

2001 MAFMA Final Report

Project Title **A Comparison of Isolated Components and Whole Foods: An Investigation of Vitamin E and α -tocopherol.**

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1. Objective Summary

The overall objective of this study was to determine effects of a food matrix on the bioavailability of vitamin E, and specifically α -tocopherol, in a whole animal model. Swine were used as the animal model because of strong similarities with human cardiovascular and digestive systems. The project successfully demonstrated that swine provide a useful whole animal model for assessing availability of bioactive components from whole foods and dietary supplements.

2. Objective Accomplishments

Pigs were fed purified α -tocopherol, semi purified α -tocopherol in an oil-based dietary supplement, mixed tocopherol isoforms in processed wheat germ oil, and a whole food containing vitamin E (broccoli). Blood samples were collected from the jugular vein and the hepatic portal vein. Contents of the distal small intestine, feces, and urine were also collected. Plasma α -tocopherol concentrations were determined by HPLC.

Plasma α -tocopherol concentrations were greatest during the 24 hour period following ingestion, with a dose-relationship to oral doses greater than 3500 IU purified α -tocopherol. A doubling of the dose from 3500 to ~7000 IU resulted in a two-fold increase in the plasma concentration. When lower doses were administered, plasma concentrations were increased, but the total areas under the curves for increased concentrations of α -tocopherol during the 24 hour period were not consistently associated with dose.

The availability of α -tocopherol from two oils was compared; one contained 98% α -tocopherol and the other contained a mixture of all isoforms of tocopherol. Concentrations of α -tocopherol in the plasma were not different even though the semi purified oil with 98% α -tocopherol contained 125 IU compared to 60 IU α -tocopherol contributed by the wheat germ oil. This result, consistent with our observation that plasma α -tocopherol concentrations did not reflect a dose-response to oral doses < 3500 IU α -tocopherol, indicates that availability of α -tocopherol from a mixture of isoforms, and/or additional food components found in the wheat germ oil was not different from the availability of α -tocopherol from a more purified preparation. Additional components in the wheat germ oil did not interfere with α -tocopherol availability in this study.

Ingestion of broccoli resulted in increased concentrations of α -tocopherol in the plasma, but the 24-hour plasma increases were not related to dose. Since all broccoli-derived plasma values were below those derived from 3500 IU purified tocopherol, this finding is in agreement with our earlier statement that below a dose of 3500 IU, resulting plasma tocopherol concentrations were not dose-related. Broccoli, although high in vitamin E relative to other vegetables, did not appear to contribute amounts of vitamin E sufficient to establish clear dose dependent relationships.

Three specific objectives were investigated during this project.

- a. *To determine if the source and form of α -tocopherol affects the concentration in the plasma.*

Six pigs were fitted with indwelling catheters in the hepatic portal and the external jugular veins. A single dose of α -tocopherol was given to each animal once per week such that a wash-out period of 7 days between doses could be strictly observed. Concentrations of α -tocopherol returned to non-detectable, baseline levels in the plasma between each treatment in all animals regardless of the dietary source of α -tocopherol. Six dietary sources of α -tocopherol were fed such that one animal received each treatment in a single replicate of the experiment. Over the 6 week period of the trial, all animals were fed each of the dietary sources of α -tocopherol, allowing comparisons between treatments within a single animal. Purified α -tocopherol was administered in a single dose of ~450 IU. Concentration of α -tocopherol in the plasma was determined at 0, 1, 2, 3, 6, 9, 12 and 24 hours following treatment and plotted against time. The total area under the curve was calculated and compared with the concentrations in the plasma of the same pig fed an oil-based dietary supplement containing ~125 IU α -tocopherol, wheat germ oil containing ~ 60 IU, and fresh cooked broccoli containing ~ 2-8 IU.

Dietary sources of α -tocopherol were chosen to reflect normal levels expected to be consumed in a human's diet either as serving sizes of foods or as the recommended dose for over the counter dietary supplements. Pigs were fed ~450 IU α -tocopherol from a purified chemical source (equal ~ 1 supplement capsule of 400 IU), ~ 60 IU of α -tocopherol in processed wheat germ oil (68 ml or ~1/4 cup), 125 IU from a semi-purified vitamin E oil containing 98% α -tocopherol (determined by HPLC analysis of a dietary supplement and chosen to compare to a pre-trial estimate of the α -tocopherol content of wheat germ oil), or fresh, cooked broccoli at 50, 100, or 200 grams fresh weight of vegetable estimated to contain ~2, 4, and 8 IU α -tocopherol respectively.

The concentrations of α -tocopherol in the plasma of pigs ingesting the 2 oils were of the same order of magnitude over the 24 hour sampling period. The concentrations were roughly 1/4 the concentration observed with the purified α -tocopherol indicating that the additional components in the oils did not interfere with the availability of α -tocopherol. Absorption of the purified form of α -tocopherol appeared more rapid than absorption of α -tocopherol from wheat germ oil, as peak concentrations were detected nearer the time of

ingestion. The longer time to peak plasma concentrations following ingestion of the less purified oils suggests that α -tocopherol from these supplements enters the blood stream less rapidly. However, when the total areas under the curve for the 24 hour period were compared, there were no statistically significant differences ($p < 0.05$) in the total area under the curves when equalized doses of purified α -tocopherol were compared with doses from the oils.

Doses of α -tocopherol from broccoli were low in this study but transient increases in plasma content of α -tocopherol were detected by HPLC. Increases were not consistently different when associated with increased amounts of broccoli consumed. In order to establish a dose response for α -tocopherol from a whole food, a food with a higher natural tocopherol concentration may need to be used. In this study, it was not possible to feed more than 200 g of fresh cooked broccoli in a single meal. Quantities of fresh cooked broccoli used in this study were chosen to be comparable to those expected to be consumed by humans in a normal serving at a single meal (100 – 200 g fresh, cooked broccoli).

b. To compare the time-related concentrations of α -tocopherol in the plasma, ileum and feces following ingestion of the compound from various sources.

A preliminary study was conducted using 2 pigs fitted with indwelling vascular catheters. Pigs were fed vitamin E at 5 doses to establish a dose/time relationship of α -tocopherol in the plasma. Doses of vitamin E ranged from approximately 400 IU (calculated potency from HPLC standards = 447 IU) to over 7,000 IU (7152 IU α -tocopherol) given as a single dietary dose. Dietary supplements typically sold over the counter to humans contain 400 IU vitamin E/capsule with recommendations to ingest 1 to 2 capsules, 1 or 2 times per day. The following doses (as calculated by HPLC and related to numbers of capsules sold as dietary supplements) of α -tocopherol were fed to the pigs in a single dose; 447 IU (1 capsule), 894 IU (2 capsules), 1788 IU (4 capsules), 3576 IU (8 capsules), 7152 IU (16 capsules). Blood samples were collected at 0, 1, 2, 3, 6, 9, 12 and 24 hours after ingestion of the dietary vitamin E. The concentration of α -tocopherol in the plasma was analyzed using HPLC and plotted over time to generate a total area under the curve for each dose. Doses of α -tocopherol up to 1788 IU (4 capsules) were not associated with an increased concentration in the plasma over the 24 hour period following ingestion. Although increased concentrations of α -tocopherol in the plasma were detected at single points in time following the lowest dose (447 IU), the increases were not sustained over a 24 hour period. When single doses of 3576 IU (8 capsules) and 7152 IU (16 capsules) were given and concentrations α -tocopherol in the plasma measured, there was a linear relationship between the dose administered and the increased total area under the curve for 24 hours following ingestion. These results suggest that α -tocopherol given in a single oral dose up to 1788 IU, increases plasma concentrations but this increase is not sustained for 24 hours. Doses as great as 7152 IU α -tocopherol sustained elevated plasma α -tocopherol levels over

the 24 hour period. By 36 hours after ingestion, the concentration of α -tocopherol in the plasma had returned to baseline levels for all doses. It is not known if repeated doses of vitamin E at 400 IU would eventually increase the plasma concentration to a sustainable level, if the tissues would store any excess vitamin E, or if the excess would be excreted in bile and lost in the feces. Further studies are needed to define the kinetic parameters of vitamin E availability.

c. *To determine if the source and form of α -tocopherol affects the ability of red blood cells to resist peroxide-induced hemolysis.*

Studies were conducted comparing the fragility of red blood cells from growing pigs depleted of vitamin E with fragility of red blood cells of normal growing pigs. Animals were approximately 150 pounds body weight when blood samples were collected. Animals were depleted of vitamin E by feeding a diet devoid of vitamin E for 10 weeks. Blood was collected from the jugular vein of three normal and three depleted animals. Whole blood was centrifuged and erythrocytes were washed twice in physiological saline (0.9%). Each animal's packed erythrocytes were suspended in distilled water to establish an absorbance value equal to 100% hemolysis and in 0.9% saline to establish a baseline value for background hemolysis in the absence of osmotic stress. Packed erythrocytes were suspended in saline titrated to create concentrations from 0.6 to 0.9% in a 96-well microtitre plate and allowed to rest at room temperature for 30 minutes. Absorbance for each titration was recorded in triplicate for each animal. Results indicated that erythrocytes from animals fed a vitamin E deficient diet for 10 weeks hemolysed more readily than erythrocytes from pigs fed a diet with adequate vitamin E. Erythrocytes from normal pigs exhibited 15% and 20% hemolysis at 0.65% and 0.6% saline, respectively. Erythrocytes from vitamin E deficient pigs exhibited 48% and 72% hemolysis at 0.65% and 0.6% saline, respectively. These results indicate that fragility of red blood cells may be useful as a biomarker for vitamin E status in pigs.

Erythrocytes were collected from catheterized jugular veins of 6 pigs used to study the bioavailability of vitamin E from the 6 dietary sources. A blood sample was collected from each animal prior to ingestion of vitamin E following the 7 day wash-out period. Timed blood samples were collected during 12 hours following ingestion of vitamin E. Erythrocyte fragility in titrated saline did not differ between pre-treatment (baseline) samples and samples collected at the time when α -tocopherol concentration was greatest in the peripheral blood. Although a long-term association between fragility of red blood cells from pigs with depleted vitamin E status was observed in the pretrial study, the same association was not detected within 12 hours of a single dose of dietary vitamin E. We conclude that although fragility of circulating erythrocytes in pigs is associated with vitamin E status, this appears to be a long-term, rather than short-term effect. It is possible that a single dose of vitamin E, or the amount of vitamin E used in this study, were insufficient to overcome the depleted status of circulating erythrocytes within

a 12 hour period. Further studies would be required to determine if changes can be made in circulating erythrocytes by a single dose of vitamin E or if longer exposures may be required. It is also possible that vitamin E status of the animal may affect the fragility of erythrocytes during maturation from stem cells in the bone marrow but not alter their fragility after they are released into the circulatory system. There are no studies available on short-term and long-term effects on erythrocytes of supplementing vitamin E to deficient individuals.

3. Unexpected Findings

When large single oral doses of vitamin E (>7100 IU) were administered to the animals, the resulting increases in α -tocopherol in the plasma were surprisingly short-lived. Increased concentrations in the plasma returned to baseline levels in fewer than 36 hours. This result emphasizes the need to critically examine all recommendations for oral dose and duration of vitamin supplement therapy with vitamin E. Although two-fold increases in plasma concentrations over a 24 hour period were observed when 7100 IU were administered compared to 3500 IU, this increase was not sustained for a longer period of time, suggesting that clearance from the blood was not delayed in proportion to dose, but that clearance from the blood increased with increasing plasma levels. Infrequent use of high oral doses of vitamin E may increase the concentrations in the plasma but may not provide long-term therapeutic value. Further work is needed to understand the dynamics of vitamin E metabolism, the effect of depletion of body storage sites, and the time necessary to impact metabolic functions associated with this bioactive molecule.

4. Practical Impacts of Research

a. Short Term Impacts

This research allows comparisons between availability of α -tocopherol from a purified source and from less purified food products such as wheat germ oil. The presence of additional components in the wheat germ oil had no apparent negative effect on appearance of α -tocopherol in the plasma of these animals. This result suggests that α -tocopherol from wheat germ oil, although not as highly concentrated as found in supplement form, is still available to the body. Further work needs to be done to define the kinetics of appearance in the blood and disappearance from the body of α -tocopherol from purified, semi-purified or whole food forms. The National Academy of Sciences has recommended an upper limit of 1,000 mg α -tocopherol daily. Considering that humans and pigs are similar in body weight and body water, as well as in digestive and cardiovascular parameters, this research shows that a dose of < 1,000 mg daily, taken as synthetic, purified, or as a component in wheat germ oil, would provide sufficient α -tocopherol to elevate plasma concentrations.

b. Long Term Impacts

A model to study the bioavailability of dietary supplements and components of whole foods was successfully developed. Swine were successfully fitted with vascular catheters and a digestive fistula and these modifications were maintained for up to 12 weeks during this project. The model provides an opportunity to track appearance and disappearance of bioactive components from foods and supplements in a whole animal model. The ability to monitor blood simultaneously from both the hepatic portal vein and the external jugular vein is expected to open opportunities for assessing the role of the liver in metabolism of bioactive components from foods. This model will allow sensitive methods to be further developed for tracking a number of important molecules through labeling of bioactive components with stable isotopes. The use of swine provides a practical way to monitor biological effects of ingested food components or nutraceuticals over a period of 12 weeks or more.