

The Role of Dietary Safflower Oil in the Management of Glucose Levels in Obese Postmenopausal Women with Type 2 Diabetes Mellitus

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ABSTRACT

With more than 8% of people in the United States diagnosed with type 2 diabetes, it is imperative to develop treatments that improve glycemic control (1). Previously, we reported that dietary safflower oil (8 g qd) significantly decreased trunk adipose mass, increased adiponectin levels, and decreased HbA_{1c} levels in post-menopausal, obese women with type 2 diabetes mellitus (T2DM) (2). However, the specific components of safflower oil that contributed to altering adipose mass distribution and improving glycemic control are not known. Safflower oil (SAF) contains several compounds (e.g., tocopherols, tocotrienols, and linoleic acid) that may improve insulin sensitivity. The objectives of this study were to determine if supplementation with SAF (8 g qd for 16 weeks) increases alpha tocopherol or gamma tocopherol levels in post-menopausal women with T2DM and to measure the relationship of serum tocopherols or linoleic acid with the insulin sensitizing cytokine, adiponectin, HbA_{1c}, and trunk adipose. Tocopherols were extracted from frozen serum samples via solid phase extraction and analyzed using high performance liquid chromatography (HPLC) for gamma and alpha tocopherol concentrations at the baseline and the endpoints of the (SAF) supplementation. While not all of the subjects exhibited increased tocopherol, the subjects who showed an increase in tocopherol concentration had a significant increase in plasma adiponectin and a significant decrease in trunk adipose mass and HbA_{1c}. Overall, the data suggest that alpha and gamma tocopherol may contribute to the improvements in markers of glycemia in obese post-menopausal women with T2DM.

Confounding factors that may have contributed to variability within our observations are related to the nature of this study being a secondary analysis derived from a primary study (2). The number of observations (n) was limited due to the primary objective (2). In addition, these considerations include possible exposure of samples to more than one freeze-thaw cycle, unregulated exposure to light, and duration of storage in -80C freezer. This study provides evidence that in addition to linoleic acid, tocopherols found in dietary SAF may contribute to improved glycemic control in women with (T2DM).

INTRODUCTION

Currently, it is estimated that more than 25.8 million (or 8.3%) of people in the United States alone have been diagnosed with diabetes (1). Due to the rising trend in obesity, the prevalence of type 2 diabetes mellitus (T2DM) continues to increase at a staggering rate. The primary factor in the development of T2DM is centralized (android pattern) obesity coupled with substantial weight gain leading to impaired glucose tolerance and fasting glucose ultimately resulting in insulin resistance. While healthy eating, moderate exercise, and weight loss significantly improve glycemic control, weight loss is not always a viable or realistic option for many obese patients with diabetes. However, current research continues to reveal more effective and less costly drug therapy and dietary supplement options to curb T2DM and provide stable glycemic control.

In particular, safflower oil, a cost-effective and widely available natural dietary supplement, has been the subject of several T2DM studies for improving insulin sensitivity and glycemic control. However, the specific components of safflower oil that contributed to altering adipose mass distribution and improving glycemic control have yet to be characterized.

Safflower oil is a colorless and flavorless dietary oil that is rich in the omega-6 (n-6) fatty acid, linoleic acid, and vitamin E. The polyunsaturated fatty acid (PUFA) n-6 linoleic acid (18:2n6) found in safflower oil promotes heart health and was recently found to improve body weight and body composition (3). Vitamin E refers to a group of compounds that include tocopherols and tocotrienols that are known antioxidants yet their effects on weight loss and adipose distribution have yet to be fully understood. In a previous study (The Women’s Diabetes Study), safflower oil decreased trunk adipose mass, lowered fasting glucose, and increased adiponectin levels in postmenopausal women with T2DM (2).

The results of the study suggested that the tocopherols and/or linoleic acid provided by the safflower oil may mimic the mechanism of a class of prevalent diabetes drugs, thiazolidinediones (TZDs), though the implications of this hypothesis are still unclear. TZDs act by binding peroxisome proliferator-activated receptor γ (PPAR γ) (4). Binding to the PPAR γ induces secretion of adipokines (particularly adiponectin) by the adipocytes resulting in significant increases in insulin sensitivity (Figure 1).

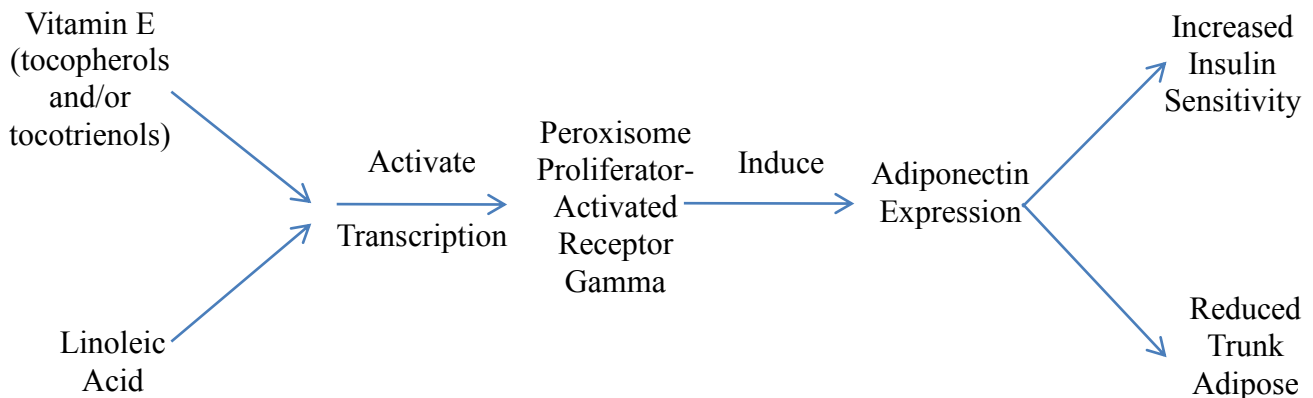


FIGURE 1: Proposed Mechanism of Tocopherols and/or Linoleic Acid

Unfortunately, TZDs have recently been scrutinized for possible weight gain, and more importantly, potential life-threatening cardiac and liver complications (5). The problems with synthetic pharmaceutical TZDs have been attributed to their extremely high affinity for PPAR γ and/or the slow rate of metabolism. In contrast, naturally occurring ligands for PPAR γ such as fatty acids and eicosanoids bind and activate PPAR γ with lower affinity and have more rapid turnover through metabolism; both advantages of naturally occurring ligands then offer a lower likelihood of toxicity. Tocopherols were recently shown to promote PPAR γ -mediated gene expression and phenotypic outcomes in experimental animals and cell culture (6). The results of the Women's Diabetes Study showing improved glycemic control with SAF oil supplementation coupled with previous studies demonstrating the ability of safflower oil to increase adiponectin levels and improve glycemic control may be attributed to the tocopherol components promoting a PPAR γ -mediated mechanism similar to TZDs but without the potency and the side effects of the synthetic drug (2).

This study is secondary analysis of the data and frozen serum samples from the Women's Diabetes Study with following two-pronged objective: 1) To determine the extent that supplementation with safflower oil increases serum tocopherol levels in post-menopausal women with T2DM; 2) To quantify the strength of the relationship between plasma tocopherol levels and improved glycemia (reduced fasting glucose) via a PPAR γ binding mechanism.

MATERIALS AND METHODS

WDS Overview

The study was a 36-wk randomized, double blind, cross-over design that was approved by the Ohio State University Institutional Review Board and CRC Advisory Board. Fifty-five

postmenopausal women with T2DM received SAF or CLA (8 g qd) during two 16-week diet periods separated by a 4-week washout period (Figure 2).

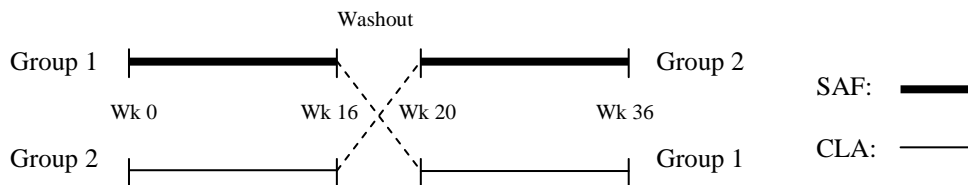


FIGURE 2: Experimental design of the Women’s Diabetes Study

Dual-energy X-ray absorptiometry (DXA) was used to determine body composition. Fasting blood samples were collected from subjects every 16 weeks and plasma samples were analyzed for glucose, insulin, leptin, and adiponectin by the General CRC Laboratory Core. Serum linoleic acid was analyzed by gas chromatography in the Belury Laboratory and described previously (7).

Study Design

Frozen serum samples from the primary study were selected for secondary analysis. Only samples that represented the baseline and endpoints of the safflower supplementation were analyzed for serum α -tocopherol and γ -tocopherol concentrations. Serum tocopherol concentrations as well as previously reported linoleic acid levels were then compared to HbA_{1c}, adiponectin, and trunk adipose using least squares linear regression and matched paired t-test.

Tocopherol Standard Preparation

Stock solutions of α -tocopherol and γ -tocopherol (Supelco) were prepared in chloroform at 50 and 12.5 mg/mL respectively. A stock solution of Rac-tocol (Matreya, LLC) was prepared in methanol at 500uM. Subsequent dilutions were performed into methanol to produce mixed tocopherol standards at 50uM and 500uM, and a Rac-tocol internal spike of 50uM. These

standards were used to make the daily calibration standards for quantification ranging from 1 to 100uM with Rac-tocol at 25uM.

Sample Preparation

Frozen serum samples were thawed on ice at room temperature and an internal standard (50 uL, 50 uM tocol) was added with 250uL of serum deproteinized by cooled ethanol (650 uL, 8 min, 4°C). After centrifugation (2000 xg, 15 min, 4°C), the supernatant was separated and acidified to pH 3 (10 uL, 1M, HCl). Prior to solid phase extraction (SPE), the SPE column was conditioned with 3 mL of methanol, distilled water, and hydrochloric acid (1M). The serum was extracted with ethyl acetate (3 mL), dried by nitrogen steam, and re-constituted with methanol (100 uL) in preparation for analysis (8).

Tocopherol HPLC Method

Chromatography was performed on a Shimadzu HPLC system (Columbia, MD) with detection by UV absorption at 295 nm. Separation was achieved using an isocratic elution on a Chromolith Performance RP-18e, 100x4.6mm monolithic column (Merck). The mobile phase was acetonitrile: 1% ammonium acetate in water, pH7 at a ratio of (95:5) and a flow rate of 1.5mL/min for 7 minutes. The column was then flushed at a ratio of acetonitrile: 1% ammonium acetate (98:2) with an increased flow rate of 3.2 mL/min for 4 minutes then was allowed to re-equilibrate for 2 minutes. A column heater was used to maintain ambient temperature at 25°C. The injection volume was 20 uL (8).

Statistical Analysis

All statistical analyses were done using SPSS Software Version 19

Analysis of Variance: Comparisons between baseline and endpoint concentrations of α -tocopherol, γ -tocopherol, and linoleic acid were analyzed using one-way ANOVA with $p < 0.10$

considered significantly different. Bonferroni method was used to adjust for multiple comparisons.

Least Squares Linear Regression: Serum concentrations of α -tocopherol, γ -tocopherol, and linoleic acid were analyzed by multiple least squares linear regression models with the change in α -tocopherol, γ -tocopherol, and linoleic acid as the independent variable and the change in adiponectin, HbA_{1c}, and trunk adipose as the dependent variable

Matched Paired T-test: A matched paired t-test was also conducted between the subjects who had increases in γ -tocopherol, α -tocopherol, and linoleic acid from the baseline to the endpoint of SAF supplementation and the aforementioned dependent variables. Data is presented as mean difference \pm standard deviation (SD) with $p < 0.10$ considered significantly different.

RESULTS

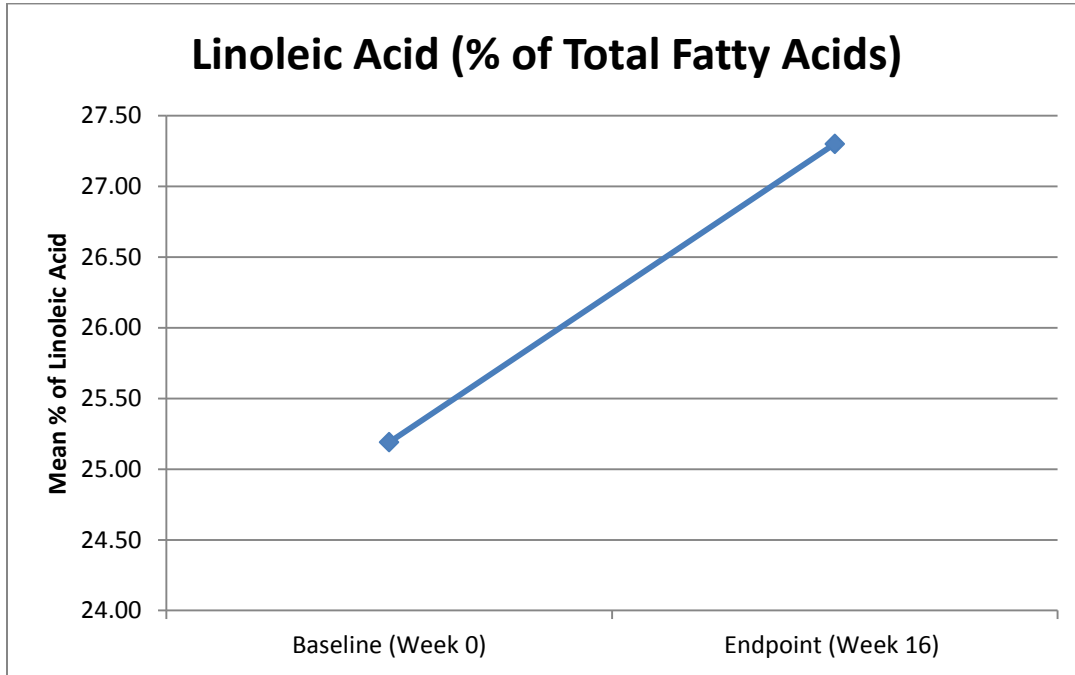
Matched frozen serum samples were found for 29 of the 40 women who completed the safflower supplementation. The data between diet period 1 (Weeks 0-16) and diet period 2 (Weeks 20-36) for the safflower supplementation showed no significant difference. Also, no differences were observed for age, ethnicity, BMI, duration of diabetes, and HbA_{1c} at baseline of the diet groups. Therefore, these data were overlapped to increase power of the statistical analyses.

Tocopherol Analysis

Serum concentrations of α -tocopherol and γ -tocopherol were analyzed via HPLC from the baseline and endpoints of safflower oil supplementation. There was no significant increase in either α -tocopherol or γ -tocopherol during either 16-week diet period ($p = 0.67$).

Linoleic Acid Analysis

Serum linoleic acid was previously analyzed by gas chromatography although the data had yet to be statistically analyzed. A significant increase in linoleic acid was observed over the course of the SAF supplementation ($p=0.10$) (Figure 3).



Descriptive Statistics				95% Confidence Interval		p-value
	Mean	Std. Deviation	n	Lower Bound	Upper Bound	
Baseline (Week 0)	25.19	4.83	15	22.52	27.87	0.10
Endpoint (Week 16)	27.30	3.55	15	25.33	29.27	

FIGURE 3: Change in Linoleic Acid Level from Baseline to Endpoint of SAF Supplementation. Linoleic acid is expressed as the % of total identified fatty acids.

Anthropometric Variables

The previous study reported no effect on total adipose mass, but a significantly reduced trunk adipose mass ($p=0.04$) and increased lean tissue mass ($p=0.04$) during the SAF supplementation. However, linear regression analysis showed no significant correlation between

change in α -tocopherol, γ -tocopherol, or linoleic acid and change trunk adipose mass from baseline to endpoint. A matched paired t-test was performed in which only subjects who had an increase in α -tocopherol, γ -tocopherol, and linoleic acid (respectively) were compared to trunk adipose mass. This analysis also showed no difference between the baseline and endpoint of SAF supplementation.

Biochemical Measurements

Changes between α -tocopherol, γ -tocopherol, or linoleic acid and adiponectin or HbA_{1c} were analyzed by least squares linear regression models (Table 1). There was, however, a significant correlation between change in α -tocopherol and change in HbA_{1c} (p=0.02) (Figure 4).

Linear Regression Summary			
Dependent Variable	Independent Variable	Linear Regression – R ²	Significance (p-value)
ADIPONECTIN (ug/mL)	γ -TOCOPHEROL (uM)	0.02	0.56
HbA _{1c} (%)	γ -TOCOPHEROL (uM)	0.02	0.52
TRUNKADIPOSE (g)	γ -TOCOPHEROL (uM)	0.00	0.80
ADIPONECTIN (ug/mL)	α -TOCOPHEROL (uM)	0.02	0.53
HbA _{1c} (%)	α -TOCOPHEROL (uM)	0.08	0.02*
TRUNKADIPOSE (g)	α -TOCOPHEROL (uM)	0.02	0.49
ADIPONECTIN (ug/mL)	LINOLEIC ACID (%)	0.05	0.30
HbA _{1c} (%)	LINOLEIC ACID (%)	0.01	0.60
TRUNKADIPOSE (g)	LINOLEIC ACID (%)	0.00	0.90

TABLE 1: Linear Regression Summary.

The independent and dependent variables are expressed as the difference between baseline and endpoint of safflower supplementation. Values for each measurement that have (*) are significantly different at p<0.10

Change in α -tocopherol (uM) is Negatively Correlated to Change in HbA_{1c} (%)

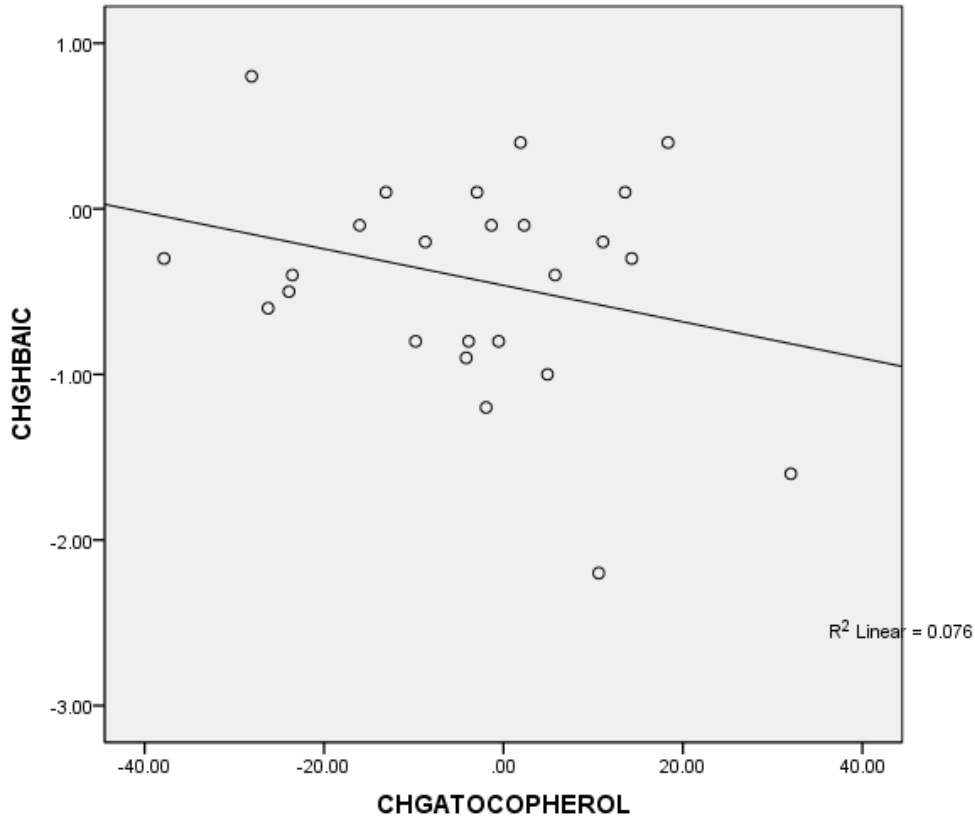


FIGURE 4: Change in α -tocopherol (uM) negatively correlated to change in HbA_{1c} (%) ($p=0.02$) at the $p=0.10$ level

To analyze the correlation between subjects who had increases in α -tocopherol, γ -tocopherol, and linoleic acid to adiponectin and HbA_{1c}, only subjects who had increases in α -tocopherol, γ -tocopherol, and linoleic acid were included in a matched paired t-test taking the difference in means from baseline to endpoint of SAF supplementation for adiponectin and HbA_{1c} (Table 2). α -tocopherol, γ -tocopherol, and linoleic acid had no statistically significant effects on adiponectin. However, both α -tocopherol and γ -tocopherol showed p-values that were marginal to the $p=0.10$ level ($p=0.12$, $p=0.12$ respectively) (Figure 5, 6). By contrast, there was a significant decrease in HbA_{1c} when compared to α -tocopherol, γ -tocopherol, and linoleic acid (Figure 7, 8, 9).

Match Paired T-Test Summary						
Dependent Variable	Independent Variable	n	Mean/Std Dev	Mean/Std Dev	Mean Difference	Significance (p-value)
			Endpoint	Baseline		
γ- TOCOPHEROL (uM)	ADIPONECTIN (ug/mL)	12	11.4 +/- 8.5	9.2 +/- 6.6	2.2	0.12
	HbA _{1c} (%)	11	7.0 +/- 1.2	8.2 +/- 2.0	-1.2	0.08*
	TRUNK ADIPOSE (g)	11	21788.2 +/- 5356.0	23418.9 +/- 6382.4	-1630.7	0.17
α- TOCOPHEROL (uM)	ADIPONECTIN (ug/mL)	13	10.0 +/- 7.0	7.8 +/- 3.1	2.2	0.12
	HbA _{1c} (%)	13	7.2 +/- 1.2	8.3 +/- 1.9	-1.1	0.06*
	TRUNK ADIPOSE (g)	11	24229.5 +/- 6021.3	25099.5 +/- 6088.8	-870.0	0.45
LINOLEIC ACID (%)	ADIPONECTIN (ug/mL)	15	7.9 +/- 6.1	6.4 +/- 3.3	1.5	0.17
	HbA _{1c} (%)	16	7.0 +/- 0.9	7.5 +/- 1.2	-0.5	0.07*
	TRUNK ADIPOSE (g)	14	24305.0 +/- 7531.5	25012.5 +/- 7359.2	-707.5	0.48

TABLE 2: Match Paired T-Test Summary

The independent and dependent variables are expressed as the difference between baseline and endpoint of safflower supplementation. Endpoint (Wk 16) and Baseline (Wk 0) are represented as the mean difference \pm standard deviation. Values for each measurement that have (*) are significantly different at $p < 0.10$

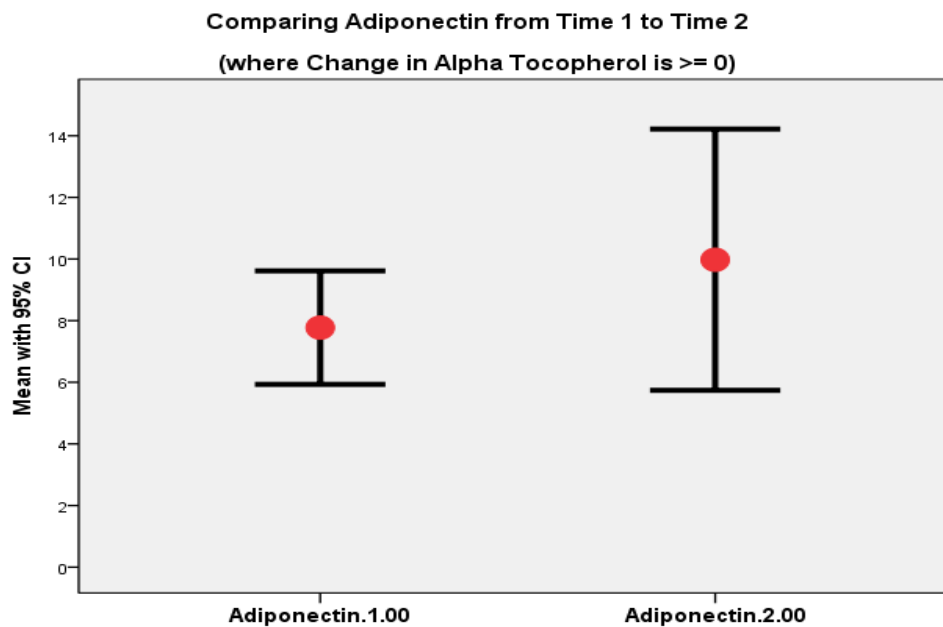


FIGURE 5: Mean difference of adiponectin (ug/mL) from baseline to endpoint where change in α -tocopherol (uM) >0 ($p = 0.12$).

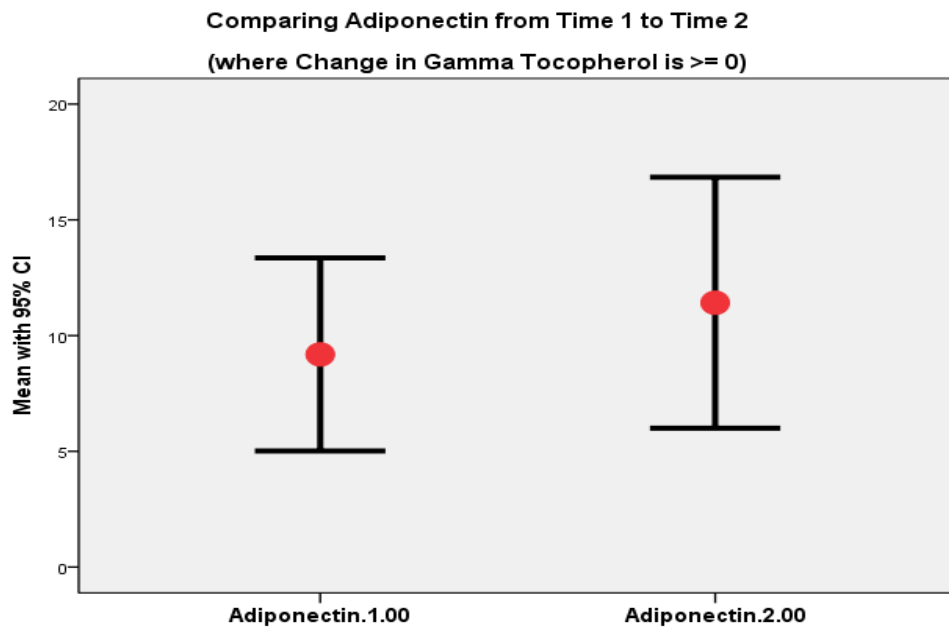


FIGURE 6: Mean difference of adiponectin (ug/mL) from baseline to endpoint where change in γ -tocopherol (uM) >0 ($p=0.12$).

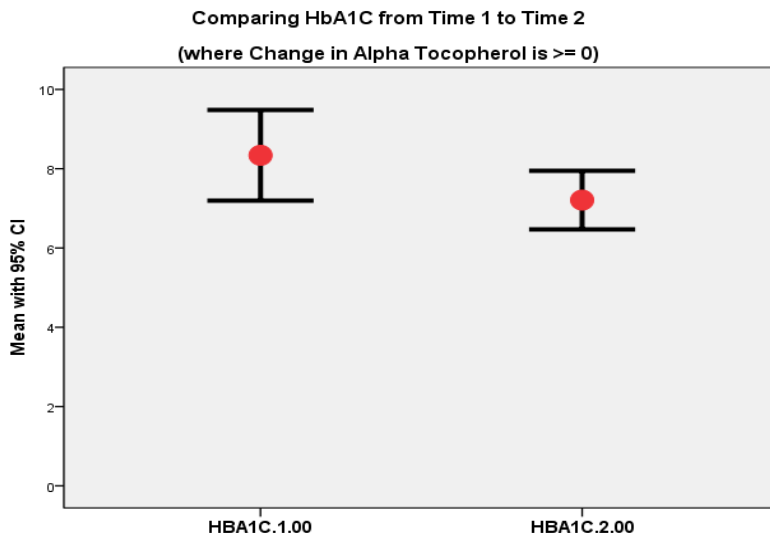


FIGURE 7: Mean difference in HbA_{1c} (%) from baseline to endpoint where change in α -tocopherol (uM) >0 (p=0.06).

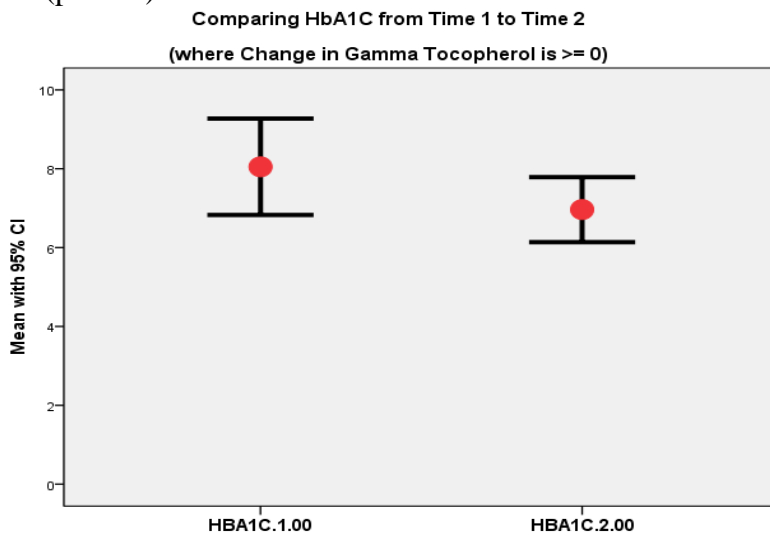


FIGURE 8: Mean difference in HbA_{1c} (%) from baseline to endpoint where change in α -tocopherol (uM) >0 (p=0.08).

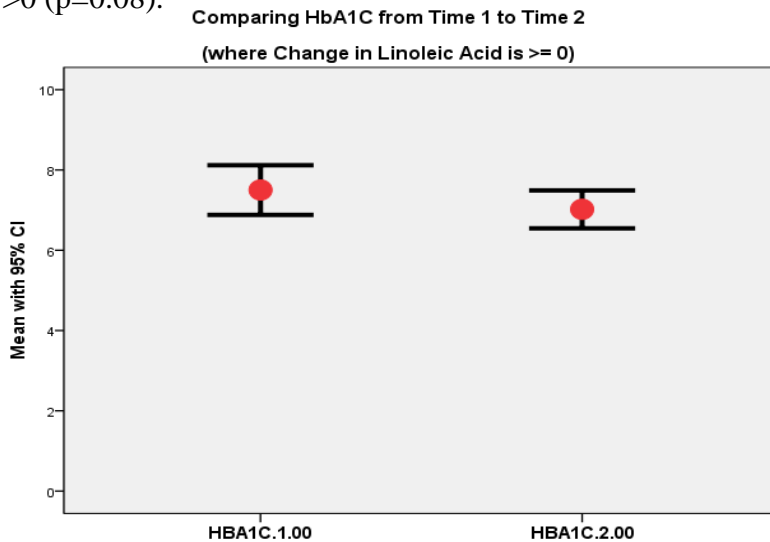


FIGURE 9: Mean difference in HbA_{1c} (%) from baseline to endpoint where change in linoleic acid (% of total fatty acids) >0 (p=0.07).

DISCUSSION

The results of this study suggest that alpha and gamma tocopherol may contribute to the improvements in markers of glycemia (particularly plasma glucose as measured by HbA_{1c}) in postmenopausal women with T2DM. While the results were inconclusive in regards to whether safflower oil increases serum concentrations of tocopherol, there were significant increases in the % of linoleic acid (of total fatty acids) from baseline to end point of SAF supplementation.

Although there was not a significant increase in α -tocopherol and γ -tocopherol throughout SAF supplementation, those subjects who did have an increase in α -tocopherol, γ -tocopherol, as and linoleic acid showed improved HbA_{1c} and adiponectin expression indicating improved insulin sensitivity. Adiponectin regulates glucose metabolism by increasing glucose uptake and decreasing gluconeogenesis resulting in decreased HbA_{1c} (9). Because adiponectin transcription is responsive to PPAR γ ligands, the increase in adiponectin expression may be due to linoleic acid which is a weak ligand for PPAR γ . While it has not yet been determined if tocopherols are a direct ligand to PPAR γ , some animal studies suggest that tocopherols facilitate activation of PPAR γ via stimulation of 15-deoxy- Δ 12,14-prostaglandin J₂ (15d-PGJ₂), a known PPAR γ ligand (6). 15d-PGJ₂ seems to enhance binding of PPAR γ to the PPAR γ response element (PPRE) in the promoter region of the adiponectin gene therefore facilitating adiponectin expression (10). Furthermore, α -tocopherol, γ -tocopherol, and linoleic acid did not show any correlation to a decrease in trunk adipose tissue even after women who did not have increases in tocopherol and linoleic acid were removed from statistical analysis.

In the Women's Diabetes study, SAF supplementation resulted in a decrease in trunk adipose mass and an increase in lean tissue mass resulting in no change in total BMI. This phenomenon could be due to the insulin sensitizing effects of PPAR γ . The proposed mechanism

for α -tocopherol, γ -tocopherol, and linoleic acid to improve insulin sensitivity is through PPAR γ activation. PPAR γ is a receptor for a popular diabetes drug, thiazolidinediones (TZDs) which triggers adipocyte differentiation improving glucose uptake and therefore, insulin sensitivity (11). However, animal studies and clinical trials have shown that TZDs result in moderate weight gain and edema, possibly due to an increase in fat cell number and a decrease in fat cell size (12). TZDs, however, bind PPAR γ with high affinity so it is likely that a weaker ligand would result in milder side effects (little or no overall weight gain). Because linoleic acid and tocopherols in safflower oil may function via a PPAR γ dependent mechanism, the evidence of no change in BMI throughout safflower supplementation is consistent with the findings of other PPAR γ ligands, specifically TZDs.

There were some limitations to this study, many of which were due to the nature of this study being a secondary analysis of a study whose primary objective was not specific to the components of safflower oil. First, the number of observations (n) was limited because only 29 of the 40 women who finished the safflower supplementation had matched samples that were viable for analysis. Therefore, a small number of women who may naturally not respond to safflower supplementation or absorb tocopherol from safflower oil would have had a strong effect on the outcome of the study. The limited number of observations also affected the power of the statistical analysis since the matched paired t-test has lower power than some other statistical measures. Also, the 16-week cross over design may not have provided adequate time to show maximum results of the safflower supplementation. In addition, some confounding variables contributing to the observed tocopherol concentrations include possible exposure of samples to multiple freeze-thaw cycles, light exposure, and duration of storage in -80C freezer.

Overall, this study shows that α -tocopherol, γ -tocopherol, and linoleic acid are likely candidates for the insulin-sensitizing effects of safflower oil. Also, while the mechanisms of linoleic acid and tocopherols that contribute to insulin sensitivity have yet to be fully understood, findings from this study indicate that α -tocopherol, γ -tocopherol, and linoleic acid may function through a PPAR γ - mediated process. Future studies should focus on prolonging the duration of the intervention and determining whether tocopherols and/or linoleic acid are responsible for improved glucose metabolism. A method to compare the effectiveness of tocopherol versus linoleic acid would be to supplement vitamin E or linoleic acid to two randomly assigned treatment groups of postmenopausal women with T2DM. An intervention of this nature would be useful in not only determining to what extent tocopherols and/or linoleic acid contribute to glucose metabolism, but would also be beneficial in determining the mechanisms of tocopherols and/or linoleic acid.

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