists between individuals [23, 166].

2.5.1.3 Blood lipids

Blood lipids such as cholesterol and triacylglycerides, which are the major carriers of $\alpha$-tocopherol in the blood, have consistently been shown to be positively correlated and associated with plasma $\alpha$-tocopherol concentration [15-18, 91, 167-170]. Conditions that increase or decrease blood lipids therefore, can alter plasma $\alpha$-tocopherol concentration.
independent of α-tocopherol intake [171]. Consequently, it has been suggested that plasma α-tocopherol concentration corrected for blood lipid levels provides a better indication of plasma α-tocopherol status [171].

2.5.1.4 Age

Plasma α-tocopherol concentration has been reported to increase with increasing age [15, 17, 18, 21, 163, 172-178]. Part of the positive association can be explained by the increasing cholesterol concentrations with age as adjustment for blood lipids attenuates this positive association [15, 163, 174, 179]. However, some studies still show a significant positive association between plasma α-tocopherol concentration and age even after lipid adjustment [176-178]. It has been suggested that alterations in lipoprotein metabolism and enzyme activity between young and older individuals can also help explain the positive association between plasma α-tocopherol and age [178]. For example, a slower chylomicron clearance in older subjects compared to younger subjects, has previously been reported [180]. LPL activity has also been shown to decrease with age, which may explain the slower chylomicron clearance observed in older subjects [178, 181].

2.5.1.5 Body mass index (BMI)

The relationship between BMI and plasma α-tocopherol concentration is unclear. Several studies have reported a negative association between BMI and plasma α-tocopherol concentration [18-20, 91, 182-185]. A few studies, however, have reported a positive association [163, 168, 169], while others have reported no effect [170, 172], even after adjustment for factors such as energy intake, age and blood lipids. Possible explanations for
the negative association between BMI and plasma α-tocopherol concentration include a
dilution effect, where the larger body mass and plasma volume in individuals with a higher
BMI dilutes the concentration of α-tocopherol in plasma [91, 182]. It is also possible that
more of the lipid-soluble α-tocopherol is being stored in the adipose tissue in obese compared
to leaner individuals and therefore, less α-tocopherol is available for the plasma [168, 169]. A
further potential explanation is a greater demand for antioxidants such as α-tocopherol in
individuals with greater adiposity thereby resulting in greater depletion of plasma α-
tocopherol [169].

2.5.1.6 Dietary and plasma ascorbic acid

The antioxidant ascorbic acid (vitamin C) regenerates the α-tocopheryl radical,
formed from the reaction between α-tocopherol and a lipid free radical, back to α-tocopherol
[31-33]. Plasma ascorbic acid, therefore, can influence plasma α-tocopherol concentration
and some studies have shown a positive relationship between plasma α-tocopherol and
ascorbic acid [91, 186, 187].

2.5.1.7 Smoking

Smoking results in an increased amount of oxidative stress and thus, smokers may use
up more α-tocopherol compared with non-smokers in order to defend against this oxidative
stress [188, 189]. In one study, smokers and non-smokers were given 50 mg of labeled RRR-
α-tocopherol and RRR-α-tocopheryl acetate [190]. Although no difference in plasma α-
tocopherol concentration was observed between smokers and non-smokers, the labeled
plasma α-tocopherol concentration (indicating newly absorbed α-tocopherol) was
significantly lower in smokers than non-smokers. From the study, however, it was not clear whether the lower labeled \( \alpha \)-tocopherol concentration in smokers was due to decreased absorption or increased clearance. Subsequent studies that have tried to address this question demonstrated that smokers have a quicker fractional disappearance rate of \( \alpha \)-tocopherol as well as a shorter plasma \( \alpha \)-tocopherol half-life compared with non-smokers [191-193]. The faster plasma \( \alpha \)-tocopherol disappearance rate was not explained by an increased rate of catabolism to \( \alpha \)-CEHC metabolites since smokers had significantly lower plasma and urinary \( \alpha \)-CEHC than non-smokers [191, 193, 194]. It was, therefore, suggested that the increased fractional plasma \( \alpha \)-tocopherol disappearance rate was due to the oxidation of \( \alpha \)-tocopherol that resulted from smoking-induced oxidative stress [193, 194]. In support of this hypothesis is the inverse association between plasma \( \alpha \)-tocopherol fractional disappearance rate and plasma ascorbic acid concentration in smokers, but not in non-smokers [191]. In addition, the fractional plasma \( \alpha \)-tocopherol disappearance rate was attenuated in smokers, but not in non-smokers by ascorbic acid supplementation [193]. It has been suggested that ascorbic acid regenerates the \( \alpha \)-tocopheryl radical, thereby reducing the fractional disappearance of \( \alpha \)-tocopherol [193, 195]. It may also be possible that ascorbic acid is sparing \( \alpha \)-tocopherol in that once ascorbic acid is depleted, \( \alpha \)-tocopherol begins to defend against oxidative stress [195].

A few epidemiological studies have found significantly lower plasma \( \alpha \)-tocopherol concentrations in smokers compared with non-smokers [21, 185, 196]. Most studies, however, have not found an effect between smoking status and plasma \( \alpha \)-tocopherol concentration [18, 96, 172, 197-200]. Possible explanations for the discrepancies may be the
ascorbic acid status of the populations examined or the mobilization of \( \alpha \)-tocopherol from adipose tissue stores to plasma to replenish the plasma \( \alpha \)-tocopherol that has been oxidized thereby maintaining the plasma \( \alpha \)-tocopherol concentration [191, 193].

### 2.5.1.8 Polyunsaturated fatty acid (PUFA) content of cellular membranes

The amount of PUFA in cellular membranes may also influence plasma \( \alpha \)-tocopherol concentration. Since \( \alpha \)-tocopherol is required to help prevent lipid peroxidation of PUFAs in cellular membranes, \( \alpha \)-tocopherol is mobilized from the plasma to cellular membranes as the PUFA content in cellular membranes increases. Therefore, as the \( \alpha \)-tocopherol content in cellular membranes increases, the amount of \( \alpha \)-tocopherol in the plasma decreases if it is not replenished [201].

### 2.5.1.9 Other determinants

The aforementioned factors help explain variation in plasma \( \alpha \)-tocopherol concentration. However, even after the adjustment for these factors, there is still a proportion of variability in plasma \( \alpha \)-tocopherol concentration that is not explained by these variables [21, 163, 168, 170, 183]. Therefore, further investigation is required in order to uncover other factors that can influence plasma \( \alpha \)-tocopherol concentration. Genetics may, in part, be able to explain some of this unaccounted variation in plasma \( \alpha \)-tocopherol concentration and its response to diet.
2.5.2 Variation in vitamin E metabolism genes and plasma α-tocopherol

Plasma α-tocopherol concentration is a function of the amount of α-tocopherol absorbed from the diet, the amount taken into and released from cells and the amount that is utilized, metabolized and excreted. It is therefore possible that variations in genes involved in these different processes in α-tocopherol metabolism can influence plasma α-tocopherol concentration. To date, there have been only a few studies that have examined whether polymorphisms in vitamin E metabolism genes, particularly those that are also involved in lipid metabolism, can alter α-tocopherol concentrations in the blood [202-208].

A recent study looked at whether the -1131T>C polymorphism of the apolipoprotein (apo) A5 gene influences plasma α-tocopherol concentration in a group of 297 healthy, non-smoking males between the ages of 20 and 75 years [202]. Apolipoprotein A-V, the protein product of apoA5, is involved in triglyceride metabolism and has been reported to be a strong determinant for circulating triglyceride concentrations [202]. Although the exact function of apoA-V is unknown, in vitro studies have shown that it may influence plasma triglyceride concentrations by downregulating VLDL synthesis by the liver and stimulating LPL activity [202]. Sundl et al. found that carriers of the C allele had significantly higher plasma α-tocopherol concentrations compared with homozygotes for the T allele [202]. This difference in plasma α-tocopherol concentration, however, was no longer significant after adjusting for plasma cholesterol and triglycerides (significantly higher in C allele carriers compared to T allele homozygotes), which transport α-tocopherol in the blood [202]. Therefore, the effect observed was entirely due to the role of the apoA5 polymorphism in lipid metabolism. This
study highlights the importance of adjusting plasma α-tocopherol concentration by blood lipids.

This -1131T>C apoA5 polymorphism was also tested in a type 2 diabetic population consisting of 169 non-smoking males and females [203]. Similar to the Sundl et al. study, carriers of the C allele had significantly higher plasma α-tocopherol concentrations compared to TT subjects [203]. The prevalence of the CC+CT genotype was also 2.6-fold significantly higher among individuals who were categorized as having a high plasma α-tocopherol concentration (defined as 38.8 μmol/L in males and 41.6 μmol/L in females) compared with TT subjects [203]. Unlike the Sundl et al. study, however, plasma α-tocopherol adjusted for total cholesterol (as the plasma α-tocopherol to total cholesterol ratio) remained significantly higher in C allele carriers compared with T allele homozygotes [203]. This discrepancy may be due to differences in the populations (healthy vs. type 2 diabetic, males only vs. males and females).

Several studies have examined the relationship between plasma α-tocopherol concentration and the Cys112Arg and Arg158Cys polymorphism of apoE, a protein which is involved in triglyceride-rich lipoprotein remnant (such as chylomicron remnant) clearance through hepatic binding, uptake and catabolism [204-206]. This polymorphism results in the E2, E3 and E4 alleles which form 6 different phenotypes that have been shown to influence plasma lipid levels [204, 205]. The E4 allele has higher, while the E2 allele has lower, total and LDL-cholesterol concentrations compared with the E3 allele [204, 205]. Ortega et al. found that the lipid adjusted plasma α-tocopherol concentration in E2/E2 subjects was
significantly higher compared with subjects with all the other genotypes except for the E4/E2 genotype (which had the second highest concentration) in a population of 926 children aged 6 to 8 years [204]. Conversely, the Gomez-Coronado et al. study did not find a significant association between the apoE polymorphism and plasma α-tocopherol concentration in a sample consisting of 361 adults [205]. Borel et al. reported that subjects possessing the E2/E2 genotype had significantly lower, while the E4/2 genotype had significantly higher plasma α-tocopherol concentrations compared with the other apoE genotypes (E4/E3, E3/E3, E3/E2, E4/E4) [206]. This is in contrast to the Ortega et al. study, however, after adjustment for cholesterol, the apoE polymorphism was no longer significantly related to plasma α-tocopherol concentration in the total population (P = 0.057), but remained significant in males [206]. The reason for the discrepancies between these studies is unclear. Differences in the populations, samples sizes, age of subjects and comparison groups may be potential explanations. While Ortega et al. [204] and Borel et al. [206] compared all genotypes with each other, Gomez-Coronado et al. [205] compared three groups of subjects. These groups included the E2 group (consisting of E2/E2 and E3/E2 genotypes), the E3 group (consisting of the E3/E3 genotype) and the E4 group (consisting of E4/E4 and E4/E3 genotypes), while individuals with the E4/E2 genotype were not included in the analysis [205].

In addition to the apoE polymorphism, Borel et al. also examined whether other polymorphisms in various lipid metabolism genes (apoA-IV, apoB, LPL and SR-BI) influence plasma α-tocopherol concentration [206]. A polymorphism (Ser-347) in apoA-IV, a protein which is secreted by the intestine and associated with chylomicrons, was significantly associated with plasma α-tocopherol concentration, but only in females [206]. This effect,
however, was no longer significant after adjustment for cholesterol [206]. The SR-BI polymorphism located in exon 8 significantly altered plasma \( \alpha \)-tocopherol concentration, but only in males [206]. This association in males persisted after adjustment for cholesterol [206]. There was no effect with the apoB (a protein involved in hepatic VLDL secretion and LDL clearance) and LPL polymorphisms examined on plasma \( \alpha \)-tocopherol concentration [206].

Polymorphisms in other lipid metabolism genes including apoC-III (inhibits triacylglycerol from the plasma), cholesteryl ester transfer protein (responsible for the transfer of cholesteryl esters and triacylglycerol between lipoproteins), hepatic lipase (responsible for lipolysis of lipoprotein triacylglycerol), intestinal fatty acid binding protein (intracellular transport of fatty acids in the small intestine) and microsomal triacylglycerol transfer protein (incorporates triacylglycerol in chylomicrons) were also studied for a potential role in influencing plasma \( \alpha \)-tocopherol concentration [207]. After lipid adjustment, only the apoC-III polymorphism (S1/S2 C>G) was significantly associated with plasma \( \alpha \)-tocopherol concentration, but only in females [207]. Furthermore, several polymorphisms in \( \alpha \)-TTP and TAP have also been studied and some of these polymorphisms were found to influence fasting serum \( \alpha \)-tocopherol concentration in a group of middle-aged male smokers [208].

These gene-plasma association studies indicate that polymorphisms in genes involved in lipid and vitamin E metabolism may influence \( \alpha \)-tocopherol levels in the blood. These studies, however, have not examined whether these genes can influence the relationship between dietary and plasma \( \alpha \)-tocopherol. Thus, studies are needed to determine whether
genetic variations in these and other vitamin E metabolism genes alter the plasma α-tocopherol response to dietary α-tocopherol.
Chapter THREE: Rationale, Hypothesis and Objectives
3.1 Rationale

There is a lot of variability in plasma α-tocopherol concentration which arises from several determinants. While dietary α-tocopherol is the main determinant for plasma α-tocopherol concentration, studies have shown it to be weakly correlated to plasma α-tocopherol concentration and there is a lot of inter-individual variation in the plasma α-tocopherol response to dietary α-tocopherol [22, 23, 164, 165]. Intra-individual variation in response to the same α-tocopherol dose has been shown to be relatively stable over time [23]. This suggests that environmental, lifestyle and genetic factors may explain some of this variability between individuals [23, 166]. Adjustment for other determinants of plasma α-tocopherol such as plasma cholesterol and lipids, age, body mass index, plasma antioxidants and smoking do not fully explain all the variation in plasma α-tocopherol concentration [16-21]. Therefore, further investigation is required in order to uncover other factors that influence plasma α-tocopherol concentration.

Genetics may, in part, explain some of this unaccounted variability in plasma α-tocopherol concentration and its response to diet. Since plasma α-tocopherol concentration is a function of the amount of dietary α-tocopherol that gets absorbed, the amount taken up by and released from cells and the amount that is utilized, metabolized and excreted, it may be possible that variation in genes involved in any one of these processes may influence plasma α-tocopherol concentration or its response to diet. Therefore, a candidate gene approach examining polymorphisms in vitamin E metabolism genes may reveal a genetic basis for the variability in plasma α-tocopherol concentration. To date, only a few studies have examined whether polymorphisms in vitamin E metabolism genes, particularly those involved in lipid
metabolism, influence plasma $\alpha$-tocopherol concentration [202-208]. These studies, however, have not addressed whether the genetic variation also influences the plasma response to dietary $\alpha$-tocopherol. Therefore, further studies are warranted to examine whether polymorphisms in these and other vitamin E metabolism genes modify the relationship between dietary and plasma $\alpha$-tocopherol.

The $\alpha$-tocopherol transfer protein ($\alpha$-TTP) is a liver cytosolic protein that facilitates the selective incorporation of the $\alpha$-tocopherol form of vitamin E into very low density lipoproteins (VLDLs) [8, 9]. The importance of a functional $\alpha$-TTP protein in maintaining an adequate plasma $\alpha$-tocopherol concentration is demonstrated in individuals with ataxia with vitamin E deficiency (AVED). AVED, which is transmitted through an autosomal recessive mode of inheritance, results from rare variations in the $\alpha$-TTP gene which lead to severely low plasma $\alpha$-tocopherol concentration (<5 $\mu$mol/L; deficiency <11.6 $\mu$mol/L), cerebellar ataxia and symptoms of central and peripheral axonopathy [12, 209-212]. It may be possible, therefore, that common polymorphisms in this gene can explain some of the more subtle inter-individual differences in plasma $\alpha$-tocopherol concentration or its response to dietary $\alpha$-tocopherol.

The tocopherol associated protein (TAP) is ubiquitously expressed in many cell types, binds to vitamin E in the cell and may be involved in vitamin E cellular trafficking [151, 152]. It may be possible that common TAP polymorphisms influence plasma $\alpha$-tocopherol concentration by affecting its ability to bind and retain $\alpha$-tocopherol in the cell. A recent study showed that certain polymorphisms in $\alpha$-TTP and TAP influence plasma $\alpha$-tocopherol
concentration in a group of middle-aged male smokers [208]. This study, however, did not examine whether the variations in the \(\alpha\)-TTP and TAP genes modify the relationship between dietary and plasma \(\alpha\)-tocopherol. It is also not known whether the results can be generalized to younger populations consisting of predominantly non-smokers or to females.

The enzyme CYP4F2, a member of the cytochrome P450 (CYP P450) family of enzymes, is involved in vitamin E catabolism [10, 11]. CYP4F2 catalyzes the \(\omega\)-hydroxylation of the vitamin E side chain, which is the first step in the formation of the carboxyethyl hydroxychroman (CEHC) vitamin E metabolite [10, 11]. Variation in this gene may potentially influence plasma \(\alpha\)-tocopherol concentration by influencing the extent of \(\alpha\)-tocopherol catabolism and therefore, its clearance from the blood. In addition to vitamin E catabolism, CYP4F2 is also involved in arachidonic acid [213], leukotriene B4 [214] and vitamin K [215] catabolism, functions that have been shown to be influenced by genetic variation in CYP4F2. A single nucleotide polymorphism (SNP) in CYP4F2 (Val433Met) has been reported to alter arachidonic acid [216, 217] and vitamin K [215] metabolism. Since vitamin K is involved in blood clotting, the effect of the Val433Met polymorphism on vitamin K catabolism has been shown to translate into differences in the required dose of the anti-coagulants warfarin [218, 219] and acenocoumarol [220] depending on an individual’s genotype for the Val433Met SNP. The Val433Met SNP has also been associated with disease risk, possibly due to the effect of the Val433Met SNP on 20-hydroxyeicosatetraenoic acid production, which is an arachidonic acid metabolite that is involved in blood pressure and vascular function and tone [221-224]. It is unknown, however, whether the Val433Met polymorphism also influences vitamin E catabolism.
3.2 Hypothesis

Common genetic variations in $\alpha$-TTP, TAP and CYP4F2 influence plasma $\alpha$-tocopherol concentration and modify the association between dietary and plasma $\alpha$-tocopherol.

3.3 Objectives

The objectives of this thesis were to determine whether common SNPs in genes coding for the $\alpha$-TTP (rs6994076 A>T), TAP (rs2072157 C>T and Arg11Lys (rs757660)) and CYP4F2 (Val433Met (rs2108622)) proteins 1) influence fasting plasma $\alpha$-tocopherol concentration and 2) modify the association between dietary and plasma $\alpha$-tocopherol. Details pertaining to the SNPs examined are listed in Table 3-1.

Table 3-1. Single nucleotide polymorphisms (SNPs) in vitamin E metabolism genes that were examined.

<table>
<thead>
<tr>
<th>Gene symbol</th>
<th>Protein name</th>
<th>rs number$^f$</th>
<th>Gene position</th>
<th>Nucleotide change</th>
<th>Amino acid change</th>
<th>How SNP is referred to in text</th>
</tr>
</thead>
<tbody>
<tr>
<td>TTPA</td>
<td>$\alpha$-tocopherol transfer protein ($\alpha$-TTP)</td>
<td>rs6994076</td>
<td>5’ upstream</td>
<td>A&gt;T</td>
<td>N/A</td>
<td>$\alpha$-TTP A&gt;T</td>
</tr>
<tr>
<td>SEC14L2</td>
<td>Tocopherol associated protein (TAP)</td>
<td>rs2072157</td>
<td>Intron 3</td>
<td>C&gt;T</td>
<td>N/A</td>
<td>TAP C&gt;T</td>
</tr>
<tr>
<td>SEC14L2</td>
<td>Tocopherol associated protein (TAP)</td>
<td>rs757660</td>
<td>Exon 1</td>
<td>G&gt;A</td>
<td>Arg11Lys</td>
<td>TAP Arg11Lys</td>
</tr>
<tr>
<td>CYP4F2</td>
<td>Cytochrome P450, family 4, sub-family F, polypeptide 2 (CYP4F2)</td>
<td>rs2108622</td>
<td>Exon 11</td>
<td>G&gt;A</td>
<td>Val433Met</td>
<td>CYP4F2 Val433Met</td>
</tr>
</tbody>
</table>

$^f$ The rs number is a unique identifier for each SNP.
Chapter FOUR: Methods
4.1 Participants

Participants (n=1277) were subjects from the cross-sectional Toronto Nutrigenomics and Health (TNH) study that were recruited between September 2004 and June 2009. The overall objectives of the TNH study are to investigate diet*gene interactions on biomarkers of chronic disease and to research genetic determinants of food preference and intake behaviours in a young, healthy population. The TNH study began recruitment in September 2004 and is currently ongoing. Subjects were recruited through University of Toronto e-mail bulletins, university newspaper advertisements as well as through classroom announcements and postings along the St. George campus. The sample consisted of free-living males (n=391) and females (n=886) between the ages of 20 and 29 years from diverse ethno-cultural backgrounds. Four major ethno-cultural groups were present in this population and subjects were categorized into these four groups based on their self-reported ethnicities: Caucasian (European, Hispanic or Middle-Eastern; n=603), East Asian (Chinese, Japanese, Korean, Vietnamese or Filipino; n=442), South Asian (Indian, Pakistani and Sri Lankan; n=136) and Other (African descent, First Nations or individuals from mixed (two or more) backgrounds; n=96).

Women who were pregnant or breastfeeding were ineligible for the TNH study because changes in metabolism, biochemistry, taste and food intake can occur during these life stages [225, 226]. Individuals were also excluded from the TNH study if they could not provide a blood sample or could not communicate in English since the questionnaires were only available in English. For the present study, subjects were further excluded from the analysis if they broke their fast (n=3) or had missing data for any of the variables of interest.
After exclusions, 1248 subjects remained (377 males and 871 females). For analyses involving dietary α-tocopherol, subjects were also excluded if they did not know the dose of the vitamin E supplement they were taking (n=8), did not have dietary α-tocopherol values (n=89), or may have incorrectly under- (n=25) or over-reported (n=64) total energy intakes (<800 kcal/day, >3500 kcal/day for females and >4000 kcal/day for males). These energy cut-points are those that were established by Willett [227]. After dietary exclusions were applied, the sample consisted of 1062 subjects (319 males and 743 females) for analyses involving dietary α-tocopherol.

A supplement user was defined as a subject who reported taking a vitamin E supplement, a vitamin E-containing multivitamin or both within the past month. A current smoker was defined as presently smoking at least one cigarette a day for the past month. The month that each subject provided a blood sample was used to categorize subjects into the 4 seasons of winter (December, January, February), spring (March, April, May), summer (June, July, August) and fall (September, October, November). All subjects provided written informed consent and the research protocol was approved by the Research Ethics Board at the University of Toronto.

4.2 Study protocol

Interested subjects were screened for age by phone or e-mail. Individuals who met the requirements were recruited into the TNH study and visited with a trained study coordinator at the study office on two different occasions. During the first visit, written informed consent was obtained and anthropometric measurements including height, weight, waist
circumference and blood pressure were taken. Subjects were given instructions regarding how to complete four self-administered questionnaires: a one-month, 196-item food frequency questionnaire (FFQ), a 63-item food preference checklist (FPC), a general health and lifestyle questionnaire (GHLQ) and a physical activity questionnaire (PAQ). The GHLQ and the PAQ were completed in the study office. The FFQ and the FPC were completed away from the study office to allow subjects to look at the brands and labels of the food products they consumed over the previous month. Subjects were given a venous blood sample requisition form, which was to be presented at LifeLabs Medical Laboratory Services at 180 Bloor Street West (Toronto, Ontario, Canada) between 8:00 and 10:30 a.m. following a 12-hour overnight fast. Subjects were asked to visit LifeLabs within 7 days of their initial visit to the TNH study office to ensure that the blood sample was collected as close to the FFQ time frame (previous month) as possible. Once the blood sample was drawn and the FFQ and FPC were completed, subjects returned to the study office to submit their questionnaires. The FFQ and FPC were subsequently reviewed for completeness by the study coordinator and at the end of this second visit, subjects received $20 in compensation for their participation.

4.3 Dietary assessment

Subjects completed a 196-item, semi-quantitative Toronto-modified Willett food frequency questionnaire (FFQ) in order to estimate their habitual food and supplement intake over the past month. (Please refer to Appendix 1 for sample questions included in the FFQ.) The FFQ was modified to improve the dietary assessment of whole grains, fruits and vegetables, glycemic index and caffeine. The modification involved the addition of 26 food items including 6 breads and cereals, 6 fruits, 7 vegetables, 3 tree nuts and 4 beverages. The
Toronto-modified Willett FFQ contains 24 pages consisting of questions regarding the quantity and frequency of consumption of 189 food and beverage items and 12 vitamin and mineral supplements. For most food items, subjects had to choose from 9 possible options which ranged from never to 4 or more times per day for a specified serving amount. In the vitamin and mineral supplementation section, subjects were asked whether they ever consumed a multivitamin (never, past, current), their weekly multivitamin consumption frequency (2 or less, 3-5, 6-9, 10 or more times a week), the multivitamin brand and their duration of use (0-1 year, 2-4 years, 5-9 years, 10 years or more). For vitamin E supplements, subjects were asked whether they ever consumed a supplement containing exclusively vitamin E (never, past, current), their daily dose (<100 IU, 100-250 IU, 300-500 IU, 600 IU or more, don’t know) and their duration of use (0-1 year, 2-4 years, 5-9 years, 10 years or more). In order to improve the accuracy of self-reported FFQ responses, subjects were given instructions regarding how to complete the FFQ and were shown visual aids of portion sizes. Questions related to food preparation in the home such as the type of fat used for baking, cooking and frying were asked as well. The FFQ also contained a few open-ended questions inquiring about brand names (such as the brand of cold breakfast cereal typically consumed) and other frequently consumed items that were not included in the FFQ food list.

Completed FFQs were sent to Harvard University where they were optically scanned and analyzed. The subjects’ FFQ responses were used to calculate the number of servings consumed per day for each food item. The \( \alpha \)-tocopherol content values for each serving of every food item listed in the FFQ were determined using the Harvard FFQ Database and the United States Department of Agriculture National Nutrient Database for Standard Reference [228]. To estimate \( \alpha \)-tocopherol intake from supplements or fortified food when the vitamin E
amount obtained from the food industry was expressed in IU units, the database converted the IU values to mg α-tocopherol by multiplying the IU amount by either 0.45 for \textit{all-rac-α}-tocopherol (synthetic) or 0.67 for \textit{RRR-α}-tocopherol (natural) \cite{229}. For each FFQ food and supplement item, the product of the daily consumption frequency (number of servings per day) and the α-tocopherol content value of the food serving was computed. These products were then combined to estimate subjects’ total (food and supplements combined) daily \textit{α}-tocopherol intake.

4.4 \textbf{Anthropometric measurements}

Anthropometric measurements taken include height, weight, waist circumference and blood pressure. Height was measured to the nearest 0.1 cm using a wall-mounted stadiometer (Model Seca 206, Seca Corporation, Hanover, MD, USA). A digital scale (Model Bellissima 841, Seca Corporation, Hanover, MD, USA) was used to measure body weight to the nearest 0.1 kg. Subjects wore light clothing and no shoes for these measurements. Waist circumference, to the nearest 0.1 cm, was measured two times using a flexible measuring tape at the midpoint between the lower edge of the ribs and upper edge of the pelvis. The average of the two measurements was calculated. Two resting systolic and diastolic blood pressure readings taken one minute apart were measured using the OMRON IntelliSense Blood Pressure Monitor (Model HEM-907XL, OMRON Healthcare, Vernon Hills, IL, USA). The average of these two readings was used to determine resting systolic and diastolic blood pressure. Body mass index (BMI) was calculated as weight (in kg) divided by the square of height (in m) (BMI units, kg/m²).
4.5 Physical activity assessment

The energy cost of modifiable physical activity was expressed in metabolic equivalent of task (MET)-hours per week, which is comprised of leisure and occupational activity, but does not include the sedentary hours of sleeping or sitting. One MET is equal to 1 kcal energy expended per kilogram body weight per hour while sitting quietly at rest [230]. MET-hours per week were determined from subject’s responses to a physical activity questionnaire (PAQ). The PAQ asked subjects to record the number of hours spent performing various levels of physical activity (sleeping (0.9 MET), sitting or reclining (1.0 MET), light activity (2.4 MET), moderate activity (3.6 MET) and vigorous activity (7.5 MET)) during a typical weekday and weekend day over the previous month. Examples of activities within each of these physical activity levels were provided to help subjects fill out the questionnaire. The PAQ is derived from a portion of the questionnaire developed by Paffenbarger et al. which was created for the purpose of measuring physical activity to assess health effects in a healthy, free-living population [231]. The number of MET-hours per week was calculated from the following equation:

\[
\text{MET-hr per week} = \left( 2.4 \text{ MET} \times \# \text{ weekday light activity hours} \times 5 \text{ weekday days} \right) \\
+ \left( 2.4 \text{ MET} \times \# \text{ weekend light activity hours} \times 2 \text{ weekend days} \right) \\
+ \left( 3.6 \text{ MET} \times \# \text{ weekday moderate activity hours} \times 5 \text{ days} \right) \\
+ \left( 3.6 \text{ MET} \times \# \text{ weekend moderate activity hours} \times 2 \text{ days} \right) \\
+ \left( 7.5 \text{ MET} \times \# \text{ weekday vigorous activity hours} \times 5 \text{ days} \right) \\
+ \left( 7.5 \text{ MET} \times \# \text{ weekend vigorous activity hours} \times 2 \text{ days} \right) \\
/24 \text{ hr /day}\]
4.6 Blood collection

After an overnight, minimum 12-hour fast, subjects arrived at LifeLabs Medical Laboratory Services (Toronto, Ontario, Canada) between 8:00 and 10:30 a.m. to have their blood drawn. Subjects were instructed not to consume any food or vitamin supplements during their fast, however, they were permitted to consume water. All blood samples were collected from the antecubital vein and protected from light. About 10 mL of whole blood was treated with citric acid-trisodium citrate dextrose (ACD) and was shipped to the University of Toronto at room temperature on the day of blood collection. Leukocytes extracted from the ACD-treated blood were used for DNA isolation. Serum samples, which were used for the determination of lipids, were collected in gold serum separator tubes. The blood was left to clot for 30 minutes while in a vertical position and then centrifuged at 3000 rpm for 10 minutes at room temperature. Serum samples were analyzed the same day as the blood collection. Plasma samples, which were used to determine the concentration of $\alpha$-tocopherol, were also obtained. Plasma samples were collected in a 7 mL sodium-heparin tube and centrifuged at 1500 g for 15 min at 4°C within 2 hours after collection. An aliquot of 2 mL was placed in an amber transport vial, frozen immediately and transported on dry ice to the University of Toronto. Plasma samples were stored at a temperature of -80°C until analysis.

4.7 Serum lipids

Serum lipid concentrations were determined at LifeLabs Medical Laboratory Services (Toronto, Ontario, Canada). Total serum cholesterol, high density lipoprotein- (HDL) cholesterol and triglycerides were measured using a chromatographic enzymatic method with
a Siemens Advia 2400 analyzer (Siemens Healthcare Diagnostics, Deerfield, Illinois). Low density lipoprotein- (LDL) cholesterol was calculated using the Friedewald equation for samples with triglyceride values between 0.30 and 4.52 mmol/L [232].

4.8 Plasma α-tocopherol concentration

Plasma α-tocopherol concentration was determined at the University of Toronto using reversed-phase isocratic high performance liquid chromatography (HPLC) with fluorescent detection as recently described [233]. All processes were performed under low ambient light conditions to minimize light-induced degradation. Briefly, 100 μL of mixed internal standard solution (0.25 mg/L retinyl acetate, 50 mg/L α-tocopherol acetate, and 0.2 mg/L echinenone) and a 200 μL aliquot of plasma were pipetted into an amber microfuge vial. A volume of 100 μL of ethanol-butylated hydroxytoluene (BHT) (0.0625%) was added to the mixture for deproteinization [234]. Following a 15 s vortex, 1 mL of n-hexane-BHT (0.005%) was added. The solution was then vortexed and shaken alternately for 5 min and centrifuged for 3 minutes at 2000 g. An aliquot of 900 μL of the supernatant was transferred to a 4 mL amber glass vial with a screw cap. This extraction procedure was repeated a second time and the pooled extract was evaporated to dryness under a stream of nitrogen. The extract was reconstituted in 100 μL of ethanol-BHT (0.0625%) by vortexing for 3 minutes and transferred into a microvolume HPLC vial insert. Samples were then placed into the HPLC autosampler compartment at 10°C before injection into the column. The HPLC system consisted of a Waters 2690 Alliance HPLC system (Milford, MA, USA), a Shimadzu RF 535 fluorescence detector (excitation wavelength, 285nm; emission wavelength, 325nm) (Kyoto, Japan) and a Waters Spherisorb 3μm ODS2, 4.6 × 250mm analytical column. The mobile phase was a
mixture of acetonitrile and methanol (65:35, v/v) containing 0.065% of triethylamine and was added to the column at a flow rate of 1.5 mL/min. The temperature of the column oven was 30°C and the injection volume into the column was 10 μL.

### 4.9 Genotyping

DNA was extracted from peripheral white blood cells using the salting-out procedure (GenomicPrep™ Blood DNA Isolation Kit, Amersham Pharmacia Biotech Inc., Piscataway, NJ, USA). Subjects were genotyped for the rs6994076 (α-TTP A>T), rs2072157 (TAP C>T), rs757660 (TAP Arg11Lys) and rs2108622 (CYP4F2 Val433Met) polymorphisms by real-time polymerase chain reaction (PCR) using the Applied Biosystems (ABI) Assay-On-Demand Taqman® SNP and Drug Metabolism genotyping assays (Catalogue # C___31550115_10, C__15870399_10, C_____18801_10 and C__16179493_40, respectively) (Foster City, CA). Fluorescent detection for allelic discrimination was measured with an ABI 7000 Sequence Detection System. The PCR conditions were set at 1 cycle of 95°C for 10 minutes, followed by 40 cycles of 95°C for 15 seconds and 60°C for 1 minute. Each batch of samples that were genotyped (96) consisted of about 3-4 negative controls, which contained no DNA, and 1 positive control. For quality control, a random 5-10% of samples were repeated to ensure reproducibility of the PCR assay.

### 4.10 Statistical analysis

Statistical analyses were conducted using the Statistical Analysis Systems (SAS) software program (version 9.1) (SAS Institute, Cary, NC, USA). Since vitamin E supplement users tend to have α-tocopherol intakes that are much greater than what can be achieved from
the diet alone and since these high intakes may result in the saturation of vitamin E metabolism proteins, two different groups were analyzed. These groups included the total population (consisting of both individuals who do and those who do not consume vitamin E supplements) and the group that excluded vitamin E supplement users. A supplement user was defined as a subject who reported taking a vitamin E supplement, a vitamin E-containing multivitamin or both within the past month. To meet the assumptions of various parametric statistical tests, variables that did not follow a normal distribution (dietary \( \alpha \)-tocopherol and vitamin C) were log\(_e\) transformed and the results are presented as the anti-log of the estimate.

An unpaired \( t \)-test assuming unequal variances was used for continuous variables and the \( \chi^2 \) statistic for categorical variables (sex, ethno-cultural group, season, smoking status and dietary \( \alpha \)-tocopherol adequacy) to test for differences in characteristics between supplement users and individuals not consuming supplements. Differences in characteristics between genotypes were assessed using a one-way analysis of variance for continuous variables and a \( \chi^2 \) test for categorical variables.

Departure from Hardy-Weinberg equilibrium for each SNP was assessed using the \( \chi^2 \) test with 1 degree of freedom. The HAPLOVIEW software package [235] was used to test whether the two TAP polymorphisms were in linkage disequilibrium.

Dietary \( \alpha \)-tocopherol was adjusted for total energy intake in order to correct for variation in \( \alpha \)-tocopherol consumption due to differences in total energy intake (i.e. a person may consume more \( \alpha \)-tocopherol because they are consuming more energy and not because they are consuming an \( \alpha \)-tocopherol-rich diet). Since plasma \( \alpha \)-tocopherol is highly correlated
to blood lipids such as cholesterol, which transport \(\alpha\)-tocopherol in the blood, it has been suggested that plasma \(\alpha\)-tocopherol concentration corrected for blood lipid levels is a better indicator of plasma \(\alpha\)-tocopherol status [167, 171]. This lipid-adjusted plasma \(\alpha\)-tocopherol concentration provides a measure of plasma \(\alpha\)-tocopherol concentration independent of blood lipid levels (which can be influenced by many factors) [167]. LDL- and HDL-cholesterol, serum triglycerides and total serum cholesterol were considered for such adjustment. The serum lipid that was most strongly correlated to plasma \(\alpha\)-tocopherol (total serum cholesterol) according to the Spearman rank correlation coefficient was chosen for the adjustment. These dietary and plasma \(\alpha\)-tocopherol adjustments were made utilizing both the multivariate and the residual methods [167, 227]. In the multivariate method, total energy intake and total serum cholesterol were added to the model as independent variables [227]. The residual method uses the residuals from the regression model between total energy intake (independent variable) and dietary \(\alpha\)-tocopherol (log\(_e\) transformed; dependent variable) for dietary adjustment and the model between total serum cholesterol (independent variable) and plasma \(\alpha\)-tocopherol concentration (dependent variable) for plasma adjustment in place of dietary or plasma \(\alpha\)-tocopherol, respectively [167]. The rationale for the residual method is that the residual is the component of the dependent variable (dietary or plasma \(\alpha\)-tocopherol) that is uncorrelated to the independent variable (total energy intake or total serum cholesterol, respectively). It, therefore, serves as a measure of dietary and plasma \(\alpha\)-tocopherol that is independent of total energy intake and total serum cholesterol, respectively [227]. Additionally, the results were also analyzed using the ratio of plasma \(\alpha\)-tocopherol concentration to total serum cholesterol as a substitute for plasma \(\alpha\)-tocopherol concentration.
All three adjustment methods yielded similar results, therefore, the results shown are from the multivariate adjustment method to facilitate interpretation.

An analysis of covariance was used to determine potential covariates (sex, ethnocultural group, smoking status, season, age, BMI, waist circumference, physical activity, vitamin E supplement use, dietary intakes of cholesterol, fat, alcohol and vitamin C and serum ascorbate). The potential covariates were tested, one by one, in the models that included plasma α-tocopherol concentration as the outcome variable and either dietary α-tocopherol or genotype as the independent variables. Variables that were significantly associated with plasma α-tocopherol concentration (outcome variable) and decreased the variance were included in the final model [236]. Plasma α-tocopherol concentration was also adjusted for ethno-cultural group to correct for differences in allele frequencies between the different ethno-cultural groups. Variables that were significantly associated with genotype were also added individually to the adjusted models to see if they altered the results. The results were not materially affected by the inclusion of these variables and were therefore not included in the final model. There were no significant interactions between covariates and genotype on plasma α-tocopherol concentration. In addition, there were no gene*ethno-cultural group interactions on plasma α-tocopherol concentration for any of the polymorphisms examined in either of the two groups analyzed.

To determine whether the polymorphisms studied can explain differences in plasma α-tocopherol concentration, a general linear model was employed which was adjusted for covariates. The models included plasma α-tocopherol concentration as the outcome variable
and genotype (α-TTP A>T, TAP C>T, TAP Arg11Lys or CYP4F2 Val433Met SNP) and covariates (supplement use, age, season, ethno-cultural group, physical activity, dietary vitamin C and total serum cholesterol) as the independent variables. Significant differences as determined by the general linear model were further analyzed with a post-hoc Tukey test in order to determine which genotypes were significantly different from each other. To assess whether the plasma α-tocopherol of the various genotypes responds differently to vitamin E supplementation, a general linear model including plasma α-tocopherol concentration as the outcome variable and genotype, supplement use, a supplement use*gene interaction term and covariates (age, season, ethno-cultural group, physical activity, dietary vitamin C and total serum cholesterol) as the independent variables was employed.

The Spearman rank correlation coefficient, adjusted for covariates (season, ethno-cultural group, physical activity, total energy intake and total serum cholesterol) was used to determine the strength of the association between dietary and plasma α-tocopherol for all subjects and for each genotype. A general linear model including plasma α-tocopherol concentration as the outcome variable and dietary α-tocopherol, genotype, a diet*gene interaction term and covariates (season, ethno-cultural group, physical activity, total energy intake and total serum cholesterol) as the independent variables, was used to test whether the plasma α-tocopherol response to dietary α-tocopherol is modified by genotype. The diet*gene interaction was tested using both the continuous and categorical (above and below median) dietary α-tocopherol variables. Results were not materially affected when the categorical dietary α-tocopherol variable was used. Therefore, the diet*gene interaction results shown are from the analyses using the continuous dietary α-tocopherol variable. Slopes for the
relationship between dietary and plasma \( \alpha \)-tocopherol were estimated using a general linear model stratified by genotype and adjusted for covariates (season, ethno-cultural group, physical activity, total energy intake and total serum cholesterol). The \( R^2 \) value was also determined from the model that was used to derive the slopes.

Based on some of the findings, a gene*gene interaction analysis on plasma \( \alpha \)-tocopherol concentration between the \( \alpha \)-TTP A>T and TAP Arg11Lys polymorphisms in subjects that did not use supplements was conducted using a general linear model. For this analysis, the A/A and A/T subjects of the \( \alpha \)-TTP A>T SNP were grouped together and designated as “A carriers”. For the TAP Arg11Lys SNP, Lys/Lys and Arg/Lys genotypes were grouped together and designated as “Lys carriers”. The model included plasma \( \alpha \)-tocopherol concentration as the outcome variable and \( \alpha \)-TTP A>T (grouped genotypes), TAP Arg11Lys (grouped genotypes), an \( \alpha \)-TTP A>T*TAP Arg11Lys interaction term (using the grouped genotypes for each SNP) and covariates (age, season, ethno-cultural group, physical activity, dietary vitamin C and total serum cholesterol) as the independent variables.

The effect of the various combination genotypes between the \( \alpha \)-TTP A>T and TAP Arg11Lys SNPs on plasma \( \alpha \)-tocopherol concentration was also tested in subjects not consuming supplements using a general linear model. The model included plasma \( \alpha \)-tocopherol concentration as the outcome variable and the combined \( \alpha \)-TTP A>T and TAP Arg11Lys grouped genotypes as well as covariates (age, season, ethno-cultural group, physical activity, dietary vitamin C and total serum cholesterol) as the independent variables. A post-hoc Tukey test was used to determine which combined genotypes were significantly
different from each other. To determine whether the α-TTP A>T and TAP Arg11Lys combined genotypes modify the plasma α-tocopherol response to dietary α-tocopherol, a general linear model was employed which included plasma α-tocopherol as the outcome variable and dietary α-tocopherol, the combined α-TTP A>T and TAP Arg11Lys grouped genotypes, a diet*gene interaction term (using the combined genotypes) and covariates (season, ethno-cultural group, physical activity, total energy intake and total serum cholesterol) as the independent variables. In addition, the $R^2$ value from the models that included these combined genotypes was also determined as described above for the individual genotype analyses.

Several of the analyses described above were additionally conducted stratified by ethno-cultural group to check whether the results were due to population admixture or population stratification. For the purpose of this analysis, the South Asian and other ethno-cultural groups were combined to form the “Others” category. P-values were 2-sided and significance was set at $P \leq 0.05$. 
Chapter FIVE: Results
5.1 Subject characteristics

Subject characteristics according to vitamin E-containing supplement use are shown in Table 5-1. A supplement user was defined as an individual who reported taking a vitamin E supplement, a vitamin E-containing multivitamin or both within the previous month. There were no differences between supplement users and individuals not using supplements for most of the characteristics including season of blood draw, physical activity, smoking status, body mass index, waist circumference, systolic and diastolic blood pressure, total serum cholesterol, energy intake and intakes of dietary carbohydrate, fat and protein. There was also no difference in the distribution of genotype frequencies between subjects who used supplements and those who did not for the $\alpha$-TTP A>T, TAP C>T, TAP Arg11Lys and CYP4F2 Val433Met polymorphisms examined.

The allele and genotype frequencies for the $\alpha$-TTP A>T, TAP C>T, TAP Arg11Lys and CYP4F2 Val433Met polymorphisms in the Caucasian and East Asian ethno-cultural groups were comparable to those previously reported [208, 218, 223, 237-240]. The two TAP polymorphisms as well as the CYP4F2 Val433Met polymorphism were in Hardy-Weinberg equilibrium (HWE) in the total population and in subjects not using supplements (data not shown). Similar results were also obtained when these two groups were stratified by ethno-cultural group (data not shown). The $\alpha$-TTP A>T polymorphism was not in HWE in the total population or individuals who did not use supplements (data not shown). However, the $\alpha$-TTP A>T polymorphism was in HWE in each of the ethno-cultural groups examined. To correct for differences in allele frequencies between the various ethno-cultural groups, subsequent analyses were either adjusted for or stratified by ethno-cultural group.
Females were more likely to consume supplements than males (32.4% versus 23.1%, P = 0.001). Supplement users were also significantly older than subjects who did not consume supplements, however, this age difference was minimal (22.9 ± 0.1 years versus 22.5 ± 0.1 years, P = 0.002). The distribution of ethno-cultural groups differed between the supplement use categories (P = 0.001), with a lower proportion of East Asian subjects reporting consumption of vitamin E-containing supplements within the past month. Serum HDL concentration was significantly higher (P = 0.001), while serum LDL concentration was significantly lower (P = 0.02), in supplement users than in individuals not using supplements. The difference in HDL concentration observed may be attributed to the greater proportion of females in the TNH study population as females in this population have significantly higher HDL concentrations than males (data not shown).

As expected, users of vitamin E-containing supplements ingested significantly greater amounts of α-tocopherol compared with subjects who did not use supplements (25.6 ± 1.0 mg α-tocopherol versus 8.3 ± 1.0 mg α-tocopherol, P<0.0001). In addition, a greater percentage of supplement users met or exceeded the Recommended Dietary Allowance for vitamin E of 15 mg α-tocopherol per day compared with individuals who did not consume supplements (71.6% versus 28.4%, P<0.0001). The significantly higher α-tocopherol intakes in subjects who took supplements translated into the significantly higher plasma α-tocopherol concentration observed in supplement users compared with individuals not using supplements (33.1 ± 0.7 μmol/L versus 28.6 ± 0.4 μmol/L, P<0.0001). Since plasma α-tocopherol concentration is significantly correlated to blood lipids, it has been suggested that correction
for blood lipids provides a better indicator for plasma $\alpha$-tocopherol status [171]. Thus, the plasma $\alpha$-tocopherol to total cholesterol ratio (a method for blood lipid adjustment) was also compared between supplement users and non-users and was found to be significantly higher in users of vitamin E-containing supplements compared with subjects who did not consume supplements ($P<0.0001$).

Due to the much higher $\alpha$-tocopherol intakes in supplement users that are difficult to achieve through the diet alone, and which may result in the saturation of proteins involved in $\alpha$-tocopherol metabolism, subsequent analyses were conducted in both the total population (including vitamin E-containing supplement users) and in the sub-group that did not report consuming vitamin E-containing supplements. The supplement user population was not large enough to draw any meaningful conclusions. In addition, the range of intakes for dietary $\alpha$-tocopherol in the supplement user group was not very continuous after about 80 mg of dietary $\alpha$-tocopherol. After about 80 mg of $\alpha$-tocopherol, there were gaps in the intakes of dietary $\alpha$-tocopherol between subjects. For example, a few subjects had $\alpha$-tocopherol intakes between 200 and 230 mg or intakes between 400 and 450 mg, however no subjects had $\alpha$-tocopherol intakes between 230 and 400 mg. Thus, since it may be possible that these few subjects with $\alpha$-tocopherol intakes greater than 80 mg may skew results in this sub-group and since the sample size of supplement users was relatively small, the results of this sub-group are not shown.
5.2 Plasma α-tocopherol concentration by α-TTP A>T genotype

Crude and adjusted plasma α-tocopherol concentration by α-TTP A>T genotype for the total population and after the exclusion of supplement users is shown in Table 5-2. In the total population, plasma α-tocopherol concentration did not differ between the various α-TTP A>T genotypes. Conversely, when vitamin E-containing supplement users were excluded from the analysis, a significant difference in plasma α-tocopherol concentration between the different α-TTP genotypes was observed (P = 0.04). Subjects with the T/T genotype had significantly lower (26.4 ± 0.9 μmol/L) plasma α-tocopherol concentration compared with subjects with the A/A genotype (29.0 ± 0.6 μmol/L), while A/T subjects had a plasma α-tocopherol concentration that was intermediate (28.6 ± 0.5 μmol/L). A similar analysis was also conducted stratified by ethno-cultural group to assess whether the results were due to population admixture or population stratification (Table 5-2). When subjects were stratified by ethno-cultural group, no significant difference in plasma α-tocopherol concentration was observed between the α-TTP genotypes, regardless of supplement use. A trend in the Caucasian group (the largest of the ethno-cultural groups) was observed when supplement users were excluded. In this group, a similar pattern to the unstratified sample was observed, with a lower plasma α-tocopherol concentration in T/T subjects, however, this did not reach statistical significance (0.09).

5.3 Plasma α-tocopherol concentration by TAP C>T genotype

The crude and adjusted plasma α-tocopherol concentration for each TAP C>T genotype for the total population and individuals not consuming supplements, both stratified
and unstratified by ethno-cultural group, is presented in **Table 5-3**. While there was no significant difference in plasma α-tocopherol concentration between the TAP C>T genotypes in the total population, there was a non-significant trend for lower plasma α-tocopherol concentration in C/C subjects (P = 0.06) when supplement users were excluded. This trend was also observed in the Others group (P = 0.07) when supplement users were excluded. In addition, the trend was observed in the East Asian subjects (P = 0.06) in the total sample and reached statistical significance in this ethno-cultural group when supplement users were excluded (P = 0.02). East Asian subjects homozygous for the C allele had significantly lower plasma α-tocopherol concentration (26.6 ± 0.8 μmol/L) compared with T/T subjects (30.9 ± 1.5 μmol/L), while C/T subjects had plasma α-tocopherol concentrations that were intermediate (28.8 ± 0.8 μmol/L). In Caucasian subjects, no difference in plasma α-tocopherol concentration between the TAP C>T genotypes was observed, regardless of supplement use.

### 5.4 Plasma α-tocopherol concentration by TAP Arg11Lys genotype

Plasma α-tocopherol concentration, crude and adjusted, by TAP Arg11Lys genotype both including and excluding vitamin E-containing supplement users is reported in **Table 5-4**. Although the TAP Arg11Lys polymorphism was significantly associated with plasma α-tocopherol concentration in the total population (P = 0.05), a post-hoc analysis did not reveal any significant differences between the different TAP Arg11Lys genotypes. However, Arg/Arg subjects tended to have lower plasma α-tocopherol concentrations compared with
the other genotypes. The significant association between plasma α-tocopherol concentration and the TAP Arg11Lys polymorphism remained statistically significant after supplement users were removed from the analysis (P = 0.02). In this sub-group, subjects with the Arg/Arg genotype had significantly lower plasma α-tocopherol concentrations (27.4 ± 0.5 μmol/L) compared with the Lys/Lys subjects (29.9 ± 0.9 μmol/L). Subjects with the Arg/Lys genotype had plasma α-tocopherol concentrations that were intermediate (28.9 ± 0.5 μmol/L). When the analysis was repeated stratified by ethno-cultural group (Table 5-4), a similar pattern was observed in East Asians. In this ethno-cultural group, there was a trend in the total population (Arg/Arg had non-statistically significant lower plasma α-tocopherol concentration, P = 0.07) that reached statistical significance when supplement users were excluded (P = 0.01). Similarly to the unstratified analysis, Arg/Arg East Asians had significantly lower plasma α-tocopherol concentration (26.5 ± 0.8 μmol/L) than Lys/Lys East Asians (31.3 ± 1.5 μmol/L) and plasma α-tocopherol concentration in Arg/Lys subjects was intermediate (28.7 ± 0.8 μmol/L) in subjects not using supplements. In contrast, there was no difference in plasma α-tocopherol concentration between the TAP Arg11Lys genotypes in the Caucasian and Others ethno-cultural groups in both the sample including and the sample excluding vitamin E-containing supplement users.

5.5 Plasma α-tocopherol concentration by CYP4F2 Val433Met genotype

As demonstrated in Table 5-5, there was no difference in either the crude or adjusted plasma α-tocopherol concentration between the different CYP4F2 Val433Met genotypes in
both the total population and after the exclusion of supplement users. Similarly, no significant differences in plasma $\alpha$-tocopherol concentration were observed in any of the ethno-cultural groups, regardless of supplement use (Table 5-5).

### 5.6 Supplement use*gene interaction on plasma $\alpha$-tocopherol concentration

Since some of the plasma $\alpha$-tocopherol results by genotype varied according to supplement use, a supplement use*gene interaction on plasma $\alpha$-tocopherol concentration was tested in order to determine whether genotype alters the plasma $\alpha$-tocopherol response to supplement use. The results of this analysis for the $\alpha$-TTP A>T, TAP C>T, TAP Arg11Lys and CYP4F2 Val433Met SNPs for the total population as well as stratified by ethno-cultural group are presented in Table 5-6. There was no significant supplement use*gene interaction on plasma $\alpha$-tocopherol concentration for any of the polymorphisms examined in the unstratified sample. Interestingly, the $\alpha$-TTP A>T polymorphism was associated with altered plasma $\alpha$-tocopherol concentration but only when supplement users were excluded (Table 5-2). When the supplement use*gene analysis was carried out stratified by ethno-cultural group, no significant supplement use*gene interaction on plasma $\alpha$-tocopherol concentration was found for any of the SNPs examined with a couple of exceptions. The supplement use*gene interaction term was statistically significant in the Others group for the two TAP polymorphisms ($P = 0.006$ and $P = 0.03$ for the C>T and Arg11Lys SNPs, respectively).
5.7 Spearman correlation coefficients for the relationship between dietary and plasma α-tocopherol

To determine whether the genotypes of the polymorphisms under study influenced the strength of the association between dietary and plasma α-tocopherol, the Spearman rank correlation coefficients were computed. The results of this analysis for all subjects and stratified by genotype in both the total population and after excluding supplement users are listed in Table 5-7. In the total population, the adjusted Spearman rank correlation coefficient between dietary and plasma α-tocopherol was 0.15 (P<0.0001). The correlation coefficients according to genotype for the α-TTP A>T, TAP C>T and TAP Arg11Lys polymorphisms were statistically significant and similar to that of the entire population (0.15). When stratified by α-TTP A>T genotype, the correlation coefficients for the A/T and T/T genotypes were similar, while the correlation coefficient of the A/A genotype was a little lower than the other two genotypes. The correlation coefficients for the Val/Val and Val/Met CYP4F2 genotypes were also significant and were similar to that of the total population. The correlation coefficient of the Met/Met CYP4F2 genotype, however, was not statistically significant. It may be possible that this non-significant relationship between dietary and plasma α-tocopherol is due to the smaller sample size of the Met/Met group. When supplement users were excluded from the analysis, the correlation coefficients between dietary and plasma α-tocopherol were not statistically significant for all subjects combined or for any of the genotypes examined.
5.8 Slopes for the relationship between dietary and plasma α-tocopherol and diet*gene interaction on plasma α-tocopherol concentration

To determine whether the α-TTP A>T, TAP C>T, TAP Arg11Lys and CYP4F2 Val433Met genotypes modify the relationship between dietary and plasma α-tocopherol, a diet*gene interaction term on plasma α-tocopherol concentration was assessed. The crude and adjusted slopes for the relationship between dietary and plasma α-tocopherol for all subjects and stratified by genotype and supplement use are presented in Table 5-8, along with the diet*gene interaction p-values. When supplement users were included, the slopes for the relationship between dietary and plasma α-tocopherol for the different α-TTP A>T, TAP C>T, TAP Arg11Lys and CYP4F2 Val433Met polymorphisms were similar to that of the entire population (3.0 ± 0.5, P<0.0001). Within each polymorphism, the slopes were also similar to each other, therefore resulting in no significant diet*gene interaction on plasma α-tocopherol concentration for any of the SNPs examined. When supplement users were removed from the analysis, the slopes for the relationship between dietary and plasma α-tocopherol approached significance for the entire population (P = 0.055). These slopes were also not significant for any of the polymorphisms studied with a few exceptions (Arg/Lys and Val/Val genotypes).

The crude and adjusted p-values for the diet*gene interaction term on plasma α-tocopherol concentration according to ethno-cultural group and supplement use are shown in Table 5-9. The diet*gene interaction was not significant for any of the ethno-cultural groups.
for the $\alpha$-TTP A>T, TAP C>T or CYP4F2 Val433Met SNPs, both when including and excluding supplement users. The diet*gene interaction was significant, however, for the TAP Arg11Lys polymorphism but only in the Others ethno-cultural group when supplement users were included in the analysis.

5.9 The proportion of the variance in plasma $\alpha$-tocopherol concentration explained by genotype

The $R^2$ values for the models with plasma $\alpha$-tocopherol concentration as the outcome variable and dietary $\alpha$-tocopherol and covariates as the independent variables were calculated in order to determine the proportion of the variation in plasma $\alpha$-tocopherol concentration explained by the independent variables. The results of this analysis are presented in Table 5-10. In both the total population and after the exclusion of supplement users, the $R^2$ value was much greater in the adjusted models compared with the crude models which only contained dietary $\alpha$-tocopherol as the independent variable. Very little of the variance in plasma $\alpha$-tocopherol concentration was explained by dietary $\alpha$-tocopherol alone (crude models). Only 3% of the total variance was explained with the crude model in the total population. This was reduced to only 0.1% after the removal of supplement users. After further adjustment for season, ethno-cultural group, physical activity, total energy intake and total serum cholesterol, the $R^2$ values increased to 0.21 in the total population and 0.23 in subjects not using supplements. The removal of supplement users did not change the proportion of variation explained by the crude or adjusted models.
To determine whether the $\alpha$-TTP, TAP and CYP4F2 SNPs alter the amount of variation in plasma $\alpha$-tocopherol concentration explained by the crude and adjusted models, each polymorphism was entered, one at a time, into the various models (Table 5-10). The addition of genotype to either the crude or adjusted models did not change the $R^2$ value by very much in both the total population and in subjects not using supplements. This indicates that genotype does not further contribute to explaining the variance in plasma $\alpha$-tocopherol concentration.

The $R^2$ value was also determined stratified by genotype for each of the polymorphisms examined in order to determine whether the $R^2$ value is altered in the different genotype groups (Table 5-10). The removal of supplement users did not change the $R^2$ value by very much for each genotype, however, these values did tend to be slightly higher in individuals not using supplements. For the $\alpha$-TTP A>T polymorphism, the adjusted model for subjects with the A/A genotype explained most of the variance in plasma $\alpha$-tocopherol concentration compared with the other $\alpha$-TTP genotypes in both the total population and after excluding supplement users. In the total population, the proportion of variance in $\alpha$-tocopherol concentration explained by the adjusted model in A/A subjects was about 10% higher than that explained by the adjusted model for subjects with the T/T genotype (27% in A/A subjects versus 18% in T/T subjects). After supplement users were excluded, the proportion of the variance explained by the adjusted model in A/A subjects was about 10% higher than that explained in A/T and T/T subjects (32%, 19% and 22% for A/A, A/T and T/T subjects, respectively). The $R^2$ values were similar between the different TAP C>T and TAP Arg11Lys genotypes in both groups (total population and in subjects not consuming
supplements) and ranged between 0.20 and 0.28. The adjusted models for subjects with the Met/Met genotype explained the most (about 40%), while the adjusted models for subjects with the Val/Met genotype explained the least (15%) amount of variance in plasma α-tocopherol concentration compared with the other CYP4F2 genotypes in both the total population and after excluding supplement users. However, due to the small sample size of the Met/Met genotype, these results should be interpreted with caution.

5.10 Combined α-TTP A>T and TAP Arg11Lys genotype results

Since both the α-TTP A>T and TAP Arg11Lys SNPs significantly altered plasma α-tocopherol concentration, particularly in subjects not using supplements, a gene*gene interaction on plasma α-tocopherol concentration was conducted in subjects not using supplements. The results from this analysis are presented in Table 5-11. There was no significant gene*gene interaction on plasma α-tocopherol concentration (P = 0.4), which suggests that one polymorphism does not modify the effect of the other polymorphism on plasma α-tocopherol concentration. Therefore, the two SNPs are acting independently of each other.

The α-TTP A>T and TAP Arg11Lys genotypes were then combined and the effect of these combined genotypes on plasma α-tocopherol concentration and its response to dietary α-tocopherol was examined in subjects not consuming supplements. These findings are listed in Table 5-11. Before genotypes were combined, for each SNP, genotypes with high and intermediate plasma α-tocopherol concentrations were grouped together. For the α-TTP polymorphism, the A/A and A/T genotypes were grouped together and were designated “A
carriers”. The Lys/Lys and Arg/Lys genotypes of the TAP Arg11Lys SNP were grouped together and were designated “Lys carriers”. The high and intermediate plasma α-tocopherol concentration genotypes were grouped together because the effects of each SNP appeared to be most apparent for homozygotes of the T and Arg allele for the α-TTP A>T and TAP Arg11Lys SNPs, respectively. Therefore, by combining the high and intermediate plasma α-tocopherol concentration genotypes together for each polymorphism, the effects of possessing zero, one and two low plasma α-tocopherol concentration genotypes (T/T and Arg/Arg) on plasma α-tocopherol concentration could be determined.

The combined α-TTP A>T and TAP Arg11Lys genotype was significantly associated with plasma α-tocopherol concentration in individuals not consuming supplements (P = 0.003). In the individual SNP analysis, the T/T and Arg/Arg genotypes were associated with lower plasma α-tocopherol concentrations (Tables 5-2 and 5-4). Subjects possessing both of these low plasma α-tocopherol concentration genotypes (T/T + Arg/Arg) had plasma α-tocopherol concentrations that were significantly lower than subjects possessing two of the high plasma α-tocopherol genotypes (A carriers + Lys carriers) (24.6 ± 1.3 μmol/L versus 29.4 ± 0.5 μmol/L). Subjects with only one of these low plasma α-tocopherol concentration genotypes (the Arg/Arg genotype in A carriers + Arg/Arg subjects and the T/T genotype in subjects with the T/T + Lys carriers combined genotype) had intermediate plasma α-tocopherol concentrations (27.9 ± 0.6 μmol/L and 27.7 ± 1.1 μmol/L, respectively). The difference between the highest (in subjects with the A carriers + Lys carriers combined genotype) and lowest (in subjects with the T/T + Arg/Arg combined genotype) plasma α-tocopherol concentrations was 16.3%. This difference was roughly double that from the
individual genotype analysis (9.0% for the $\alpha$-TTP A>T SNP and 8.4% for TAP Arg11Lys SNP), which suggests a potential additive effect of these two low plasma $\alpha$-tocopherol concentration genotypes on plasma $\alpha$-tocopherol concentration.

A diet*gene interaction on plasma $\alpha$-tocopherol concentration using the combined $\alpha$-TTP A>T and TAP Arg11Lys genotypes was also tested in subjects not using supplements and results are shown in Table 5-11. Similar to the individual SNP analysis, there was no significant diet*gene interaction on plasma $\alpha$-tocopherol concentration with the combined genotypes ($P = 0.3$). The proportion of variation in plasma $\alpha$-tocopherol concentration explained by the addition of the $\alpha$-TTP A>T and TAP Arg11Lys combined genotype to the crude and adjusted models with plasma $\alpha$-tocopherol concentration as the outcome variable and dietary $\alpha$-tocopherol and covariates (for adjusted model) as the independent variables was also examined (Table 5-11). The addition of the combined genotype as an independent variable to the adjusted model increased the $R^2$ value compared with the model that did not contain the combined genotype variable (0.25 versus 0.23 (Table 5-10) in subjects not using supplements). Although this increase in the $R^2$ value was small (0.2), it was larger than the $R^2$ values when the $\alpha$-TTP A>T or TAP Arg11Lys genotypes were added individually to the adjusted model (0.24 for both SNPs; Table 5-10). Therefore, the combined $\alpha$-TTP A>T and TAP Arg11Lys genotypes explain slightly more of the variation in plasma $\alpha$-tocopherol concentration compared with each genotype alone.
5.11 Linkage disequilibrium analysis for the two TAP polymorphisms

Based on the similarity in the results of the TAP C>T and TAP Arg11Lys SNPs, a linkage disequilibrium analysis was conducted on these two TAP polymorphisms and the findings from this analysis are listed in Table 5-12. The two TAP polymorphisms were found to be highly linked in the total population as well as in each ethno-cultural group. The $D'$ was 0.98 or greater and the $r^2$ ranged from 0.85 to 0.95 in the different ethno-cultural groups. There does not appear to be a difference in the extent of linkage disequilibrium between the different ethno-cultural groups.
<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Supplement use</th>
<th></th>
<th></th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No</td>
<td>Yes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Subjects [n (% of total)]</td>
<td>879 (70.4)</td>
<td>369 (29.6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sex [n (%)]</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>290 (76.9)</td>
<td>87 (23.1)</td>
<td></td>
<td>0.001</td>
</tr>
<tr>
<td>Female</td>
<td>589 (67.6)</td>
<td>282 (32.4)</td>
<td></td>
<td>0.002</td>
</tr>
<tr>
<td>Age (y)</td>
<td>22.5 ± 0.1</td>
<td>22.9 ± 0.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ethno-cultural group [n (%)]</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caucasian</td>
<td>395 (67.5)</td>
<td>190 (32.5)</td>
<td></td>
<td>0.001</td>
</tr>
<tr>
<td>East Asian</td>
<td>336 (77.2)</td>
<td>99 (22.8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Others</td>
<td>148 (64.9)</td>
<td>80 (35.1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Season [n (%)]</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Winter</td>
<td>245 (69.2)</td>
<td>109 (30.8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spring</td>
<td>259 (73.2)</td>
<td>95 (26.8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Summer</td>
<td>221 (68.9)</td>
<td>100 (31.2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fall</td>
<td>154 (70.3)</td>
<td>65 (29.7)</td>
<td></td>
<td>0.6</td>
</tr>
<tr>
<td>Physical activity (MET-h/wk)</td>
<td>7.5 ± 0.1</td>
<td>7.9 ± 0.2</td>
<td></td>
<td>0.1</td>
</tr>
<tr>
<td>Smoking status [n (%)]</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current smoker</td>
<td>65 (77.4)</td>
<td>19 (22.6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Past and never smoker</td>
<td>814 (69.9)</td>
<td>350 (30.1)</td>
<td></td>
<td>0.1</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>22.8 ± 0.1</td>
<td>22.8 ± 0.2</td>
<td></td>
<td>0.996</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>74.2 ± 0.3</td>
<td>73.6 ± 0.5</td>
<td></td>
<td>0.3</td>
</tr>
<tr>
<td>Systolic blood pressure (mm Hg)</td>
<td>114.0 ± 0.4</td>
<td>113.2 ± 0.6</td>
<td></td>
<td>0.3</td>
</tr>
<tr>
<td>Diastolic blood pressure (mm Hg)</td>
<td>69.0 ± 0.3</td>
<td>68.9 ± 0.4</td>
<td></td>
<td>0.9</td>
</tr>
<tr>
<td>Total serum cholesterol (mmol/L)</td>
<td>4.25 ± 0.03</td>
<td>4.24 ± 0.04</td>
<td></td>
<td>0.9</td>
</tr>
<tr>
<td>Serum HDL cholesterol (mmol/L)</td>
<td>1.54 ± 0.01</td>
<td>1.62 ± 0.02</td>
<td></td>
<td>0.001</td>
</tr>
<tr>
<td>Serum LDL cholesterol (mmol/L)</td>
<td>2.27 ± 0.02</td>
<td>2.18 ± 0.03</td>
<td></td>
<td>0.02</td>
</tr>
<tr>
<td>Plasma α-tocopherol [µmol/L]</td>
<td>28.6 ± 0.4</td>
<td>33.1 ± 0.7</td>
<td></td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Plasma α-tocopherol: total serum cholesterol ratio (µmol/mmol)</td>
<td>6.8 ± 0.1</td>
<td>7.9 ± 0.2</td>
<td></td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Energy (kcal/d)</td>
<td>2072 ± 31</td>
<td>1998 ± 41</td>
<td></td>
<td>0.2</td>
</tr>
<tr>
<td>Dietary α-tocopherol adequacy [n (%)]</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;RDA</td>
<td>773 (88.3)</td>
<td>102 (11.7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥RDA</td>
<td>106 (28.4)</td>
<td>267 (71.6)</td>
<td></td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>α-TTP A&gt;T genotype [n (%)]</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A/A</td>
<td>356 (40.5)</td>
<td>128 (34.7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A/T</td>
<td>373 (42.4)</td>
<td>174 (47.2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T/T</td>
<td>150 (17.1)</td>
<td>67 (18.2)</td>
<td></td>
<td>0.2</td>
</tr>
<tr>
<td>TAP C&gt;T genotype [n (%)]</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C/C</td>
<td>394 (44.8)</td>
<td>158 (42.8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C/T</td>
<td>384 (43.7)</td>
<td>168 (45.5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T/T</td>
<td>101 (11.5)</td>
<td>43 (11.7)</td>
<td></td>
<td>0.8</td>
</tr>
<tr>
<td>TAP Arg11Lys genotype [n (%)]</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arg/Arg</td>
<td>375 (42.7)</td>
<td>151 (40.9)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arg/Lys</td>
<td>386 (43.9)</td>
<td>165 (44.7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lys/Lys</td>
<td>118 (13.4)</td>
<td>53 (14.4)</td>
<td></td>
<td>0.8</td>
</tr>
<tr>
<td>CYP4F2 Val433Met genotype [n (%)]</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Val/Val</td>
<td>444 (50.6)</td>
<td>197 (53.4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Val/Met</td>
<td>362 (41.2)</td>
<td>144 (39.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Met/Met</td>
<td>72 (8.2)</td>
<td>28 (7.6)</td>
<td></td>
<td>0.7</td>
</tr>
</tbody>
</table>

1 A supplement user was defined as a subject who reported taking a vitamin E supplement, a multivitamin or both within the past month. Differences between supplement users and individuals not using supplements were assessed using an unpaired t-test for continuous variables and a χ² test for categorical variables. Abbreviations: α-TTP, α-tocopherol transfer protein; BMI, body mass index; CYP4F2, cytochrome P450, family 4, sub-family
F, polypeptide 2; HDL, high density lipoprotein; LDL, low density lipoprotein; MET-h/wk, metabolic equivalent of task-hours per week; RDA, Recommended Dietary Allowance; TAP, tocopherol associated protein.

2 Mean ± standard error (all such values).

3 P-value was obtained from the analysis using the loge transformed variable and the means presented are the anti-logs.

4 The RDA for vitamin E is 15 mg α-tocopherol per day for adult males and females aged 19 years and older.
Table 5-2. Crude and adjusted plasma α-tocopherol concentration by α-TTP A>T genotype, ethno-cultural group and supplement use.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>All subjects</th>
<th>Excluding supplement users</th>
<th>P-value</th>
<th>Excluding supplement users</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n (%)</td>
<td>Plasma α-tocopherol</td>
<td></td>
<td>n (%)</td>
</tr>
</tbody>
</table>
|                     |              | concentration (μmol/L)
|                     |              | Crude | Adjusted | Crude | Adjusted |
| α-TTP A>T           |              | 29.9 ± 0.5 | 30.8 ± 0.5 | 29.9 ± 0.6 | 30.8 ± 0.6 | 0.8 |
| A/A                 | 484 (38.8)   | 356 (40.5) | 29.1 ± 0.6 | 29.0 ± 0.6 | 0.3 |
| A/T                 | 547 (43.8)   | 373 (42.4) | 28.5 ± 0.6 | 28.6 ± 0.5 | 0.04 |
| T/T                 | 217 (17.4)   | 150 (17.1) | 27.4 ± 0.9 | 26.4 ± 0.9 | 0.09 |
| P-value             | 0.5          | 0.4           | 0.3     | 0.09 |

Ethno-cultural group

Caucasian

<table>
<thead>
<tr>
<th></th>
<th>n (%)</th>
<th>Plasma α-tocopherol</th>
<th></th>
<th>n (%)</th>
<th>Plasma α-tocopherol</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Crude</td>
<td>Adjusted</td>
<td></td>
<td>Crude</td>
<td>Adjusted</td>
</tr>
<tr>
<td>A/A</td>
<td>131 (22.4)</td>
<td>30.5 ± 1.0</td>
<td>31.3 ± 0.9</td>
<td>92 (23.3)</td>
<td>29.3 ± 1.2</td>
<td>29.4 ± 1.0</td>
</tr>
<tr>
<td>A/T</td>
<td>289 (49.4)</td>
<td>31.1 ± 0.7</td>
<td>32.3 ± 0.6</td>
<td>187 (47.3)</td>
<td>29.7 ± 0.8</td>
<td>30.1 ± 0.7</td>
</tr>
<tr>
<td>T/T</td>
<td>165 (28.2)</td>
<td>29.7 ± 0.9</td>
<td>30.9 ± 0.8</td>
<td>116 (29.4)</td>
<td>27.8 ± 1.0</td>
<td>27.6 ± 0.9</td>
</tr>
<tr>
<td>P-value</td>
<td>0.5</td>
<td>0.4</td>
<td></td>
<td>0.3</td>
<td>0.09</td>
<td></td>
</tr>
</tbody>
</table>

East Asian

<table>
<thead>
<tr>
<th></th>
<th>n (%)</th>
<th>Plasma α-tocopherol</th>
<th></th>
<th>n (%)</th>
<th>Plasma α-tocopherol</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Crude</td>
<td>Adjusted</td>
<td></td>
<td>Crude</td>
<td>Adjusted</td>
</tr>
<tr>
<td>A/A</td>
<td>265 (60.9)</td>
<td>29.6 ± 0.7</td>
<td>30.3 ± 0.7</td>
<td>204 (60.7)</td>
<td>28.8 ± 0.8</td>
<td>28.3 ± 0.7</td>
</tr>
<tr>
<td>A/T</td>
<td>151 (34.7)</td>
<td>28.6 ± 0.9</td>
<td>30.5 ± 0.9</td>
<td>119 (35.4)</td>
<td>27.0 ± 1.0</td>
<td>27.7 ± 0.9</td>
</tr>
<tr>
<td>T/T</td>
<td>19 (4.4)</td>
<td>30.3 ± 2.7</td>
<td>29.4 ± 2.4</td>
<td>13 (3.9)</td>
<td>27.7 ± 3.0</td>
<td>26.4 ± 2.7</td>
</tr>
<tr>
<td>P-value</td>
<td>0.7</td>
<td>0.9</td>
<td></td>
<td>0.4</td>
<td>0.8</td>
<td></td>
</tr>
</tbody>
</table>

Others

<table>
<thead>
<tr>
<th></th>
<th>n (%)</th>
<th>Plasma α-tocopherol</th>
<th></th>
<th>n (%)</th>
<th>Plasma α-tocopherol</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Crude</td>
<td>Adjusted</td>
<td></td>
<td>Crude</td>
<td>Adjusted</td>
</tr>
<tr>
<td>A/A</td>
<td>88 (38.6)</td>
<td>30.1 ± 1.2</td>
<td>31.0 ± 1.2</td>
<td>60 (40.5)</td>
<td>29.7 ± 1.5</td>
<td>29.6 ± 1.4</td>
</tr>
<tr>
<td>A/T</td>
<td>107 (46.9)</td>
<td>29.5 ± 1.1</td>
<td>29.7 ± 1.0</td>
<td>67 (45.3)</td>
<td>28.0 ± 1.4</td>
<td>27.7 ± 1.3</td>
</tr>
<tr>
<td>T/T</td>
<td>33 (14.5)</td>
<td>27.5 ± 2.0</td>
<td>27.9 ± 1.8</td>
<td>21 (14.2)</td>
<td>25.3 ± 2.5</td>
<td>25.9 ± 2.3</td>
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<tr>
<td>P-value</td>
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<td>0.3</td>
<td></td>
<td>0.3</td>
<td>0.3</td>
<td></td>
</tr>
</tbody>
</table>

1 Mean ± standard error.

2 Adjusted for supplement use, age, season, ethno-cultural group (when not stratified by ethno-cultural group), physical activity, dietary vitamin C and total serum cholesterol.

3 Adjusted for age, season, ethno-cultural group (when not stratified by ethno-cultural group), physical activity, dietary vitamin C and total serum cholesterol.

ab Different letters indicate statistical significance (p≤0.05) as determined using a post-hoc Tukey test.
<table>
<thead>
<tr>
<th>Genotype</th>
<th>All subjects</th>
<th>Excluding supplement users</th>
<th>n (%)</th>
<th>Plasmatic α-tocopherol concentration (μmol/L)</th>
<th>n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Crude Adjusted</td>
<td>Crude Adjusted</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C/C</td>
<td>552 (44.2) 29.5 ± 0.5 30.0 ± 0.5</td>
<td>394 (44.8) 28.0 ± 0.6 27.5 ± 0.5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C/T</td>
<td>552 (44.2) 30.2 ± 0.5 31.1 ± 0.5</td>
<td>384 (43.7) 29.1 ± 0.6 29.0 ± 0.5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T/T</td>
<td>144 (11.5) 30.1 ± 1.0 31.4 ± 0.9</td>
<td>101 (11.5) 28.9 ± 1.1 29.5 ± 1.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P-value</td>
<td>0.6 0.1</td>
<td>0.4 0.06</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Ethno-cultural group

Caucasian

<table>
<thead>
<tr>
<th>Genotype</th>
<th>n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C/C</td>
<td>272 (46.5) 30.7 ± 0.7 31.4 ± 0.7</td>
</tr>
<tr>
<td>C/T</td>
<td>259 (44.3) 30.4 ± 0.7 31.8 ± 0.7</td>
</tr>
<tr>
<td>T/T</td>
<td>54 (9.2) 30.8 ± 1.6 32.7 ± 1.4</td>
</tr>
<tr>
<td>P-value</td>
<td>0.9 0.7</td>
</tr>
</tbody>
</table>

East Asian

<table>
<thead>
<tr>
<th>Genotype</th>
<th>n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C/C</td>
<td>195 (44.8) 28.2 ± 0.8 29.0 ± 0.8</td>
</tr>
<tr>
<td>C/T</td>
<td>184 (42.3) 30.4 ± 0.9 31.4 ± 0.9</td>
</tr>
<tr>
<td>T/T</td>
<td>56 (12.9) 29.5 ± 1.6 31.4 ± 1.4</td>
</tr>
<tr>
<td>P-value</td>
<td>0.2 0.06</td>
</tr>
</tbody>
</table>

Others

<table>
<thead>
<tr>
<th>Genotype</th>
<th>n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C/C</td>
<td>85 (37.3) 29.0 ± 1.2 29.5 ± 1.2</td>
</tr>
<tr>
<td>C/T</td>
<td>109 (47.8) 29.6 ± 1.1 30.2 ± 1.0</td>
</tr>
<tr>
<td>T/T</td>
<td>34 (14.9) 29.8 ± 2.0 30.0 ± 1.8</td>
</tr>
<tr>
<td>P-value</td>
<td>0.9 0.9</td>
</tr>
</tbody>
</table>

1 Mean ± standard error.

2 Adjusted for supplement use, age, season, ethno-cultural group (when not stratified by ethno-cultural group), physical activity, dietary vitamin C and total serum cholesterol.

3 Adjusted for age, season, ethno-cultural group (when not stratified by ethno-cultural group), physical activity, dietary vitamin C and total serum cholesterol.

a,b Different letters indicate statistical significance (p≤0.05) as determined using a post-hoc Tukey test.
Table 5-4. Crude and adjusted plasma $\alpha$-tocopherol concentration by TAP Arg11Lys genotype, ethno-cultural group and supplement use.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>All subjects</th>
<th>Excluding supplement users</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$n$ (%)</td>
<td>Plasma $\alpha$-tocopherol concentration ($\mu$mol/L)$^1$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Crude</td>
</tr>
<tr>
<td>TAP Arg11Lys</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arg/Arg</td>
<td>526 (42.1)</td>
<td>29.4 ± 0.5</td>
</tr>
<tr>
<td>Arg/Lys</td>
<td>551 (44.2)</td>
<td>30.1 ± 0.5</td>
</tr>
<tr>
<td>Lys/Lys</td>
<td>171 (13.7)</td>
<td>30.7 ± 0.9</td>
</tr>
<tr>
<td>P-value</td>
<td>0.4</td>
<td><strong>0.05</strong></td>
</tr>
<tr>
<td>Ethno-cultural group</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caucasian</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arg/Arg</td>
<td>258 (44.1)</td>
<td>30.4 ± 0.7</td>
</tr>
<tr>
<td>Arg/Lys</td>
<td>255 (43.6)</td>
<td>30.3 ± 0.7</td>
</tr>
<tr>
<td>Lys/Lys</td>
<td>72 (12.3)</td>
<td>31.7 ± 1.4</td>
</tr>
<tr>
<td>P-value</td>
<td>0.7</td>
<td>0.3</td>
</tr>
<tr>
<td>East Asian</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arg/Arg</td>
<td>189 (43.4)</td>
<td>28.1 ± 0.8</td>
</tr>
<tr>
<td>Arg/Lys</td>
<td>188 (43.2)</td>
<td>30.2 ± 0.8</td>
</tr>
<tr>
<td>Lys/Lys</td>
<td>58 (13.3)</td>
<td>30.1 ± 1.5</td>
</tr>
<tr>
<td>P-value</td>
<td>0.2</td>
<td>0.07</td>
</tr>
<tr>
<td>Others</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arg/Arg</td>
<td>79 (34.6)</td>
<td>29.3 ± 1.3</td>
</tr>
<tr>
<td>Arg/Lys</td>
<td>108 (47.4)</td>
<td>29.4 ± 1.1</td>
</tr>
<tr>
<td>Lys/Lys</td>
<td>41 (18.0)</td>
<td>29.7 ± 1.8</td>
</tr>
<tr>
<td>P-value</td>
<td>0.98</td>
<td>0.9</td>
</tr>
</tbody>
</table>

$^1$ Mean ± standard error.

$^2$ Adjusted for supplement use, age, season, ethno-cultural group (when not stratified by ethno-cultural group), physical activity, dietary vitamin C and total serum cholesterol.

$^3$ Adjusted for age, season, ethno-cultural group (when not stratified by ethno-cultural group), physical activity, dietary vitamin C and total serum cholesterol.

$^a, b$ Different letters indicate statistical significance ($p \leq 0.05$) as determined using a post-hoc Tukey test.
Table 5-5. Crude and adjusted plasma α-tocopherol concentration by CYP4F2 Val433Met genotype, ethno-cultural group and supplement use.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>All subjects</th>
<th></th>
<th>Excluding supplement users</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n (%)</td>
<td>Plasma α-tocopherol concentration (μmol/L)</td>
<td>Adjusted&lt;sup&gt;2&lt;/sup&gt;</td>
<td>n (%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Crude</td>
<td>Adjusted&lt;sup&gt;3&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Val/Val</td>
<td>641 (51.4)</td>
<td>29.7 ± 0.5</td>
<td>30.3 ± 0.5</td>
<td>444 (50.6)</td>
</tr>
<tr>
<td>Val/Met</td>
<td>506 (40.6)</td>
<td>29.8 ± 0.5</td>
<td>30.8 ± 0.5</td>
<td>362 (41.2)</td>
</tr>
<tr>
<td>Met/Met</td>
<td>100 (8.0)</td>
<td>31.9 ± 1.2</td>
<td>32.3 ± 1.1</td>
<td>72 (8.2)</td>
</tr>
<tr>
<td>P-value</td>
<td>0.2</td>
<td>0.2</td>
<td></td>
<td>0.4</td>
</tr>
<tr>
<td>P-value</td>
<td>0.2</td>
<td>0.2</td>
<td></td>
<td>0.4</td>
</tr>
</tbody>
</table>

Ethno-cultural group

**Caucasian**

| Val/Val           | 286 (48.9)   | 30.3 ± 0.7   | 31.3 ± 0.6                 | 185 (46.8)   | 28.6 ± 0.8   | 28.7 ± 0.7                 |
| Val/Met           | 245 (41.9)   | 30.4 ± 0.8   | 31.7 ± 0.7                 | 171 (43.3)   | 29.1 ± 0.8   | 29.2 ± 0.7                 |
| Met/Met           | 54 (9.2)     | 32.5 ± 1.6   | 34.1 ± 1.4                 | 39 (9.9)     | 30.8 ± 1.8   | 31.4 ± 1.6                 |
| P-value           | 0.4          | 0.2          |                           | 0.5          | 0.3          |                           |

**East Asian**

| Val/Val           | 267 (61.4)   | 29.3 ± 0.7   | 30.1 ± 0.7                 | 203 (60.4)   | 28.3 ± 0.8   | 27.9 ± 0.7                 |
| Val/Met           | 148 (34.0)   | 29.0 ± 1.0   | 30.5 ± 1.0                 | 118 (35.1)   | 27.9 ± 1.0   | 28.1 ± 0.9                 |
| Met/Met           | 20 (4.6)     | 31.1 ± 2.6   | 31.5 ± 2.4                 | 15 (4.5)     | 28.0 ± 2.8   | 27.8 ± 2.5                 |
| P-value           | 0.7          | 0.8          |                           | 0.9          | 0.98         |                           |

**Others**

| Val/Val           | 88 (38.8)    | 28.7 ± 1.2   | 29.2 ± 1.2                 | 56 (38.1)    | 28.0 ± 1.5   | 28.2 ± 1.4                 |
| Val/Met           | 113 (49.8)   | 29.7 ± 1.1   | 30.4 ± 1.0                 | 73 (49.7)    | 28.0 ± 1.4   | 28.2 ± 1.3                 |
| Met/Met           | 26 (11.5)    | 31.3 ± 2.3   | 30.4 ± 2.1                 | 18 (12.2)    | 30.9 ± 2.7   | 28.3 ± 2.5                 |
| P-value           | 0.6          | 0.7          |                           | 0.6          | 0.999        |                           |

<sup>1</sup> Mean ± standard error.

<sup>2</sup> Adjusted for supplement use, age, season, ethno-cultural group (when not stratified by ethno-cultural group), physical activity, dietary vitamin C and total serum cholesterol.

<sup>3</sup> Adjusted for age, season, ethno-cultural group (when not stratified by ethno-cultural group), physical activity, dietary vitamin C and total serum cholesterol.
Table 5-6. Crude and adjusted p-values for the supplement use*gene interaction on plasma α-tocopherol concentration by ethnocultural group for the α-TTP, TAP and CYP4F2 polymorphisms.

<table>
<thead>
<tr>
<th>Polymorphism</th>
<th>α-TTP A&gt;T</th>
<th>TAP C&gt;T</th>
<th>TAP Arg11Lys</th>
<th>CYP4F2 Val433Met</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Crude</td>
<td>Adjusted</td>
<td>Crude</td>
<td>Adjusted</td>
</tr>
<tr>
<td>All subjects</td>
<td>0.3</td>
<td>0.1</td>
<td>0.6</td>
<td>0.6</td>
</tr>
<tr>
<td>Caucasian</td>
<td>0.5</td>
<td>0.2</td>
<td>0.7</td>
<td>0.6</td>
</tr>
<tr>
<td>East Asian</td>
<td>0.3</td>
<td>0.4</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Others</td>
<td>0.6</td>
<td>0.9</td>
<td><strong>0.02</strong></td>
<td><strong>0.006</strong></td>
</tr>
</tbody>
</table>

1 Adjusted for age, season, ethno-cultural group (for all subjects only), physical activity, dietary vitamin C and total serum cholesterol.
Table 5-7. Crude and adjusted Spearman correlation coefficients for the relationship between dietary and plasma $\alpha$-tocopherol by genotype and supplement use.

<table>
<thead>
<tr>
<th></th>
<th>All subjects</th>
<th>Excluding supplement users</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n (%)</td>
<td>Spearman correlation</td>
</tr>
<tr>
<td></td>
<td></td>
<td>coefficient (p-value)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Crude</td>
</tr>
<tr>
<td>Total</td>
<td>1062 (100)</td>
<td>0.15 ($&lt;0.0001$)</td>
</tr>
<tr>
<td>$\alpha$-TTP A&gt;T</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A/A</td>
<td>410 (38.6)</td>
<td>0.11 (0.03)</td>
</tr>
<tr>
<td>A/T</td>
<td>460 (43.3)</td>
<td>0.20 ($&lt;0.0001$)</td>
</tr>
<tr>
<td>T/T</td>
<td>192 (18.1)</td>
<td>0.15 (0.04)</td>
</tr>
<tr>
<td>TAP C&gt;T</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C/C</td>
<td>470 (44.3)</td>
<td>0.15 (0.001)</td>
</tr>
<tr>
<td>C/T</td>
<td>472 (44.4)</td>
<td>0.13 (0.004)</td>
</tr>
<tr>
<td>T/T</td>
<td>120 (11.3)</td>
<td>0.24 (0.009)</td>
</tr>
<tr>
<td>TAP Arg11Lys</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arg/Arg</td>
<td>445 (41.9)</td>
<td>0.16 (0.001)</td>
</tr>
<tr>
<td>Arg/Lys</td>
<td>475 (44.7)</td>
<td>0.14 (0.003)</td>
</tr>
<tr>
<td>Lys/Lys</td>
<td>142 (13.4)</td>
<td>0.20 (0.02)</td>
</tr>
<tr>
<td>CYP4F2 Val433Met</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Val/Val</td>
<td>558 (52.6)</td>
<td>0.17 ($&lt;0.0001$)</td>
</tr>
<tr>
<td>Val/Met</td>
<td>416 (39.2)</td>
<td>0.15 (0.002)</td>
</tr>
<tr>
<td>Met/Met</td>
<td>87 (8.2)</td>
<td>0.01 (0.9)</td>
</tr>
</tbody>
</table>

$^1$ Adjusted for season, ethno-cultural group, physical activity, total energy intake and total serum cholesterol.
Table 5-8. Crude and adjusted slopes for the relationship between dietary and plasma α-tocopherol and diet*gene interaction on plasma α-tocopherol concentration by genotype and supplement use.1

<table>
<thead>
<tr>
<th></th>
<th>All subjects</th>
<th></th>
<th>Excluding supplement users</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Crude</td>
<td>Adjusted*</td>
<td>Crude</td>
<td>Adjusted*</td>
</tr>
<tr>
<td>Total</td>
<td>2.8 ± 0.5 (&lt;0.0001)</td>
<td>3.0 ± 0.5 (&lt;0.0001)</td>
<td>0.8 ± 0.9 (0.3)</td>
<td>1.9 ± 1.0 (0.055)</td>
</tr>
<tr>
<td>α-TTP A&gt;T</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A/A</td>
<td>2.1 ± 0.8 (0.006)</td>
<td>2.4 ± 0.7 (0.001)</td>
<td>-0.01 ± 1.4 (0.99)</td>
<td>1.3 ± 1.6 (0.4)</td>
</tr>
<tr>
<td>A/T</td>
<td>3.6 ± 0.7 (&lt;0.0001)</td>
<td>3.2 ± 0.7 (&lt;0.0001)</td>
<td>2.1 ± 1.4 (0.1)</td>
<td>2.7 ± 1.6 (0.09)</td>
</tr>
<tr>
<td>T/T</td>
<td>2.7 ± 1.1 (0.01)</td>
<td>3.9 ± 1.1 (0.003)</td>
<td>-0.07 ± 1.9 (0.97)</td>
<td>1.2 ± 2.4 (0.6)</td>
</tr>
<tr>
<td>Interaction p-value</td>
<td>0.4</td>
<td>0.6</td>
<td>0.5</td>
<td>0.8</td>
</tr>
<tr>
<td>TAP C&gt;T</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C/C</td>
<td>2.8 ± 0.7 (&lt;0.0001)</td>
<td>2.7 ± 0.6 (&lt;0.0001)</td>
<td>0.1 ± 1.3 (0.9)</td>
<td>1.1 ± 1.6 (0.5)</td>
</tr>
<tr>
<td>C/T</td>
<td>2.6 ± 0.7 (0.0002)</td>
<td>3.1 ± 0.7 (&lt;0.0001)</td>
<td>1.0 ± 1.2 (0.4)</td>
<td>2.7 ± 1.4 (0.058)</td>
</tr>
<tr>
<td>T/T</td>
<td>4.5 ± 1.8 (0.01)</td>
<td>4.9 ± 1.8 (0.007)</td>
<td>3.8 ± 3.3 (0.2)</td>
<td>1.6 ± 4.2 (0.7)</td>
</tr>
<tr>
<td>Interaction p-value</td>
<td>0.6</td>
<td>0.3</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>TAP Arg11Lys</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arg/Arg</td>
<td>3.0 ± 0.7 (&lt;0.0001)</td>
<td>2.8 ± 0.7 (&lt;0.0001)</td>
<td>0.04 ± 1.3 (0.98)</td>
<td>-0.2 ± 1.6 (0.9)</td>
</tr>
<tr>
<td>Arg/Lys</td>
<td>2.5 ± 0.7 (0.0004)</td>
<td>2.8 ± 0.7 (&lt;0.0001)</td>
<td>1.3 ± 1.3 (0.3)</td>
<td>3.4 ± 1.5 (0.02)</td>
</tr>
<tr>
<td>Lys/Lys</td>
<td>4.1 ± 1.4 (0.005)</td>
<td>4.3 ± 1.5 (0.004)</td>
<td>2.3 ± 2.9 (0.4)</td>
<td>2.2 ± 3.7 (0.5)</td>
</tr>
<tr>
<td>Interaction p-value</td>
<td>0.6</td>
<td>0.4</td>
<td>0.7</td>
<td>0.3</td>
</tr>
<tr>
<td>CYP4F2 Val433Met</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Val/Val</td>
<td>3.1 ± 0.6 (&lt;0.0001)</td>
<td>3.3 ± 0.6 (&lt;0.0001)</td>
<td>1.7 ± 1.2 (0.2)</td>
<td>2.8 ± 1.4 (0.04)</td>
</tr>
<tr>
<td>Val/Met</td>
<td>2.8 ± 0.7 (0.0002)</td>
<td>2.8 ± 0.7 (0.0001)</td>
<td>0.8 ± 1.3 (0.6)</td>
<td>2.0 ± 1.7 (0.2)</td>
</tr>
<tr>
<td>Met/Met</td>
<td>1.4 ± 2.1 (0.5)</td>
<td>1.7 ± 2.0 (0.4)</td>
<td>-4.4 ± 3.3 (0.2)</td>
<td>-5.2 ± 4.1 (0.2)</td>
</tr>
<tr>
<td>Interaction p-value</td>
<td>0.7</td>
<td>0.8</td>
<td>0.2</td>
<td>0.5</td>
</tr>
</tbody>
</table>

1 The slopes presented were obtained utilizing the loge transformed dietary α-tocopherol variable. The results are expressed as slope ± standard error (p-value). The sample sizes are identical to those listed in Table 7.

2 Adjusted for season, ethno-cultural group, physical activity, total energy intake and total serum cholesterol.
Table 5-9. Crude and adjusted p-values for the diet*gene interaction on plasma α-tocopherol concentration by ethno-cultural group for the α-TTP, TAP and CYP4F2 polymorphisms.  

<table>
<thead>
<tr>
<th>Polymorphism</th>
<th>Polymorphism</th>
<th>Polymorphism</th>
<th>Polymorphism</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-TTP A&gt;T</td>
<td>α-TTP A&gt;T</td>
<td>TAP C&gt;T</td>
<td>TAP C&gt;T</td>
</tr>
<tr>
<td>Crude</td>
<td>Adjusted$^2$</td>
<td>Crude</td>
<td>Adjusted$^2$</td>
</tr>
<tr>
<td>All subjects</td>
<td>1062 (100)</td>
<td>0.4</td>
<td>0.6</td>
</tr>
<tr>
<td>Ethno-cultural group</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caucasian</td>
<td>517 (48.7)</td>
<td>0.7</td>
<td>0.7</td>
</tr>
<tr>
<td>East Asian</td>
<td>358 (33.7)</td>
<td>0.3</td>
<td>0.4</td>
</tr>
<tr>
<td>Others</td>
<td>187 (17.6)</td>
<td>0.1</td>
<td>0.4</td>
</tr>
<tr>
<td>Excluding supplement users</td>
<td>741 (69.8)</td>
<td>0.5</td>
<td>0.8</td>
</tr>
<tr>
<td>Ethno-cultural group</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caucasian</td>
<td>348 (47.0)</td>
<td>0.97</td>
<td>0.8</td>
</tr>
<tr>
<td>East Asian</td>
<td>274 (37.0)</td>
<td>0.5</td>
<td>0.6</td>
</tr>
<tr>
<td>Others</td>
<td>119 (16.1)</td>
<td>0.3</td>
<td>0.6</td>
</tr>
</tbody>
</table>

1 The dietary α-tocopherol variable was loge transformed.

2 Adjusted for season, ethno-cultural group (when not stratified by ethno-cultural group), physical activity, total energy intake and total serum cholesterol.
Table 5-10. \( R^2 \) values for the crude and adjusted models for the relationship between dietary and plasma \( \alpha \)-tocopherol by genotype and supplement use.\(^1\)

<table>
<thead>
<tr>
<th></th>
<th>All subjects</th>
<th></th>
<th>Excluding supplement users</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Crude</td>
<td>Adjusted</td>
<td>Crude</td>
<td>Adjusted</td>
</tr>
<tr>
<td>Total</td>
<td>0.03</td>
<td>0.21</td>
<td>0.001</td>
<td>0.23</td>
</tr>
<tr>
<td>( \alpha )-TTP A&gt;T(^2)</td>
<td>0.03</td>
<td>0.21</td>
<td>0.004</td>
<td>0.24</td>
</tr>
<tr>
<td>A/A</td>
<td>0.02</td>
<td>0.27</td>
<td>0.00</td>
<td>0.32</td>
</tr>
<tr>
<td>A/T</td>
<td>0.05</td>
<td>0.21</td>
<td>0.01</td>
<td>0.19</td>
</tr>
<tr>
<td>T/T</td>
<td>0.03</td>
<td>0.18</td>
<td>0.00</td>
<td>0.22</td>
</tr>
<tr>
<td>TAP C&gt;T(^3)</td>
<td>0.03</td>
<td>0.21</td>
<td>0.003</td>
<td>0.24</td>
</tr>
<tr>
<td>C/C</td>
<td>0.04</td>
<td>0.25</td>
<td>0.00</td>
<td>0.28</td>
</tr>
<tr>
<td>C/T</td>
<td>0.03</td>
<td>0.20</td>
<td>0.002</td>
<td>0.23</td>
</tr>
<tr>
<td>T/T</td>
<td>0.05</td>
<td>0.23</td>
<td>0.02</td>
<td>0.28</td>
</tr>
<tr>
<td>TAP Arg11Lys(^4)</td>
<td>0.03</td>
<td>0.21</td>
<td>0.004</td>
<td>0.24</td>
</tr>
<tr>
<td>Arg/Arg</td>
<td>0.04</td>
<td>0.26</td>
<td>0.00</td>
<td>0.28</td>
</tr>
<tr>
<td>Arg/Lys</td>
<td>0.03</td>
<td>0.20</td>
<td>0.003</td>
<td>0.22</td>
</tr>
<tr>
<td>Lys/Lys</td>
<td>0.05</td>
<td>0.23</td>
<td>0.01</td>
<td>0.27</td>
</tr>
<tr>
<td>CYP4F2 Val433Met(^5)</td>
<td>0.04</td>
<td>0.21</td>
<td>0.003</td>
<td>0.24</td>
</tr>
<tr>
<td>Val/Val</td>
<td>0.04</td>
<td>0.24</td>
<td>0.01</td>
<td>0.29</td>
</tr>
<tr>
<td>Val/Met</td>
<td>0.03</td>
<td>0.15</td>
<td>0.001</td>
<td>0.15</td>
</tr>
<tr>
<td>Met/Met</td>
<td>0.01</td>
<td>0.38</td>
<td>0.03</td>
<td>0.43</td>
</tr>
</tbody>
</table>

\(^1\) The crude models included plasma \( \alpha \)-tocopherol as the outcome variable and dietary \( \alpha \)-tocopherol (\( \log_e \) transformed) as the independent variable. The adjusted models also included season, ethno-cultural group, physical activity, total energy intake and total serum cholesterol as independent variables. The sample sizes are identical to those listed in Table 7.

\(^2\) The crude and adjusted models additionally included \( \alpha \)-TTP A>T genotype as an independent variable.

\(^3\) The crude and adjusted models additionally included TAP C>T genotype as an independent variable.

\(^4\) The crude and adjusted models additionally included TAP Arg11Lys genotype as an independent variable.

\(^5\) The crude and adjusted models additionally included CYP4F2 Val433Met genotype as an independent variable.
Table 5-11. Crude and adjusted plasma α-tocopherol concentration by combined α-TTP A>T and TAP Arg11Lys genotypes.

<table>
<thead>
<tr>
<th>Combined genotype</th>
<th>n  (%)</th>
<th>Plasma α-tocopherol concentration (µmol/L)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Crude</td>
<td>Adjusted</td>
<td></td>
</tr>
<tr>
<td>A Carriers + Lys Carriers</td>
<td>415 (47.2)</td>
<td>29.3 ± 0.5</td>
<td>29.4 ± 0.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>A Carriers + Arg/Arg</td>
<td>314 (35.7)</td>
<td>28.1 ± 0.6</td>
<td>27.9 ± 0.6&lt;sup&gt;ab&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>T/T + Lys Carriers</td>
<td>89 (10.1)</td>
<td>28.3 ± 1.2</td>
<td>27.7 ± 1.1&lt;sup&gt;ab&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>T/T + Arg/Arg</td>
<td>61 (6.9)</td>
<td>26.2 ± 1.4</td>
<td>24.6 ± 1.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
</tbody>
</table>

- P-value (Plasma): 0.2
- Gene*gene interaction p-value: 0.6
- Diet*gene interaction p-value: 0.2
- R<sup>2</sup> value: 0.008<sup>5</sup>, 0.25<sup>6</sup>

<sup>1</sup>A Carriers = A/A + A/T genotypes for the α-TTP A>T SNP; Lys Carriers = Lys/Lys + Arg/Lys genotypes for the TAP Arg11Lys SNP.

<sup>2</sup>Mean ± standard error. Adjusted plasma α-tocopherol concentrations are adjusted for age, season, ethno-cultural group, physical activity, dietary vitamin C and total serum cholesterol.

<sup>3</sup>Adjusted for age, season, ethno-cultural group, physical activity, dietary vitamin C and total serum cholesterol.

<sup>4</sup>Adjusted for season, ethno-cultural group, physical activity, total energy intake and total serum cholesterol.

<sup>5</sup>R<sup>2</sup> value for the model containing plasma α-tocopherol concentration as the outcome variable and dietary α-tocopherol and the α-TTP A>T and TAP Arg11Lys combined genotype as the independent variables.

<sup>6</sup>R<sup>2</sup> value for the model containing plasma α-tocopherol concentration as the outcome variable and dietary α-tocopherol, the α-TTP A>T and TAP Arg11Lys combined genotype and covariates (season, ethno-cultural group, physical activity, total energy intake and total serum cholesterol) as the independent variables.

<sup>a, b</sup>Different letters indicate statistical significance (p≤0.05) as determined using a post-hoc Tukey test.
Table 5-12. $D'$ and $r^2$ values from the linkage disequilibrium analysis for the two TAP polymorphisms for the total population and for the different ethno-cultural groups.

<table>
<thead>
<tr>
<th>Ethno-cultural group</th>
<th>n (%)</th>
<th>$D'$</th>
<th>$r^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>All subjects</td>
<td>1248 (100)</td>
<td>0.99</td>
<td>0.90</td>
</tr>
<tr>
<td>Caucasian</td>
<td>585 (46.9)</td>
<td>1.0</td>
<td>0.88</td>
</tr>
<tr>
<td>East Asian</td>
<td>435 (34.9)</td>
<td>0.995</td>
<td>0.95</td>
</tr>
<tr>
<td>Others</td>
<td>228 (18.3)</td>
<td>0.98</td>
<td>0.85</td>
</tr>
</tbody>
</table>
Chapter SIX:
Discussion, Limitations and Future Directions
6.1 Discussion

There is a lot of variability in plasma α-tocopherol concentration and its response to dietary α-tocopherol between individuals that is unexplained even after adjusting for determinants of plasma α-tocopherol concentration including plasma cholesterol and lipids, age, body mass index, plasma antioxidants and smoking [16-23, 165]. This suggests that other variables are responsible for some of this unaccounted variation in plasma α-tocopherol concentration and its response to dietary α-tocopherol. Since plasma α-tocopherol concentration at any one time is a function of the amount of dietary α-tocopherol absorbed, the amount taken up by cells, the amount released from cells and the amount that is utilized, metabolized and excreted, it is possible that variation in genes involved in any one of these processes in α-tocopherol metabolism may explain some of the inter-individual differences in plasma α-tocopherol concentration. Some evidence for a role of genetic variation in explaining variability in plasma α-tocopherol concentration stems from studies that have shown large inter-individual variation in the plasma response to dietary α-tocopherol, but much lower intra-individual variation that is stable over time [23]. In addition, previous studies have also reported that polymorphisms in α-tocopherol metabolism genes, mainly those that are also involved in lipid metabolism, can influence α-tocopherol concentration in the blood [202-208]. These studies, however, have not examined whether these genetic variations can modify the plasma response to dietary α-tocopherol. Therefore, the current thesis aimed to determine whether common variation in α-tocopherol metabolism genes influence plasma α-tocopherol concentration or modify the plasma response to dietary α-tocopherol.
The candidate genes chosen for this study were the \( \alpha \)-tocopherol transfer protein (\( \alpha \)-TTP) which is responsible for the selective retention of \( \alpha \)-tocopherol by the body, tocopherol associated protein (TAP), a cellular \( \alpha \)-tocopherol binding protein and CYP4F2, which catabolizes \( \alpha \)-tocopherol [8-11, 151]. The results of the present thesis suggest that a single nucleotide polymorphism in the \( \alpha \)-TTP (rs6994076 A>T) and TAP (Arg11Lys (rs757660)) genes influence fasting plasma \( \alpha \)-tocopherol concentration. The \( \alpha \)-TTP A>T SNP only altered plasma \( \alpha \)-tocopherol concentrations in individuals who do not consume vitamin E-containing supplements, while the TAP Arg11Lys SNP was associated with plasma \( \alpha \)-tocopherol concentration in both the total population and after excluding supplement users. In subjects not using supplements, the combined \( \alpha \)-TTP A>T and TAP Arg11Lys genotypes also influenced plasma \( \alpha \)-tocopherol concentration, possibly through an additive effect of these two SNPs. The results also suggest that none of the polymorphisms examined modify the relationship between dietary and plasma \( \alpha \)-tocopherol. These findings are summarized in Table 6-1.

**Table 6-1.** Overall summary of thesis results.

<table>
<thead>
<tr>
<th>Polymorphism</th>
<th>( \alpha )-TTP A&gt;T</th>
<th>TAP C&gt;T</th>
<th>TAP Arg11Lys</th>
<th>CYP4F2 Val433Met</th>
</tr>
</thead>
<tbody>
<tr>
<td>Significant association with plasma ( \alpha )-tocopherol</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>All subjects</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Excluding supplement users</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Significant diet*gene interaction on plasma ( \alpha )-tocopherol concentration</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>All subjects</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Excluding supplement users</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
</tbody>
</table>
6.1.1 The effect of genetic variation in α-TTP, TAP and CYP4F2 on plasma α-tocopherol concentration

6.1.1.1 α-TTP rs6994076 A>T SNP

The role of α-TTP in maintaining plasma α-tocopherol concentration is demonstrated in individuals with ataxia with vitamin E deficiency (AVED). These individuals have rare variations in the α-TTP gene that lead to severely low plasma α-tocopherol concentrations (<5 µmol/L; deficiency <11.6 µmol/L) [12, 209-212]. It is therefore possible that variations in this gene that occur more frequently in the population may be able to explain some of the subtle differences in plasma α-tocopherol concentration between individuals. Indeed, the results of the present study suggest that the rs6994076 A>T polymorphism influences fasting plasma α-tocopherol concentration in a group of relatively healthy young adults between the ages of 20 and 29 years (Table 5-2). This polymorphism, however, only appeared to have an effect in the group of subjects who did not consume vitamin E-containing supplements. In this group, subjects homozygous for the T allele had significantly lower plasma α-tocopherol concentrations compared with A/A subjects, while A/T subjects had an intermediate plasma α-concentration. In the total population, which included supplement users, there was no significant difference in plasma α-tocopherol concentration between the different α-TTP A>T genotypes.

One possible explanation for the differential effects of the α-TTP A>T polymorphism on plasma α-tocopherol concentration depending on the presence or absence of vitamin E-containing supplement users may be that the higher amounts of α-tocopherol ingested by
supplement users saturate the α-TTP [130, 241]. Under this condition, α-TTP would be working at its maximum capacity and, therefore, no longer able to secrete any additional α-tocopherol into VLDLs [130]. The α-tocopherol that is not secreted into VLDLs would be metabolized and excreted [130]. Consequently, it would be difficult to further elevate plasma α-tocopherol concentration regardless of the functional ability of the different α-TTP variants. This explanation is supported by α-tocopherol supplementation studies showing that plasma α-tocopherol concentration does not increase more than about three to four-fold its baseline value, regardless of the supplement dose, frequency or duration [241, 242].

A further explanation may be differences in the expression of α-TTP between supplement users and those not consuming supplements. Vitamin E has been reported to influence α-TTP expression, at least in vitamin E repleted animals that were previously vitamin E deficient [39]. Therefore, if this were the case, a constant supply of high α-tocopherol doses in supplemented individuals may result in lowered α-TTP expression, thereby decreasing the number of α-TTP units that are able to secrete α-tocopherol into VLDL. This would further result in saturation of the α-TTP system, leading to an inability to increase circulating α-tocopherol concentrations by very much.

The difference in plasma α-tocopherol concentration between the T/T and A/A subjects is not very large (2.6 μmol/L), which may suggest that the significant finding arose due to chance. However, the influence of the α-TTP A>T polymorphism on plasma α-tocopherol concentration observed in the present study is in agreement with a recent study by Wright et al [208]. The Wright et al. study also reported that T/T subjects had significantly
lower serum α-tocopherol concentrations compared with A/A subjects in a sample of Finnish middle-aged male smokers 50 to 69 years with similar α-tocopherol concentrations as the Toronto Nutrigenomics and Health (TNH) study population [208]. The percent difference in plasma α-tocopherol concentration between the T/T and A/A subjects was small in both studies, although it was a bit larger in the TNH population (-9.0% versus -3.1%) [208]. The Finnish population was comprised of mainly individuals who did not use supplements and subjects who were not willing to discontinue their use of vitamin E supplements in excess of 20 mg were ineligible for the study [208, 243, 244]. Therefore, since the results from the Wright et al. study are from individuals with α-tocopherol intakes that are achievable from the diet alone, the results are comparable to the TNH sub-group that did not consume supplements. The similar findings between the present and the Wright et al. study lend credence to the effect of the α-TTP A>T polymorphism on fasting plasma α-tocopherol concentration in individuals with α-tocopherol intakes obtainable from dietary sources.

The small difference in plasma α-tocopherol concentration between the T/T and A/A genotypes of the α-TTP A>T polymorphism could also mean that this SNP is in linkage disequilibrium with another polymorphism that has a much greater effect on plasma α-tocopherol concentration. The study design of the present and Wright et al. studies do not allow one to definitively conclude that the α-TTP A>T polymorphism is indeed the polymorphism that is influencing plasma α-tocopherol concentration. This issue would have to be resolved with the use of a functional assay.
Nevertheless, the small difference in plasma α-tocopherol concentration between the T/T and A/A genotypes of the α-TTP A>T polymorphism does not come as a major surprise. Variations in the α-TTP gene that cause severely low plasma α-tocopherol concentrations and lead to AVED are very rare [210, 212]. Other than in individuals with AVED or with fat malabsorption diseases, very low plasma α-tocopherol concentrations do not typically occur [25, 83]. Therefore, it appears that there is a tendency for the selection of variations in the α-TTP gene that do not lead to such drastic reductions in plasma α-tocopherol concentrations. Under this hypothesis, variations that lead to small differences in plasma α-tocopherol concentration would occur more commonly in the population.

To determine whether the results were due to population admixture or population stratification, the analysis was also conducted stratified by ethno-cultural group (Table 5-2). No significant difference in plasma α-tocopherol concentration was observed between the different α-TTP A>T genotypes for each ethno-cultural group, regardless of supplement use. Therefore, it may be possible that no significant difference in plasma α-tocopherol concentration between the α-TTP genotypes was observed in the analysis stratified by ethno-cultural group because of the small sample sizes of these groups. If a difference truly exists between the different α-TTP A>T genotypes, it appears that the difference is small. Thus, a larger sample size would be required in order to detect a difference in plasma α-tocopherol concentration between the α-TTP A>T genotypes if it did exist. Indeed, in the largest of the three ethno-cultural groups, the Caucasians, a trend was observed that approached significance. Further investigation using a larger sample size for various ethno-cultural groups is thus required in order to determine whether the effect of the α-TTP A>T SNP observed in
the unstratified sub-group consisting of subjects not using supplements was attributed to the trend in the Caucasian group and also to determine whether the non-significant findings in the stratified analysis was due to the small sample sizes of the ethno-cultural groups or to an actual lack of an effect of the $\alpha$-TTP A$>$T polymorphism in these groups.

The mechanism for the altered plasma $\alpha$-tocopherol concentrations between the $\alpha$-TTP A$>$T genotypes in individuals who do not consume supplements cannot be elucidated from the present study. However, potential mechanisms can be speculated based on the location of the polymorphism in the $\alpha$-TTP gene. The rs6994076 A$>$T single nucleotide polymorphism is located 5’ upstream of the $\alpha$-TTP gene and therefore may be affecting mRNA transcription. The Functional Analysis and Selection Tool for SNP in Large Scale Association Study (FASTSNP) bioinformatic database predicts that the rs6994076 A$>$T SNP occurs in the promoter or regulatory region of the $\alpha$-TTP gene at a transcription factor binding site [245]. The A allele seems to occur at the binding site for the Pbx-1 transcription factor, while the T allele does not appear to be a binding site for this transcription factor. In addition, the T allele, but not the A allele, appears to be a binding site for the GATA-3 transcription factor. Although this finding should be confirmed, an alteration in transcription factor binding could result in differences in the number of mRNA transcripts being produced between the different rs6994076 A$>$T variants. This could, in turn, alter the number of protein products of $\alpha$-TTP that get synthesized. If this polymorphism does influence transcription factor binding, the results of the present and Wright et al. [208] studies suggest that the T allele would result in less transcription of $\alpha$-TTP mRNA, possibly leading to fewer $\alpha$-TTP protein products than the A allele. Consequently, a smaller quantity of $\alpha$-TTP protein
products would decrease the capacity to secrete $\alpha$-tocopherol into VLDLs, leading to a lower $\alpha$-tocopherol concentration in the plasma. This speculation should be investigated in future studies to determine whether the rs6994076 A>T SNP affects the transcription and translation of $\alpha$-TTP.

### 6.1.1.2 TAP rs2072157 C>T and Arg11Lys SNPs

The tocopherol associated protein (TAP), which is found ubiquitously in many cell types, has a sequence similarity to the $\alpha$-tocopherol transfer protein [151]. Similarly to $\alpha$-TTP, TAP has been shown to bind to $\alpha$-tocopherol in the cell [151]. However, it has also been shown to have a binding affinity for other compounds such as phosphatidylinositol, phosphatidylcholine and phosphatidylglycerol [246, 247]. TAP is also involved in cholesterol synthesis (TAP is also referred to as supernatant protein factor (SPF) when it is involved in this role) [248-250]. Furthermore, TAP has been shown to display a tumor suppressor function through modulation of the phosphoinositide 3-kinase (PI3K)/Akt signaling pathway [246, 251]. PI3K is involved in cellular processes such as cell proliferation, cell survival and apoptosis [246]. The exact role of TAP in $\alpha$-tocopherol metabolism, however, has not been completely elucidated. It may be acting as a cellular $\alpha$-tocopherol binding protein, keeping $\alpha$-tocopherol in the cell. It may also be involved in $\alpha$-tocopherol cellular trafficking, transporting $\alpha$-tocopherol to various cellular compartments [25, 246]. TAP may also play a role in secretory granule assembly [246]. An additional potential role for TAP is that of a molecular chaperone that functions to protect $\alpha$-tocopherol from metabolism [25]. One study suggested that TAP may act as a transcription factor as it transported $\alpha$-tocopherol from the cytosol to the nucleus, upon cellular incubation with $\alpha$-tocopherol, and activated the
transcription of a reporter gene [252]. This finding, however, was not confirmed in a subsequent study [246]. Nonetheless, a genetic association study examining whether variations in the TAP gene influence plasma α-tocopherol concentration could help to establish whether TAP plays an important role in α-tocopherol metabolism.

Indeed, the Arg11Lys TAP polymorphism was shown to be significantly associated with fasting plasma α-tocopherol concentration in both the total population and after excluding supplement users (Table 5-4). Although there were no significant differences between the different TAP Arg11Lys genotypes in the total population, there was a trend demonstrating a lower plasma α-tocopherol concentration in Arg/Arg subjects compared with the other genotypes. In individuals who did not consume vitamin E-containing supplements, the plasma α-tocopherol concentration of Arg/Arg subjects was significantly lower than Lys/Lys subjects, while the plasma α-tocopherol concentration of Arg/Lys subjects was intermediate (P = 0.02).

The association of the TAP Arg11Lys SNP with plasma α-tocopherol concentration is in agreement with a previous study conducted in middle-aged male smokers residing in Finland who obtained their α-tocopherol from the diet [208]. Similar to the present study, the Wright et al. study found that Arg/Arg individuals had significantly lower serum α-tocopherol concentration than Lys/Lys subjects [208]. Unlike the present study, however, the Wright et al. study showed that subjects with the Arg/Lys genotype had a serum α-tocopherol concentration that was significantly higher than Arg/Arg subjects [208]. The reason for this discrepancy may be due to differences in the statistical tests used.
Likewise with the $\alpha$-TTP A>T SNP and in agreement with the Wright et al. study [208], the difference in plasma $\alpha$-tocopherol concentration between the TAP Arg11Lys genotype with the highest (Lys/Lys) and lowest (Arg/Arg) plasma $\alpha$-tocopherol concentration is not very large (2 $\mu$mol/L when supplement users are included and 2.5 $\mu$mol/L when supplement users are excluded from the analysis). This could suggest that the finding was due to chance, although, the similar results observed in the Wright et al. study [208] appears to refute this possibility. Due to the design of the present study, a significant association cannot be interpreted as a true effect of the polymorphism. Although the TAP Arg11Lys polymorphism occurs in a coding region, which may result in a functional SNP effect, it is possible that this TAP polymorphism is in linkage disequilibrium with another polymorphism that has a larger effect on plasma $\alpha$-tocopherol concentration. Future investigation is therefore required to determine whether this is the case.

The analysis with the TAP Arg11Lys polymorphism was also repeated stratified by ethno-cultural group (Table 5-4). There was a non-significant trend in the East Asian sample that included supplement users, which attained statistical significance when supplement users were removed from the analysis. The pattern observed in East Asians corresponded to that observed in the unstratified population. Among East Asian subjects who did not use supplements, those with the Arg/Arg genotype had significantly lower plasma $\alpha$-tocopherol concentration than Lys/Lys subjects. In contrast, there were no significant differences in plasma $\alpha$-tocopherol concentration between the TAP Arg11Lys genotypes in the Caucasian and Others ethno-cultural groups. The reason for this discrepancy is unclear. It does not appear to be due to sample size differences since the Caucasian group is the largest of the
ethno-cultural groups. An East Asian specific effect also does not seem to be a likely explanation since the Wright et al. study, which involved Caucasian subjects, also found a significant association between the TAP Arg11Lys SNP and plasma α-tocopherol concentration [208]. In addition, although not significant, the genotype pattern observed in the Caucasians and Others ethno-cultural groups (lower plasma α-tocopherol in Arg/Arg subjects) is consistent with that observed in the East Asian group and in the total population. Further study, perhaps utilizing a larger sample size, is therefore warranted to discern this issue.

Since the exact function of TAP in α-tocopherol metabolism has not been completely elucidated, the mechanism for the observed result with the Arg11Lys polymorphism is unclear. Since the polymorphism occurs in an exon region, it is possible that the SNP results in an altered protein conformation. This may, in turn, alter the ability of TAP to bind α-tocopherol in the cell. If binding to TAP helps prevent α-tocopherol from leaving the cell or promotes α-tocopherol utilization, an increased ability to bind α-tocopherol could result in fewer α-tocopherol molecules exiting the cell or more α-tocopherol molecules being utilized. This would have the effect of lowering plasma α-tocopherol concentration. Based on the present results as well as the results from the Wright et al. study [208], the Arg variant would be expected to have a greater binding ability for α-tocopherol. Conversely, if binding to TAP helps prevent α-tocopherol utilization, a decreased ability to bind α-tocopherol could result in a greater utilization of α-tocopherol. This may, in turn, lower plasma α-tocopherol concentration. If this were the case, the present results would indicate that the Arg variant has
a lower binding affinity for α-tocopherol compared with the Lys variant. These hypotheses, however, need to be tested in future studies.

The TAP rs2072157 C>T SNP was also examined for a potential role in altering plasma α-tocopherol concentration (Table 5-3). Unlike the Arg11Lys polymorphism, this SNP did not significantly alter plasma α-tocopherol concentration in either the total population or after the exclusion of supplement users. A non-significant trend, however, was observed in subjects not consuming supplements (P = 0.06). Subjects with the C/C genotype had a lower plasma α-tocopherol concentration than the other TAP C>T genotypes. A similar trend was also observed in the Others group (P = 0.07) when supplement users were excluded and in the East Asian group (P = 0.06) when supplement users were included in the analysis. This trend was significant in East Asian subjects that did not consume supplements (P = 0.02). East Asian subjects possessing the C/C genotype who did not use supplements had significantly lower plasma α-tocopherol concentrations compared with T/T subjects. There was no significant difference in plasma α-tocopherol concentration between the TAP C>T genotypes in Caucasians, regardless of supplement use.

A closer look at the results from the TAP C>T plasma α-tocopherol analysis reveals similar results to the TAP Arg11Lys polymorphism. It appears that the C/C genotype of the C>T SNP corresponds to the Arg/Arg genotype of the Arg11Lys polymorphism. The similar plasma α-tocopherol concentration by genotype results as well as the similarities in genotype frequencies between these two SNPs prompted a linkage disequilibrium analysis for these two TAP polymorphisms (Table 5-10). The results indicated that the C>T and Arg11Lys SNPs in
the TAP gene are highly linked, meaning that they tend to be inherited together more often than what would be expected [253]. Specifically, the C allele of the C>T SNP tends to be inherited with the Arg allele of the Arg11Lys SNP, while the T allele tends to be inherited with the Lys allele. The linkage disequilibrium analysis therefore, suggests that the results observed for the C>T polymorphism probably arise from its linkage with the Arg11Lys polymorphism and probably not from a functional implication of the TAP C>T SNP.

6.1.1.3 CYP4F2 Val433Met SNP

The enzyme CYP4F2 catalyzes the first step in the formation of the α-carboxyethyl hydroxychroman (α-CEHC) α-tocopherol catabolite [10, 11]. CYP4F2 specifically catalyzes the addition of a hydroxyl group to the terminal methyl group of the α-tocopherol side chain (ω-hydroxylation) [10, 11]. It is, therefore, possible that variation in this gene may affect α-tocopherol catabolism, which would have the effect of influencing α-tocopherol clearance from the blood and consequently, alter plasma α-tocopherol concentration. The CYP4F2 polymorphism examined in the present study results in a valine to methionine amino acid substitution at position 433 of the CYP4F2 protein (Val433Met) [240]. This polymorphism was chosen as it has been reported to alter other CYP4F2 functions including arachidonic acid [216, 217] and vitamin K [215] catabolism. Additionally, the CYP4F2 Val433Met polymorphism has also been shown to influence the dose of the anti-coagulants warfarin [218, 219] and acenocoumarol [220] and has been associated with disease risk [221-224]. The role of this SNP in α-tocopherol catabolism is not yet known.
These previous studies suggest that the Met variant is less functional. It would therefore be hypothesized that if the Val433Met variant influences α-tocopherol catabolism, the Met variant would result in a lowered α-tocopherol catabolism. This would have the effect of increasing circulating α-tocopherol in the blood. The results of the present analysis, however, indicate that the Val433Met CYP4F2 polymorphism does not alter plasma α-tocopherol concentration in either the total population or after excluding supplement users, unstratified or stratified by ethno-cultural group (Table 5-5). Although no significant effect was found for this polymorphism, it is interesting to note that in several of the groups analyzed, a higher plasma α-tocopherol concentration was observed in individuals homozygous for the Met allele. This observation is consistent with what would be expected if the CYP4F2 SNP indeed influenced plasma α-tocopherol concentration. It would, therefore, be interesting to repeat the analysis in a larger sample size to determine whether no significant effects were observed because the sample size was not large enough to detect a difference if it truly exists. A large enough sample size is important given that the Met/Met genotype occurs at a relatively low frequency.

6.1.1.4 α-TTP rs6994076 A>T and TAP Arg11Lys combined genotypes

Since the α-TTP A>T and TAP Arg11Lys polymorphisms were significantly associated with plasma α-tocopherol concentration, particularly in subjects not using supplements, a gene*gene interaction between these two SNPs on plasma α-tocopherol concentration was tested in the group of subjects excluding supplement users (Table 5-11). This gene*gene interaction was not significant, suggesting that one SNP is not modifying the effect of the other SNP on plasma α-tocopherol concentration and indicating that the two
SNPs are acting independently. Therefore, the effect of various combinations of these two SNPs on plasma α-tocopherol concentration was investigated (Table 5-11). In subjects that possessed both of the low plasma α-tocopherol concentration genotypes (T/T + Arg/Arg), as determined from the individual SNP analysis, plasma α-tocopherol concentrations were significantly lower compared with subjects possessing both of the high plasma α-tocopherol genotypes (A carriers + Lys carriers). The A carriers + Arg/Arg subjects and the T/T + Lys carriers subjects, individuals possessing only one of the low plasma α-tocopherol concentration genotypes (the Arg/Arg genotype in A carriers + Arg/Arg subjects and the T/T genotype in subjects with the T/T + Lys carriers combined genotype), had plasma α-tocopherol concentrations that were intermediate. Interestingly, the difference in plasma α-tocopherol concentration between subjects with the highest (A carriers + Lys carriers) and lowest (T/T + Arg/Arg) plasma α-tocopherol concentrations was almost double that observed when the α-TTP A>T and TAP Arg11Lys SNPs were studied independently (16.3% for the combined genotype versus 9.0% for the α-TTP A>T SNP and 8.4% for TAP Arg11Lys SNP). It, therefore, appears that the T/T and Arg/Arg genotypes may be exerting an additive effect on plasma α-tocopherol concentration when they inherited together.

6.1.1.5 Summary: The effect of genetic variation in α-TTP, TAP and CYP4F2 on plasma α-tocopherol concentration

To summarize the results of the genotype-plasma α-tocopherol concentration analysis, neither the TAP rs2072157 C>T nor the CYP4F2 Val433Met polymorphisms were associated with fasting plasma α-tocopherol concentration. However, the α-TTP rs6994076 A>T polymorphism influenced fasting plasma α-tocopherol concentration in individuals who did
not consume vitamin E-containing supplements. The TAP Arg11Lys SNP was also associated with fasting plasma \( \alpha \)-tocopherol concentration in the total population and in subjects not using supplements. These \( \alpha \)-TTP A>T and TAP Arg11Lys polymorphisms seem to exert a small effect on plasma \( \alpha \)-tocopherol concentration (6% to 9% difference in plasma \( \alpha \)-tocopherol concentration), which could mean that these were chance findings as a result of multiple comparisons. However, these results are consistent with a previous study, which makes the idea of a chance finding seem less likely. The design of the present and the Wright et al. [208] studies do not imply that any significant associations are due to a direct result of the SNPs. It may also be possible that the effects were small because these polymorphisms are not functional but are in linkage disequilibrium with another functional polymorphism that has a greater effect on plasma \( \alpha \)-tocopherol concentration.

A potential explanation for the small effects observed for the \( \alpha \)-TTP A>T and TAP Arg11Lys SNPs is that average plasma \( \alpha \)-tocopherol concentrations of adult populations who do not use supplements tend to fall between 20 to 29 \( \mu \)mol/L [164, 168, 170, 254]. Thus, if a genetic variant that is relatively common, such as the \( \alpha \)-TTP A>T and TAP Arg11Lys polymorphisms, modifies plasma \( \alpha \)-tocopherol concentration, the mean concentration for the particular variant would be expected to fall within the 20-29 \( \mu \)mol/L range, which is a fairly narrow range. In addition, the plasma \( \alpha \)-tocopherol concentration appears to be regulated. It does not increase more that three- to four-fold of its baseline value, regardless of the supplement dose, frequency or duration [241, 242]. These supplementation studies suggest that the plasma \( \alpha \)-tocopherol concentration is regulated and may plateau once a certain concentration is reached. Under this assumption, once the plasma \( \alpha \)-tocopherol concentration
approaches the concentration where it begins to plateau, little changes in plasma α-tocopherol concentration would occur. It may be possible that the plasma α-tocopherol concentrations of most subjects were near this plateau region. Therefore, even if the polymorphisms exert a fairly large effect, the effect of the polymorphisms would be small in this plasma α-tocopherol concentration range.

It may also be possible that several polymorphisms combined exert a large effect on plasma α-tocopherol concentration as opposed to one polymorphism that exerts a large effect. This was seen when the α-TTP A>T and TAP Arg11Lys genotypes were combined. When supplement users were excluded, this combined genotype significantly altered plasma α-tocopherol concentrations. From the individual SNP analysis, the T/T and Arg/Arg genotypes were associated with lower plasma α-tocopherol concentrations relative to the A/A and Lys/Lys genotypes, respectively. The combined genotype analysis revealed that subjects possessing both of these low plasma α-tocopherol concentration genotypes (T/T + Arg/Arg) had significantly lower plasma α-tocopherol concentrations compared with subjects possessing both of the high plasma α-tocopherol concentration genotypes (A carriers + Lys carriers). Interestingly, this difference was almost two-fold greater than that observed from the individual SNP analysis, suggesting that these two low plasma α-tocopherol concentration genotypes may be working additively to influence plasma α-tocopherol concentration when they are inherited together.
6.1.1.6 Biological significance of observed effects

The small effects of plasma $\alpha$-tocopherol concentration observed for the $\alpha$-TTP A>T and TAP Arg11Lys polymorphisms begs the question whether these small effects are biologically significant. Data from the $\alpha$-tocopherol $\beta$-carotene cancer prevention (ATBC) clinical trial revealed an inverse relationship between baseline serum $\alpha$-tocopherol concentration (prior to treatment with either $\alpha$-tocopherol, $\beta$-carotene, both or placebo) and total- (excluding accidental) and cause-specific mortality in middle-aged Finnish male smokers [244]. The optimum relative reduction in total- and cause-specific mortality occurred in subjects with baseline serum $\alpha$-tocopherol concentrations between about 30-32.5 $\mu$mol/L [244]. These optimum serum $\alpha$-tocopherol concentrations may not be applicable to all populations such as younger individuals, non-smokers or females, however, the inverse associations between baseline serum $\alpha$-tocopherol concentration and total- and cause-specific mortality were stronger in younger and leaner subjects who smoked less [244]. Nevertheless, if a plasma $\alpha$-tocopherol concentration of 30 $\mu$mol/L is in fact protective, it would suggest that the $\alpha$-TTP A>T and TAP Arg11Lys polymorphisms could have a biologically relevant effect. From dietary intakes of $\alpha$-tocopherol that can be achieved through food alone, the mean plasma $\alpha$-tocopherol concentration of individuals homozygous for the A allele for the $\alpha$-TTP A>T SNP and homozygous for the Lys allele for the TAP Arg11Lys SNP is roughly 30 $\mu$mol/L. Therefore, more of these A/A and Lys/Lys individuals, as well as subjects possessing both the A/A and Lys/Lys genotypes (A/A + Lys/Lys), would have plasma $\alpha$-tocopherol concentrations that meet or exceed the 30 $\mu$mol/L threshold compared with T/T and Arg/Arg individuals. This would put these A/A and Lys/Lys individuals at a potentially lower risk for total- and cause-specific mortality if a plasma $\alpha$-tocopherol concentration of 30
μmol/L is indeed protective. However, further study is warranted to determine other potential biologically relevant effects of these two polymorphisms, if any.

6.1.2 **Supplement use*gene interaction on plasma α-tocopherol concentration**

Since the α-TTP A>T polymorphism only influenced plasma α-tocopherol concentration in individuals who did not consume supplements, a supplement use*gene interaction on plasma α-tocopherol concentration was tested in order to determine whether the different genotypes of this as well as the TAP C>T, TAP Arg11Lys and CYP4F2 Val433Met polymorphisms respond differently to vitamin E supplementation (Table 5-6). No significant supplement use*gene interaction on plasma α-tocopherol concentration was observed in the unstratified sample for any of the SNPs examined. It is interesting that there was a trend with the α-TTP A>T polymorphism. This polymorphism resulted in differential effects on plasma α-tocopherol concentration depending on whether supplement users were included in the analysis (Table 5-2). When supplement users were included, no effect on plasma α-tocopherol concentration of this polymorphism was observed. In contrast, when supplement users were removed from the analysis, this polymorphism was significantly associated with plasma α-tocopherol concentration.

When the supplement use*gene interaction was repeated stratified by ethno-cultural group, no significant supplement use*gene interactions on plasma α-tocopherol concentration were observed in any of the ethno-cultural groups for any of the polymorphisms examined except for the two TAP SNPs in the Others group (Table 5-6). Since the interaction did not
appear in the unstratified sample and since the Others group is comprised of many different ethno-cultural groups, it may be possible that this is a chance finding. Further investigation is warranted, however.

The non-significant supplement use*gene interaction on plasma $\alpha$-tocopherol concentration in the total population for the TAP Arg11Lys SNP is in agreement with the results from the Wright et al. study [208]. Conversely, the non-significant supplement use*gene interaction on plasma $\alpha$-tocopherol concentration for the $\alpha$-TTP polymorphism disagrees with the Wright et al. study [208]. Differences in the study populations may explain this discrepancy. The TNH study population was comprised of young adult males and females (20 to 29 years) from various ethno-cultural groups who were predominantly non-smokers. The Wright et al. study [208], conversely, consisted of middle-aged (50 to 69 years) male smokers. It is possible that the plasma $\alpha$-tocopherol response to supplementation by this $\alpha$-TTP A>T SNP may vary depending on smoking status as smokers have been shown to have a faster fractional plasma $\alpha$-tocopherol disappearance rate compared with non-smokers.

Differences in study design may also explain this discrepancy. The Wright et al. study [208] examined whether the $\alpha$-TTP A>T SNP altered the serum $\alpha$-tocopherol response to supplementation with 50 mg of *all-rac*- $\alpha$-tocopheryl acetate for three years in control subjects from the ATBC study. The study found that A/T and T/T subjects had significantly lower serum $\alpha$-tocopherol responses to $\alpha$-tocopherol supplementation compared with A/A subjects. The response observed in T/T subjects was the lowest. A clinical trial, like the Wright et al. study, would be the most ideal study design to test whether genotype modifies the plasma or
serum α-tocopherol response to supplementation with α-tocopherol. The advantage of the clinical trial would be that each subject is serving as his or her own control and the same α-tocopherol doses would be compared. In the present supplement use*gene interaction analysis, on the other hand, the plasma α-tocopherol response to supplementation was compared between supplement users and those not using supplements. In addition, supplement users consumed varied doses of α-tocopherol supplements. The between-group comparison as well as the non-standardized supplement doses (supplement users did not consume identical doses of α-tocopherol) of the present study compared with the within-individual comparison involving an identical supplemental α-tocopherol dose (50 mg) of the Wright et al. study [208], may have resulted in a lot of noise, making it difficult to detect a difference in the plasma response to supplementation, if one truly existed. Since a trend was observed in the present study with the α-TTP A>T polymorphism, it would be interesting to repeat the supplement use*gene interaction in a larger sample as well as to conduct a clinical trial using the TNH population to test whether the α-TTP A>T polymorphism modifies the plasma response to supplementation with a specific α-tocopherol dose.

6.1.3 Spearman correlation coefficient between dietary and plasma α-tocopherol

In addition to examining whether the four polymorphisms under study influence the plasma α-tocopherol response to vitamin E supplementation, the strength of the association between dietary and plasma α-tocopherol was compared between the various genotypes by supplement use. This was tested with the Spearman rank correlation coefficient (Table 5-7). The significant yet weak correlation coefficient between dietary and plasma α-tocopherol
observed in the total population (including supplement users) has previously been reported [96, 164, 183]. The correlation coefficients for each of the genotypes of the α-TTP A>T, TAP C>T and TAP Arg11Lys polymorphisms were all significant and similar to each other as well as to that of the total population. These correlation coefficients ranged from 0.13 to 0.22. It, therefore, appears that these α-TTP and TAP polymorphisms do not modify the strength of the association between dietary and plasma α-tocopherol.

The correlation coefficients of the Val/Val and Val/Met genotypes of the CYP4F2 Val433Met SNP were all significant, similar to each other and similar to that of the total population. The correlation coefficient between dietary and plasma α-tocopherol for the Met/Met genotype, on the other hand, was not statistically significant. This could mean that dietary and plasma α-tocopherol are not significantly associated with each other in Met/Met individuals. This is interesting considering this is the genotype that would be expected to have a lower functional activity compared with the other Val433Met genotypes based on prior studies. A non-significant correlation in Met/Met subjects and a significant correlation in the Val/Val and Val/Met subjects, would suggest that the CYP4F2 Val433Met polymorphism modifies the strength of the association between dietary and plasma α-tocopherol. Alternatively, it may be possible that the non-significant correlation coefficient between dietary and plasma α-tocopherol is attributed to the small sample size of the Met/Met group. Future studies using a larger sample size could discern this issue.

When supplement users were removed from the correlation analysis, the Spearman rank correlation coefficients between dietary and plasma α-tocopherol were mostly close to 0
and were not statistically significant for all subjects combined or when stratified by the various \( \alpha \)-TTP, TAP and CYP4F2 genotypes. The lack of a significant correlation between dietary and plasma \( \alpha \)-tocopherol in individuals who do not consume vitamin E-containing supplements is in agreement with previous studies [16, 164, 167]. It, therefore, appears that the larger range in the intakes of \( \alpha \)-tocopherol achieved through vitamin E supplementation drive the correlation between dietary and plasma \( \alpha \)-tocopherol in supplement users.

6.1.4 Diet*gene interaction on plasma \( \alpha \)-tocopherol concentration

One of the main objectives of the present study was to determine whether any of the \( \alpha \)-TTP A>T, TAP C>T, TAP Arg11Lys or CYP4F2 Val433Met SNPs modify the plasma \( \alpha \)-tocopherol response to dietary \( \alpha \)-tocopherol (Tables 5-8 and 5-9). This was tested by comparing the slopes between the genotypes of the four polymorphisms examined and by computing a diet*gene interaction on plasma \( \alpha \)-tocopherol concentration both including and excluding supplement users. In the total population, no significant diet*gene interaction on plasma \( \alpha \)-tocopherol concentration was observed for any of the polymorphisms under study. This is evidenced by the similar slopes for the relationship between dietary and plasma \( \alpha \)-tocopherol between the genotypes of each polymorphism.

The slopes for the relationship between dietary and plasma \( \alpha \)-tocopherol were significant for the total population and for all genotypes examined except for the Met/Met genotype of the CYP4F2 Val433Met SNP. This is in agreement with the Spearman rank correlation coefficient analysis, where the correlation coefficient between dietary and plasma \( \alpha \)-tocopherol was significant for all genotypes examined except for the Met/Met genotype.
Since the diet*gene interaction for the CYP4F2 Val4433Met SNP was not significant, the non-significant slope observed in the Met/Met subjects appears to be due to the relatively small sample size of this group. However, this should be confirmed in future studies utilizing a larger sample size.

When supplement users were removed from the analysis, the slopes for the relationship between dietary and plasma α-tocopherol approached significance for all subjects (P = 0.055), and was not significant for any of the genotypes examined except for the Arg/Lys and Val/Val genotypes. It is not clear why the slopes were only significant in the Arg/Lys and Val/Val genotypes. One explanation may be the relatively larger sample sizes of these genotypes relative to the other genotypes analyzed. The non-significant slopes observed when the analysis was conducted in individuals who only obtained α-tocopherol from food sources alone is consistent with previous studies [169, 186]. A diet*gene interaction on plasma α-tocopherol concentration was also tested after removing supplement users and was not found to be significant for any of the SNPs under study or for the combined α-TTP A>T and TAP Arg11Lys genotype.

Similarly, when stratified by ethno-cultural group, no significant diet*gene interactions on plasma α-tocopherol concentration were observed for any of the polymorphisms examined, regardless of supplement use with one exception (Table 5-9). The diet*gene interaction was significant for the TAP Arg11Lys SNP in the Others ethno-cultural group in the total sample (including supplement users). Interestingly, this is in agreement with the supplement use*gene interaction analysis that found a significant interaction for the TAP
Arg11Lys polymorphism in the Others group. It is not clear why the diet*gene interaction was only significant in the Others group for the TAP Arg11Lys SNP. It does not appear to be biologically relevant since this group consists of various ethno-cultural groups and a significant interaction was not observed in the unstratified sample or in the other ethno-cultural groups.

6.1.5 Proportion of variation in plasma \(\alpha\)-tocopherol concentration explained by models

The proportion of variation in plasma \(\alpha\)-tocopherol concentration explained by the crude and adjusted models with and without supplement users was also assessed (Table 5-10). Very little variation in plasma \(\alpha\)-tocopherol concentration was explained by dietary \(\alpha\)-tocopherol (<1% to 3%), regardless of the inclusion of supplement users. This suggests that dietary \(\alpha\)-tocopherol alone does not account for very much of the variability in plasma \(\alpha\)-tocopherol concentration between individuals. Therefore, other factors must be explaining some of the residual variance in plasma \(\alpha\)-tocopherol concentration. After adjustment for season, ethno-cultural group, physical activity, total energy intake and total serum cholesterol, only about 20% of the variation in plasma \(\alpha\)-tocopherol concentration was explained in both the total population and after excluding supplement users.

Each polymorphism was then added, one at a time, to either the crude or adjusted models to determine whether the polymorphisms under study modified the proportion of variation in plasma \(\alpha\)-tocopherol concentration explained by the models (Table 5-10). The addition of genotype to the models did not appreciably affect the amount of variability
explained by the models, regardless of supplement use. This suggests that the α-TTP A>T, TAP C>T, TAP Arg11Lys and CYP4F2 Val433Met polymorphisms do not further contribute to explaining the variance in plasma α-tocopherol concentration. This is not very surprising since none of these polymorphisms resulted in a significant diet*gene interaction on plasma α-tocopherol concentration. Furthermore, the only two polymorphisms that were significantly associated with plasma α-tocopherol concentration (α-TTP A>T and TAP Arg11Lys SNPs) only resulted in a small change in plasma α-tocopherol concentration (8 to 9%). In addition, rather than one polymorphism exerting a large effect on plasma α-tocopherol concentration and thus contributing to explaining a substantial portion of plasma α-tocopherol variation between individuals, it may be possible that many polymorphisms combined (either additively, synergistically or as complex gene*gene interactions on plasma α-tocopherol concentration) contribute to explaining the between-person variability in plasma α-tocopherol concentration. When the α-TTP A>T and TAP Arg11Lys SNPs were combined, the proportion of variation in plasma α-tocopherol concentration explained by the adjusted models increased by about 2% compared with the adjusted model that did not contain the combined genotype as a covariate (Tables 5-10 and 5-11). This increase, although small, was larger than that observed when the α-TTP A>T and TAP Arg11Lys SNPs were added, one at a time, to the model. This, therefore, lends credence to the hypothesis that possibly many polymorphisms combined explain more of the variability in plasma α-tocopherol concentration between individuals rather than one individual polymorphism that explains a large proportion of this variation. This hypothesis, therefore, should be tested in future studies.
To explore the effect of genotype a little further and to determine whether opposing
effects of the different genotypes on the $R^2$ value contributed to the minimal effect of the
polymorphism on the $R^2$ value, the proportion of variation in plasma $\alpha$-tocopherol
concentration explained by the crude and adjusted models for each of the $\alpha$-TTP A$\rightharpoonup$T, TAP
C$\rightharpoonup$T, TAP Arg11Lys and CYP4F2 Val433Met SNPs was also computed (Table 5-10). Although the proportion of variation in plasma $\alpha$-tocopherol concentration explained for each
of the $\alpha$-TTP A$\rightharpoonup$T genotypes were similar to each other, the proportion explained by the A/A
genotype was about 10% higher than that explained by T/T subjects in the total population
and 10% higher than that explained by the A/T and T/T genotypes in subjects not using
supplements. The percentage of variation in plasma $\alpha$-tocopherol concentration explained by
the crude and adjusted models for each of the TAP C$\rightharpoonup$T and TAP Arg11Lys genotypes were
similar to each other, regardless of supplement use (20% to 28%). Therefore, the two TAP
polymorphisms do not appear to modify the percent of variation in plasma $\alpha$-tocopherol
concentration explained by the crude and adjusted models. There was a difference in the
amount of variability in plasma $\alpha$-tocopherol concentration explained by the different
CYP4F2 Val433Met genotypes. The Met/Met genotype explained the most (about 40%),
while the Val/Met genotype explained the least (15%) of the variation in both the total
population and in subjects not consuming supplements. It is interesting that models in the
Met/Met genotype would explain the greatest amount of variability in plasma $\alpha$-tocopherol
concentration. It was not shown to be significantly associated with plasma $\alpha$-tocopherol
concentration and the correlation and slope for the relationship between dietary and plasma $\alpha$-
tocopherol was not significant in this genotype. In addition, in the unstratified analysis, the
addition of CYP4F2 to the crude and adjusted models did not appreciably influence the
proportion of variability in plasma $\alpha$-tocopherol explained. However, since this group contains a relatively small sample size, the results should be interpreted with caution and confirmed in subsequent studies using larger sample sizes.

6.1.6 Summary of analyses involving dietary $\alpha$-tocopherol

To summarize the results of the analyses involving dietary $\alpha$-tocopherol, it does not appear that the $\alpha$-TTP A>T, TAP C>T, TAP Arg11Lys or CYP4F2 Val433Met polymorphisms modify the association between dietary and plasma $\alpha$-tocopherol. The CYP4F2 Val433Met polymorphism may be modifying the strength of the association between dietary and plasma $\alpha$-tocopherol or the proportion of variation in plasma $\alpha$-tocopherol concentration explained by the adjusted model, however, these effects may be due to the small sample size of the Met/Met group and therefore, should be interpreted with caution.

6.1.7 Overall summary

The TAP C>T and CYP4F2 Val433Met SNPs did not alter fasting plasma $\alpha$-tocopherol concentration nor did they modify the plasma $\alpha$-tocopherol response to dietary $\alpha$-tocopherol. The $\alpha$-TTP A>T (in subjects not using supplements) and TAP Arg11Lys (in the total population and after removing supplement users) SNPs were, however, significantly associated with fasting plasma $\alpha$-tocopherol concentration. In addition, the combined $\alpha$-TTP A>T and TAP Arg11Lys genotype also influenced fasting plasma $\alpha$-tocopherol concentrations in individuals not consuming supplements. The effects of the $\alpha$-TTP A>T and TAP Arg11Lys SNPs, alone or in combination, on plasma $\alpha$-tocopherol concentrations do not involve a modification of the association between dietary and plasma $\alpha$-tocopherol. One
potential explanation for the lack of a diet*gene interaction on plasma α-tocopherol concentration for these two polymorphisms may be that dietary α-tocopherol was very weakly associated with plasma α-tocopherol concentration in this population. In fact, in subjects not using supplements, the group of subjects in which the α-TTP A>T and TAP Arg11Lys SNPs exerted a greater effect, the slope for the relationship between dietary and plasma α-tocopherol approached significance, but was not significant. The effect of the α-TTP A>T and TAP Arg11Lys SNPs, individually, on plasma α-tocopherol concentration was also not very large (6% to 9%). It may therefore be difficult to significantly modify a relationship when it is fairly weak and the effects of the polymorphisms themselves are also small.

6.2 Limitations

This study has some limitations worth noting as discussed below.

6.2.1 Dietary assessment

The dietary assessment method used in this study, the food frequency questionnaire (FFQ), may not have adequately captured α-tocopherol intake. The FFQ is limited in the number of food items listed (196 items) and it may be possible that foods not included in the FFQ food list, such as ethnic foods, were greater contributors to the diets and thus α-tocopherol intake of certain subjects. Adjusting analyses by ethno-cultural group may help to control for differences in intake between the different ethno-cultural groups. In the total population ethno-cultural group was associated with dietary α-tocopherol if it was log e
transformed, but not when it was untransformed. In addition, the food list was fairly large to try to include many potential food sources of subjects.

The FFQ also relies on memory. It asked subjects to report on their habitual consumption of the foods in the FFQ food list over the previous month. Reliance on memory may make it difficult for subjects to accurately report their dietary intakes. However, the short time frame of the FFQ (one month) may not pose too much of a problem as compared with FFQs with larger time frames such as one year. In addition, since analyses are stratified by genotype, recall bias is expected to be evenly distributed between the different genotypes. No specific genotype of the polymorphisms examined is expected to influence the ability to recall the food eaten over the previous month.

It may also be possible that subjects have under- or over-reported their intakes. One of the difficulties in quantifying α-tocopherol intake is that it is found in oils and dietary fats and oils can often be under-reported due to an individual’s sensitivity to questions regarding their dietary fat intake [83, 98]. In addition, fats and oils added to foods may be difficult for subjects to quantify or recognize [13, 98]. Added fats can be easily forgotten about when filling out dietary assessments. Subjects may be unaware of hidden fats in their foods, especially if they have not prepared the meal themselves [98]. Thus, if subjects are not aware of these hidden fats, they will not be able to report on their consumption in a dietary assessment, leading to under-reporting of fat as well as α-tocopherol. Adjusting dietary α-tocopherol for total energy intake helps to control for the effects of under- and over-reporting. Furthermore, by stratifying the analysis by genotype, the effects of incorrect dietary reporting
should be evenly distributed between the different genotypes as the genotypes examined are not expected to influence intakes of specific foods or nutrients.

The quality, completeness and accuracy of the food composition database, which lists the \( \alpha \)-tocopherol content of various food items, can influence the accuracy of dietary \( \alpha \)-tocopherol [99]. Food composition databases contain average \( \alpha \)-tocopherol values for foods however, the amount of \( \alpha \)-tocopherol in a particular food item can vary depending on factors such as the product brand, harvesting, processing, storage and cooking [18, 99-104]. The FFQ also contains a few open-ended questions inquiring about brand names (such as the brand of cold breakfast cereal typically consumed (which can be fortified with \( \alpha \)-tocopherol) or oil usually added to foods) and other frequently consumed items that were not included in the FFQ food list. Coding one food item as another, such as coding for a different brand or similar product, could influence the accuracy of dietary \( \alpha \)-tocopherol since, as previously discussed, the \( \alpha \)-tocopherol content of a particular food is influenced by the product brand, harvesting, processing, storage and cooking [18, 99-104].

An implication of coding one food item as another occurs when foods are fortified with \( \alpha \)-tocopherol such as cold breakfast cereals. One brand may fortify a particular food with \( \alpha \)-tocopherol, while another may not. Additionally, the amount or form of \( \alpha \)-tocopherol (natural or synthetic, free or conjugated) added to foods may vary between different brands. All of these factors can affect the accuracy of dietary \( \alpha \)-tocopherol. For example, the conjugated forms of \( \alpha \)-tocopherol are more stable than the corresponding free forms since the conjugated forms are less prone to oxidation [1, 27]. Furthermore, if the food composition
database used in the present study obtained information from the food industry regarding the amount of \( \alpha \)-tocopherol added to foods expressed in IU units, the database converted the IU values to mg \( \alpha \)-tocopherol by multiplying the IU amount by a conversion factor \([229]\). The conversion factor differed depending on whether the \( \alpha \)-tocopherol added to the food item was natural or synthetic (0.67 was used for \( \text{RRR-} \alpha \)-tocopherol (natural) and 0.45 was used for \( \text{all-rac-} \alpha \)-tocopherol (synthetic)) \([229]\). Therefore, differences in the form of \( \alpha \)-tocopherol added to foods between different brands can greatly influence the accuracy of the estimated dietary \( \alpha \)-tocopherol if one food item or brand was coded as another.

In relation to the use of conversion factors from IU to mg \( \alpha \)-tocopherol, the FFQ asks subjects about the dose, in IU units, of the vitamin E supplement they are taking. However, the FFQ does not ask the subject to specify whether the supplement is natural or synthetic \( \alpha \)-tocopherol. This affects the accuracy of the conversion factors used to determine dietary \( \alpha \)-tocopherol intake from supplements and therefore, the accuracy of the estimated total dietary \( \alpha \)-tocopherol.

A further disadvantage of the FFQ in relation to the present study is that it did not specifically inquire about the consumption of products that contained sesame seeds or oils. Sesamin, which is a component of sesame seeds and oil, has been reported to decrease vitamin E catabolism, resulting in increased concentrations of \( \alpha \)-tocopherol and other vitamin E forms in the blood \([10, 156, 160-162]\). It is therefore an important confounding factor that should be taken into consideration in statistical analyses. While open-ended questions did provide subjects with the opportunity to write in their use of sesame oil or other sesame seed-
containing food items, only a few subjects reported the use of sesame seed-containing products. It may be possible that sesame seed and oil consumption were low in this population however, it is also possible that subjects did not remember to include these items in the open-ended questions. Thus, for this reason as well as the small number of subjects that reported consuming sesame seed-containing food items, it was not possible to adjust analyses by sesame seed or oil consumption. Consequently, it may be possible that the plasma $\alpha$-tocopherol concentration of some subjects were elevated due to the effect of sesamin on $\alpha$-tocopherol catabolism.

Since one of the main objectives of the present study was to determine whether genotype affects the plasma $\alpha$-tocopherol response to dietary $\alpha$-tocopherol, a measure of dietary intake the few days leading to blood sample donation is ideal to carry out this objective. This is because newly absorbed $\alpha$-tocopherol replaces existing circulating $\alpha$-tocopherol in the plasma [255]. In addition, $\alpha$-tocopherol has a short half-life in the plasma of normal individuals of about 48 hours [255]. Although the short time frame (one month) of the FFQ makes it appropriate to test the hypothesis, the use of diet records may be more ideal to measure recent dietary $\alpha$-tocopherol intake. Diet records require subjects to record all of the food and beverages they consumed over the recording period (usually 3 to 5 days including at least one weekend day), incorporating details such as portion sizes, preparation methods, recipes for food mixtures and brand names [227, 256]. The FFQ, on the other hand, asks subjects to report their usual frequency of consumption, over a defined period, of every food item in the FFQ food list [256]. If portion sizes for the food list items are asked by the FFQ, it is called “semi-quantitative” as is the FFQ used in the present study [256]. The FFQ is
considered to be inferior to the diet record in terms of its ability to accurately quantify food and nutrient intake because of the confined food list and because it asks less detail regarding the foods consumed, cooking methods and portion sizes [256, 257]. As opposed to measuring an individual’s actual intake, the FFQ is better suited to measure habitual intake or for ranking individuals (relative intake) [256, 257]. Since the diet record is not restricted to a limited food list as is the FFQ, the diet record is better able to estimate a subject’s actual α-tocopherol intake [256]. If foods are recorded as they are consumed, the diet record can more accurately report portion sizes and it is less likely to omit the consumption of foods relative to the FFQ which relies on memory [256]. Similar to the FFQ, under-reporting could still occur if the subject does not properly report portion sizes or if their diet records are incomplete [256]. In addition, hidden fats and oils can still be an issue with the diet record if they are not recognized. One of the disadvantages of the diet record is the large subject burden [256]. This may make it difficult to recruit a large enough sample. It may also cause individuals to change their usual diet in order to facilitate food recording [256]. However, this does not pose a problem since the study objective is concerned with determining how the plasma α-tocopherol responds to dietary α-tocopherol, regardless of whether the amount of α-tocopherol consumed is “normal” for the particular subject.

An additional option is the 24-hour recall, which is usually administered by a trained interviewer who asks a subject to recall all of the food and beverages they consumed over the previous 24 hours [256]. This has the advantage of posing less of a burden to subjects compared with the diet records [256]. The approximate 48 hour half-life of α-tocopherol in the blood would require several consecutive 24-hour recalls which would increase subject burden. Although the 24-hour recall is subjected to recall bias, having a trained interviewer
conduct the 24-hour recall can help prompt the subject to remember foods consumed, including added fats and oils, with the use of probes [256]. Similar to diet records, the 24-hour recall is a better measure for actual intake compared with the FFQ [257]. Despite the advantages of diet records and 24-hour recalls in estimating actual recent $\alpha$-tocopherol intake proceeding blood donation, the high costs and personnel burden associated with coding diet records and responses to 24-hour recalls as well as the subject burden associated with diet records, do not make them very feasible for use in large epidemiological studies involving a large sample size [256]. The ease of administration of the FFQ, the low subject burden and low cost of administering the FFQ make the FFQ suitable for large epidemiological studies including genetic association studies [256, 257].

It is important that an FFQ be validated to assess its reproducibility and validity in order to determine its effectiveness in estimating dietary intake [227, 257]. Although the FFQ used in the present study has not been validated yet for the TNH study population, it was modified from the Willett FFQ that has been validated in other populations [15, 258-260]. Furthermore, one method of validation is to calculate the correlation coefficient between the dietary variable and a biomarker for the variable [15, 260]. For $\alpha$-tocopherol, plasma or serum $\alpha$-tocopherol is often used as the biomarker in validation studies [15, 260]. Although the correlation coefficients between dietary and plasma $\alpha$-tocopherol were lower than those reported in previous studies using the Willett FFQ [15, 260], the correlation coefficients observed in the present study are similar to those previously reported [96, 164, 183]. This helps to validate the FFQ as a suitable method for measuring dietary $\alpha$-tocopherol.
6.2.2 Plasma α-tocopherol measurement

It is possible that measurement error in the plasma α-tocopherol concentration may have influenced the accuracy of plasma α-tocopherol concentrations. However, the method used to determine plasma α-tocopherol concentration has been validated [233]. The within-day coefficient of variation was between 3.7 to 9.3% and the between-day coefficient of variation was between 4.1 to 5.5% [233]. In addition, values from standard reference material (SRM 968c) from the National Institute of Standards and Technology (NIST) were compared to results with the method used in the present study to determine the accuracy of the method. There was close agreement between the values obtained from the present method and those from the standard reference material (SRM 968c) from NIST (4.4 and 7.1% difference for two concentrations (low and high, respectively) that were measured) [233].

6.2.3 Association does not imply causation

A limitation of this study design is that any associations found do not imply causation by the polymorphism itself. It may be possible that the polymorphism is in linkage disequilibrium with another polymorphism that is responsible for the observed effect. A functional assay would have to be conducted to determine whether the observed effects are in fact due to the particular polymorphism under study.

6.2.4 Missing dietary α-tocopherol values and small sample size of supplement user group

A further limitation is that some subjects were missing dietary α-tocopherol values (n = 89). This resulted in a smaller sample size for the analyses involving dietary α-tocopherol
compared with analyses that did not include dietary α-tocopherol. In addition, the supplement user population was relatively small to allow for an analysis using this sub-group. When the supplement user population is further stratified by genotype, the sample sizes of the genotypes become small and it is difficult to make any meaningful conclusions. Future studies should therefore use a larger sample size of supplement users with a more continuous range of α-tocopherol intakes to test the effect of various polymorphisms in individuals with very high α-tocopherol intakes.

6.3 Future directions

6.3.1 Replication

The results of the present study should be replicated in other populations. Although the results for the α-TTP and TAP Arg11Lys polymorphisms are in agreement with a recent study, it would be interesting to replicate the findings in other populations, ideally in populations with a larger sample size consisting of subjects with a wide age range. This would ensure that results are applicable to various types of populations. The TAP C>T SNP, although not previously studied in relation to plasma α-tocopherol or the plasma α-tocopherol response to dietary α-tocopherol, appears to be linked to the TAP Arg11Lys polymorphism. Future studies in different populations could confirm that the similar, although not significant, findings of the TAP C>T SNP in relation to the TAP Arg11Lys polymorphism are due to its linkage with the TAP Arg11Lys SNP. In addition, a haplotype of the two polymorphisms as well as other polymorphisms that are in linkage disequilibrium with these two SNPs could be analyzed instead of each polymorphism individually.
The CYP4F2 Val422Met polymorphism was not found to influence fasting plasma α-tocopherol concentration in the TNH population. However, a pattern was observed (highest plasma α-tocopherol concentration in Met/Met subjects) that is in agreement with what would be hypothesized based on previous studies. Thus, the analysis should be repeated in a larger sample in order to determine whether the null effect of the present study was due a small sample size or an actual lack of an effect. A larger sample is important since the Met/Met genotype is present at a relatively low frequency.

6.3.2 Clinical trial

Significant findings should be followed through with a clinical trial. In such a trial, subjects would be recruited based on their genotype and given a specific dose of α-tocopherol. It would be interesting to test doses that are achievable through dietary means as well as doses that can only be attained from supplements. The plasma response to the α-tocopherol dose would then be compared between the different genotypes to determine whether the plasma α-tocopherol response to dietary α-tocopherol is modified by genotype. The α-TTP A>T, TAP C>T, TAP Arg11Lys and CYP4F2 Val433Met polymorphisms may not be appropriate polymorphisms to test in a plasma response clinical trial as there were no significant diet*gene or supplement use*gene interactions on plasma α-tocopherol concentration for any of these SNPs.

It would be interesting, nonetheless, to conduct this plasma response clinical trial with the α-TTP A>T polymorphism. A trend for a supplement use*gene interaction was observed for this SNP. In addition, a previous study involving Finnish middle-aged male smokers
showed that this SNP influenced the serum $\alpha$-tocopherol response to supplementation with 50 mg $all$-$rac$-$\alpha$-tocopheryl acetate for three years [208]. The discrepancy could be due to differences in study design or to a smoking effect. The present study may have had a lot of noise compared with the previous study since comparisons in the plasma $\alpha$-tocopherol response were made between supplement users and individuals not using supplements. In the previous study each subject acted as his own control and therefore, the serum $\alpha$-tocopherol response to a specified dose of $\alpha$-tocopherol was more of a within-subject comparison between the different genotypes. Due to the differences in the results between the two studies as well as the trend in the present study, a plasma response clinical trial is warranted to either confirm or refute the results of the previous study. It is important that the sample size of such a trial be large enough and that equal amounts of smokers and non-smokers are included in the population in order to determine whether responses vary according to smoking status. Smokers have a faster plasma $\alpha$-tocopherol disappearance rate compared with non-smokers which may modify the effect (if any) of the $\alpha$-TTP A$>$T polymorphism on the plasma $\alpha$-tocopherol response to $\alpha$-tocopherol supplementation [191-193].

**6.3.3 Functional assay**

Significant results from genetic association studies and clinical trials that measure the plasma response to diet do not imply causation. It may be possible that the polymorphism under study is in linkage disequilibrium with another polymorphism that is responsible for the observed effect. A functional assay would need to be conducted in order to determine whether a polymorphism is indeed exerting its observed effect. In such a functional assay, cells would be transfected with the different gene variants of the polymorphisms. Variables such as the
number of mRNA transcripts, protein expression, protein folding, binding affinity to α-tocopherol or cellular α-tocopherol uptake and efflux would be measured and compared between the gene variants to help determine whether the observed effects are in fact due to the particular polymorphism under study. For example, to test whether the α-TTP A>T SNP, which is located 5’ upstream of the α-TTP gene, influences the transcription of α-TTP, hepatic cells could be transfected with either the A or the T variant of the polymorphism. The number of mRNA transcripts, measured using quantitative polymerase chain reaction, could be compared between the two α-TTP variants. The amount of α-TTP proteins produced by the two α-TTP variants could also be measured by a western blot and compared to determine whether the polymorphism results in differences in protein synthesis. Since the TAP Arg11Lys polymorphism occurs in an exon region, differences in protein folding and affinity for α-tocopherol could be compared between the different TAP Arg11Lys variants.

6.3.4 Examining other polymorphisms in α-TTP, TAP and CYP4F2 or in other vitamin E metabolism genes, combined genotypes and gene*gene interactions

The effects of the α-TTP A>T and TAP Arg11Lys polymorphisms on plasma α-tocopherol concentration appear to be small. Furthermore, only about 20% of the variation in plasma α-tocopherol is explained by the adjusted models that do not include the polymorphisms under study as independent variables. The addition the α-TTP A>T, TAP C>T, TAP Arg11Lys and CYP4F2 Val433Met genotypes to these models does not appear to materially alter the proportion of variation in α-tocopherol explained by the models. Thus,
there is still quite a bit of variation in plasma $\alpha$-tocopherol concentration between individuals that is unaccounted for. It may be possible that other polymorphisms in these or other genes may contribute to explain some of this unaccounted variation in plasma $\alpha$-tocopherol concentration. It may also be possible that many polymorphisms combined (either additively, synergistically or as complex gene*gene interactions on plasma $\alpha$-tocopherol concentration) contribute to explaining the between-person variability in plasma $\alpha$-tocopherol concentration as opposed to one polymorphism that exerts a large effect on plasma $\alpha$-tocopherol concentration. For example, the addition of the combined $\alpha$-TTP A>T and TAP Arg11Lys genotype as an independent variable to the adjusted model increased the proportion of variability in plasma $\alpha$-tocopherol concentration explained by the model compared with the model that did not contain the combined genotype as an independent variable. This increase in the proportion of variation in plasma $\alpha$-tocopherol concentration explained by the adjusted model including the combined $\alpha$-TTP A>T and TAP Arg11Lys genotype was larger than the proportion of variation explained by the adjusted models to which the $\alpha$-TTP A>T or TAP Arg11Lys genotypes were added individually. Therefore, future genetic association studies should be conducted to determine whether other polymorphisms can explain the unaccounted variation in plasma $\alpha$-tocopherol concentration, influence plasma $\alpha$-tocopherol concentration or modify the association between dietary and plasma $\alpha$-tocopherol. The effects of a single polymorphism, multiple polymorphisms combined as well as gene*gene interactions should be tested. It should be noted that any null effects from these genetic association studies do not imply that a gene itself does not influence plasma $\alpha$-tocopherol concentration. Not all polymorphisms exert a functional effect on a particular outcome and it is possible that other
polymorphisms within the gene may influence plasma \( \alpha \)-tocopherol concentration or its response to dietary \( \alpha \)-tocopherol.

Two approaches could identify polymorphisms that influence plasma \( \alpha \)-tocopherol concentration or its response to dietary \( \alpha \)-tocopherol. A genome-wide association study could help identify polymorphisms associated with plasma \( \alpha \)-tocopherol concentration that may not be intuitive to study in a candidate gene approach (such as the present study), which is the second approach that could be taken. If a candidate gene approach were used, ideal polymorphisms to study would be other polymorphisms in the \( \alpha \)-TTP, TAP and CYP4F2 genes or polymorphism in other \( \alpha \)-tocopherol metabolism genes.

### 6.4 Implications

The knowledge gained from such genetic association studies has several implications. Firstly, these types of studies help to determine whether there is a genetic basis for the inter-individual differences in plasma \( \alpha \)-tocopherol concentration or its response to dietary \( \alpha \)-tocopherol. These studies can also identify individuals who are high responders to dietary \( \alpha \)-tocopherol. Although the present study did not find any significant diet*gene interactions on plasma \( \alpha \)-tocopherol concentration for any of the polymorphisms examined, future genetic association studies may be able to identify responders to dietary \( \alpha \)-tocopherol. This knowledge will facilitate the efficient design of studies involving dietary \( \alpha \)-tocopherol in relation to a certain outcome. For example, in clinical trials testing the effect of \( \alpha \)-tocopherol supplementation on a particular outcome, it would be ideal to recruit individuals who are considered to be high responders to \( \alpha \)-tocopherol based on their genotype. This is because if
α-tocopherol does indeed have an effect on a particular outcome, it would be easier to observe the effect in individuals who have a larger plasma α-tocopherol response to dietary or supplemental α-tocopherol. The effect will not be nullified by the presence of non-responders in a sample. Polymorphisms could also be incorporated into the study design of clinical and epidemiological studies by adjusting for genotype or recruiting equal numbers of various genotypes, for example.

The identification of polymorphisms that influence plasma α-tocopherol concentration or its response to dietary α-tocopherol may also aid in explaining inconsistencies in previous observational and clinical trials that involved α-tocopherol. The genetic composition of the different populations under study may have influenced the study results, possibly leading to inconsistent findings between different studies.

The exact role of TAP in α-tocopherol metabolism has not been completely elucidated. In addition, TAP is involved in other functions, such as cholesterol biosynthesis, and has been shown to bind to non-vitamin E compounds such as phosphatidylinositol [246-250]. The significant association between the TAP Arg11Lys polymorphism with plasma α-tocopherol observed in this as well as the Wright et al. study [208] suggests that TAP plays an important role in α-tocopherol metabolism. Future studies are still required, however, to determine the exact role of TAP in α-tocopherol metabolism.

As a more distant objective, the knowledge acquired from these genetic association studies could translate into personalized dietary advice tailored to an individual’s genotype.
This would ensure optimal nutrition for each genotype. The results of the present study do not lead to the creation of personalized dietary advice for α-tocopherol since none of the polymorphisms examined altered the plasma α-tocopherol response to dietary α-tocopherol. However, future studies could lead to the formulation of personalized dietary guidelines based on genetics.

6.5 Overall summary and conclusions

The results of this thesis are summarized in Table 6-1 (section 6.1).

Objective 1: To determine whether common single nucleotide polymorphisms in genes coding for the α-TTP (rs6994076 A>T), TAP (rs2072157 C>T and Arg11Lys (rs757660)) and CYP4F2 (Val433Met (rs2108622)) proteins influence fasting plasma α-tocopherol concentration.

Conclusions (Objective 1): Neither the TAP rs2072157 C>T nor the CYP4F2 Val433Met polymorphisms were associated with fasting plasma α-tocopherol concentration, regardless of the inclusion of supplement users in the analysis. The α-TTP rs6994076 A>T polymorphism altered plasma α-tocopherol concentration, but only in subjects who did not report the use of vitamin E-containing supplements. Individuals homozygous for the T allele of the α-TTP SNP had significantly lower plasma α-tocopherol concentrations compared with A/A subjects. A/T subjects had a plasma α-tocopherol concentration that was intermediate. The TAP Arg11Lys polymorphism was also significantly associated with plasma α-tocopherol concentration in both the total population and after the exclusion of supplement
users. In the total population, no significant differences in plasma \( \alpha \)-tocopherol concentration between the different TAP Arg11Lys genotypes were observed. However, there was a trend indicating lower plasma \( \alpha \)-tocopherol concentration in individuals bearing the Arg/Arg genotype. In subjects not consuming supplements, Arg/Arg subjects of the TAP Arg11Lys SNP had significantly lower plasma \( \alpha \)-tocopherol concentrations compared with the Lys/Lys subjects. The plasma \( \alpha \)-tocopherol concentration of Arg/Lys subjects was intermediate. When the \( \alpha \)-TTP rs6994076 A>T and TAP Arg11Lys SNPs were combined, the combined genotype was significantly associated with plasma \( \alpha \)-tocopherol concentration, in a potentially additive fashion, in individuals not consuming supplements. Subjects possessing two of the low plasma \( \alpha \)-tocopherol concentration genotypes (T/T for the \( \alpha \)-TTP A>T SNP and Arg/Arg for the TAP Arg11Lys SNP) had significantly lower plasma \( \alpha \)-tocopherol concentrations compared with subjects with two of the high plasma \( \alpha \)-tocopherol concentration genotypes (A carriers and Lys carriers for the \( \alpha \)-TTP A>T and TAP Arg11Lys SNPs, respectively). Subjects bearing only one of the low plasma \( \alpha \)-tocopherol concentration genotypes (A carriers + Arg/Arg or T/T + Lys carriers) had intermediate plasma \( \alpha \)-tocopherol concentrations.

**Objective 2:** To determine whether common single nucleotide polymorphisms in genes coding for the \( \alpha \)-TTP (rs6994076 A>T), TAP (rs2072157 C>T and Arg11Lys (rs757660)) and CYP4F2 (Val433Met (rs2108622)) proteins modify the association between dietary and plasma \( \alpha \)-tocopherol.
Conclusions (Objective 2): There were no significant diet*gene interactions on plasma α-tocopherol concentration for any of the polymorphisms examined in both the total population and in individuals not consuming supplements. This suggests that none of the polymorphisms under study modified the relationship between dietary and plasma α-tocopherol.

Overall conclusions: Of the single nucleotide polymorphisms examined, only the α-TTP rs6994076 A>T and TAP Arg11Lys polymorphisms altered fasting plasma α-tocopherol concentration. The α-TTP rs6994076 A>T SNP modified plasma α-tocopherol concentration only in individuals who did not consume supplements, while the TAP Arg11Lys SNP exerted its effect in the total population and in subjects not using supplements. The combined α-TTP rs6994076 A>T and TAP Arg11Lys genotype was also significantly associated with fasting plasma α-tocopherol concentration in subjects not consuming supplements. None of the polymorphisms under study significantly modified the plasma α-tocopherol response to dietary α-tocopherol. Future studies should consider other candidate polymorphisms in the α-TTP, TAP and CYP4F2 genes as well as in other vitamin E metabolism genes.
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Appendix 1: Sample questions from the food frequency questionnaire

**VITAMINS**

1. Have you ever regularly taken multi-vitamins?
   - Never have
   - Have in the Past only
   - Currently take them

   a) For how many years did you take them in the past?
      - 1 year or less
      - 2–4 years
      - 5–9 years
      - 10 or more years

   b) If you currently take multi-vitamins, how many do you take per week?
      - 2 or less
      - 3–5
      - 6–9
      - 10 or more

   c) If you currently take multi-vitamins, how many do you take per week?
      - 1 year or less
      - 2–4 years
      - 5–9 years
      - 10 or more years

   c) If you currently take them, what brand do you usually use?
      (Specify exact brand and type)

2. Not counting multi-vitamins, have you ever taken any of the following specific vitamins or minerals?

   **Vitamin E**
   - Never taken
   - Taken in the past only
   - Yes, currently take it

   Dose per day?
   - Less than 100 IU
   - 100 to 250 IU
   - 300 to 500 IU
   - 600 IU or more
   - Don’t know

   How long?
   - 0–1 year
   - 2–4 years
   - 5–9 years
   - 10 years or more
### VEGETABLES

5. Please fill in your **average** total use, **during the past month**, of each specified food.

<table>
<thead>
<tr>
<th>Spinach, raw as in salad (1 cup)</th>
<th>Kale, mustard, collard or turnip greens (1/2 cup)</th>
<th>Romaine or leaf lettuce (serving)</th>
</tr>
</thead>
<tbody>
<tr>
<td>○ Never</td>
<td>○ Never</td>
<td>○ Never</td>
</tr>
<tr>
<td>○ Less than once per month</td>
<td>○ Less than once per month</td>
<td>○ Less than once per month</td>
</tr>
<tr>
<td>○ 1–3 times per month</td>
<td>○ 1–3 times per month</td>
<td>○ 1–3 times per month</td>
</tr>
<tr>
<td>○ Once per week</td>
<td>○ Once per week</td>
<td>○ Once per week</td>
</tr>
<tr>
<td>○ 2–4 times per week</td>
<td>○ 2–4 times per week</td>
<td>○ 2–4 times per week</td>
</tr>
<tr>
<td>○ 5–6 times per week</td>
<td>○ 5–6 times per week</td>
<td>○ 5–6 times per week</td>
</tr>
<tr>
<td>○ Once per day</td>
<td>○ Once per day</td>
<td>○ Once per day</td>
</tr>
<tr>
<td>○ 2–3 times per day</td>
<td>○ 2–3 times per day</td>
<td>○ 2–3 times per day</td>
</tr>
<tr>
<td>○ 4 or more times per day</td>
<td>○ 4 or more times per day</td>
<td>○ 4 or more times per day</td>
</tr>
</tbody>
</table>

### CEREALS, BREADS & STARCHES

7. Please fill in your **average** total use, **during the past month**, of each specified food.

<table>
<thead>
<tr>
<th>Cold breakfast cereal (1 cup)</th>
<th>What brand and type of cold breakfast cereal do you usually eat?</th>
</tr>
</thead>
<tbody>
<tr>
<td>○ Never</td>
<td>Specify brand &amp; type (e.g., “Ralston Rice Chex”)</td>
</tr>
<tr>
<td>○ Less than once per month</td>
<td></td>
</tr>
<tr>
<td>○ 1–3 cups per month</td>
<td></td>
</tr>
<tr>
<td>○ 1 cup per week</td>
<td></td>
</tr>
<tr>
<td>○ 2–4 cups per week</td>
<td></td>
</tr>
<tr>
<td>○ 5–6 cups per week</td>
<td></td>
</tr>
<tr>
<td>○ 1 cup per day</td>
<td></td>
</tr>
<tr>
<td>○ 2–3 cups per day</td>
<td></td>
</tr>
<tr>
<td>○ 4 or more cups per day</td>
<td>○ Don’t eat cold breakfast cereal</td>
</tr>
</tbody>
</table>

### SWEETS, BAKED GOODS & MISCELLANEOUS

9. Please fill in your **average** total use, **during the past month**, of each specified food.

<table>
<thead>
<tr>
<th>Peanut butter (1 tbs.)</th>
<th>Almond butter (1 tbs.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>○ Never</td>
<td>○ Never</td>
</tr>
<tr>
<td>○ Less than once per month</td>
<td>○ Less than once per month</td>
</tr>
<tr>
<td>○ 1–3 tbs. per month</td>
<td>○ 1–3 tbs. per month</td>
</tr>
<tr>
<td>○ 1 tbs. per week</td>
<td>○ 1 tbs. per week</td>
</tr>
<tr>
<td>○ 2–4 tbs. per week</td>
<td>○ 2–4 tbs. per week</td>
</tr>
<tr>
<td>○ 5–6 tbs. per week</td>
<td>○ 5–6 tbs. per week</td>
</tr>
<tr>
<td>○ 1 tbs. per day</td>
<td>○ 1 tbs. per day</td>
</tr>
<tr>
<td>○ 2–3 tbs. per day</td>
<td>○ 2–3 tbs. per day</td>
</tr>
<tr>
<td>○ 4 or more tbs. per day</td>
<td>○ 4 or more tbs. per day</td>
</tr>
<tr>
<td>Peanut (small packet or 1 oz.)</td>
<td>Almonds (small packet or 1 oz.)</td>
</tr>
<tr>
<td>-------------------------------</td>
<td>---------------------------------</td>
</tr>
<tr>
<td>□ Never</td>
<td>□ Never</td>
</tr>
<tr>
<td>□ Less than once per month</td>
<td>□ Less than once per month</td>
</tr>
<tr>
<td>□ 1–3 per month</td>
<td>□ 1–3 per month</td>
</tr>
<tr>
<td>□ 1 per week</td>
<td>□ 1 per week</td>
</tr>
<tr>
<td>□ 2–4 per week</td>
<td>□ 2–4 per week</td>
</tr>
<tr>
<td>□ 5–6 per week</td>
<td>□ 5–6 per week</td>
</tr>
<tr>
<td>□ 1 per day</td>
<td>□ 1 per day</td>
</tr>
<tr>
<td>□ 2 or more servings per day</td>
<td>□ 2 or more servings per day</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Walnuts (1 oz. or 14 halves)</th>
<th>Other nuts (small packet or 1 oz.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>□ Never</td>
<td>□ Never</td>
</tr>
<tr>
<td>□ Less than once per month</td>
<td>□ Less than once per month</td>
</tr>
<tr>
<td>□ 1–3 per month</td>
<td>□ 1–3 per month</td>
</tr>
<tr>
<td>□ 1 per week</td>
<td>□ 1 per week</td>
</tr>
<tr>
<td>□ 2–4 per week</td>
<td>□ 2–4 per week</td>
</tr>
<tr>
<td>□ 5–6 per week</td>
<td>□ 5–6 per week</td>
</tr>
<tr>
<td>□ 1 per day</td>
<td>□ 1 per day</td>
</tr>
<tr>
<td>□ 2 or more servings per day</td>
<td>□ 2 or more servings per day</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Olive oil added to food or bread (1 tbs.); exclude use in cooking</th>
<th>Wheat germ (1 tbs.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>□ Never</td>
<td>□ Never</td>
</tr>
<tr>
<td>□ Less than once per month</td>
<td>□ Less than once per month</td>
</tr>
<tr>
<td>□ 1–3 tbs. per month</td>
<td>□ 1–3 tbs. per month</td>
</tr>
<tr>
<td>□ 1 tbs. per week</td>
<td>□ 1 tbs. per week</td>
</tr>
<tr>
<td>□ 2–4 tbs. per week</td>
<td>□ 2–4 tbs. per week</td>
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<tr>
<td>□ 5–6 tbs. per week</td>
<td>□ 5–6 tbs. per week</td>
</tr>
<tr>
<td>□ 1 tbs. per day</td>
<td>□ 1 tbs. per day</td>
</tr>
<tr>
<td>□ 2–3 tbs. per day</td>
<td>□ 2 or more servings per day</td>
</tr>
<tr>
<td>□ 4–5 tbs. per day</td>
<td></td>
</tr>
<tr>
<td>□ 6+ tbs. per day</td>
<td></td>
</tr>
</tbody>
</table>

10. How much of the visible fat on your beef, pork or lamb do you remove before eating?
    □ Don’t eat meat
    □ Remove all visible fat
    □ Remove most
    □ Remove small part of fat
    □ Remove none

11. What kind of fat is usually used for frying and sautéing at home?
    □ Don’t fry
    □ Real butter
    □ Margarine
    □ Olive oil
    □ Vegetable oil
    □ Vegetable shortening
    □ Lard/bacon fat
    □ Pam type spray

12. What kind of fat is usually used for baking at home?
    □ Don’t bake
    □ Real butter
    □ Margarine
    □ Olive oil
    □ Vegetable oil
    □ Vegetable shortening
    □ Lard/bacon fat
    □ Pam type spray
13. How often do you eat food fried, stir-fried in oil, or sautéed at home?
   - Never
   - Less than once a week
   - Once per week
   - 2–4 times per week
   - 5–6 times per week
   - Daily

14. How often do you eat deep fried food away from home or as take out (e.g., French fries, fried chicken, fish, clams, shrimp, etc.)?
   - Never
   - Less than once a week
   - Once per week
   - 2–4 times per week
   - 5–6 times per week
   - Daily

15. What type of cooking oil is usually used at home (e.g., Wesson Corn Oil)?

   [Blank]