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Thus far there are no statistically significant differences between various apolipoprotein E genotypes in the cluster of seven responsiveness to a flaxseed oil supplement in persons with type 2 diabetes

Douglas E. Barre [1], Kazimiera A. Mizier-Barre [2], Odette Griscti [3]

[1]Department of Health Sciences, Cape Breton University, Sydney, Nova Scotia, Canada; [2]Department of Biology, Cape Breton University, Sydney, Nova Scotia, Canada; [3]School of Nursing, Cape Breton University, Sydney, Nova Scotia, Canada
E-mail: ed_barre@cbu.ca

Objective. The objective of the present study was to determine if there would be statistically significant differences among apolipoprotein E genotypes in the responsiveness of members of a cluster of seven measures in persons with type 2 diabetes consuming a flaxseed oil supplement (FOS). The cluster of seven are: abdominal obesity, hypertension, platelet hyperaggregability, hyperglycemia, dyslipidemia (decreased serum levels of high-density lipoprotein cholesterol (HDLc) and increased serum levels of triglycerides), increased serum low-density lipoprotein (LDL) oxidation (LDL conjugated dienes) and increased inflammation. All cluster members exacerbate type 2 diabetes.

Methods. Thirty-two patients with well-controlled type 2 diabetes participated in this double-blind, placebo (safflower oil supplementation (SOS))-controlled study consisting of three visits. Apolipoprotein E genotyping was done at visit one. The cluster of seven was assessed at each visit.

Results. Only platelet aggregability decreased as the result of the FOS relative to placebo while it appeared that there might be some potential for FOS to decrease LDL conjugated dienes. No apolipoprotein E genotype affected any of the cluster of seven responsiveness.

Conclusions. Identified apolipoprotein E genotypes played no role in the responsiveness of any member of the cluster of seven to FOS in this study.

Keywords: apolipoprotein E genotype polymorphisms, waist circumference, hypertension, bleeding time, serum glucose, high density lipoprotein cholesterol, triglycerides, low-density lipoprotein conjugated dienes, c-reactive protein, flaxseed oil supplementation

Utermann et al. (1977) were the first to note the relationships of various genotypic polymorphisms of apolipoprotein E (E2/E2, E2/E3, E2/E4, E3/E3, E3/E4, and E4/E4) with plasma lipid and lipoprotein-cholesterol levels. Dyslipidemia (primarily elevated serum triglycerides and low serum high-density lipoprotein cholesterol (HDLc)) in type 2 diabetes (T2D) contributes to or is exacerbated by other members of the cluster of seven (hyperglycemia, hypertension, abdominal obesity, increased low-density lipoprotein (LDL) oxidation, inflammation, and increased platelet aggregability (Nishigaki et al. 1981; Van Gaal et al. 1989; Schneider 2009; Tabatabaei-Malazy et al. 2012; Tangvarasittichai 2015; Asghar et al. 2023). Various members of the cluster of seven in T2D have varying degrees of association with apolipoprotein E polymorphisms (Barre et al. 2024a). Some apolipoprotein E genotypic polymorphisms impact the responsiveness to certain dietary and pharmaceutical interventions in T2D

Corresponding author: Dr. Ed Barre, Department of Health Sciences, Cape Breton University, P.O. Box 5300, Sydney, Nova Scotia, B1P-6L2 Canada; phone: (902) 563-1921; fax: (902) 563-1945; e-mail: ed_barre@cbu.ca.

(Tamasawa et al. 2003; Saito et al. 2004; Tavintharan et al. 2007; Sapkota et al. 2015; Yassine et al. 2020; Barre et al. 2024b). Genotypic analysis is important in the developing personalized medicine, which is key to better patient outcomes (Listgarten et al. 2014; Ramos-Lopez 2024). In that regard, statistically significant differences to flaxseed oil supplementation (FOS) among apolipoprotein E genotypes for all members of the cluster of seven in those with T2D have never been assessed when male and female data is combined to give greater statistical power. Based on these facts, it was of interest, for the first time, to assess all seven members of the cluster for statistically significant differences between apolipoprotein E genotypes in responsiveness to FOS in persons with well-controlled T2D. This paper is a secondary analysis of the study by Barre et al. (2010).

The objective of the current study was to determine if there would be statistically significant differences among apolipoprotein E genotypes in the responsiveness to FOS of one or more members of the cluster of seven in persons with well-controlled T2D.

Material and Methods

Subjects. The Cape Breton University Human Ethics Review Committee approved the protocol. The subjects had to be: 18 years of age or older, with well-controlled T2D of at least six months' duration and not involved in any physical training program, not taking insulin or any omega 3 supplement including but not limited to fish oil, not pregnant or planning on becoming pregnant and have no known sensitivity to flaxseed oil or safflower oil. Subjects (mean age ± SEM = 58.8 ± 1.6 years) responded to a Sydney, Nova Scotia newspaper advertisement and two area physicians. All patients gave informed consent. Waist circumference, blood pressure and fasting (12–14 h) blood samples were done at visit 1 and 3 months later for visit 2. From visit 1 to visit 2, subjects continued with their normal daily activities. At visit 2, patients were randomized to treatment (FOS, 60 mg ALA/kg body weight/day) or placebo (safflower oil supplementation, SOS) in the form of 1 g gelatin capsules for three months until visit 3 (all patients consumed approximately 104 mg of oil/kg body weight/day).

Laboratory methods. Apolipoprotein E genotyping was done by the method of Hixson and Vernier (1990) modified to use 2% agarose gels. Abdominal obesity (measured by waist circumference) was determined by the method of Lemieux et al. (1996), serum glucose by Wako Glucose C2 kit (Wako USA, Richmond, VA, USA), blood pressures by manual sphygmomanometry, serum HDL isolation by precipitation using Quantolip HDL kit, (Technoclone Vienna, Austria) and its cholesterol content using cholesterol E kit (Wako, Richmond, VA, USA), serum triglycerides by L-type TG H kit (Wako USA, Richmond, VA, USA), bleeding time by the Ivy bleeding time (Mielke et al. 1969) using Simplate II R device, LDL oxidation via serum maximal LDL conjugated dienes (LDL-CDs) level formation determined by the method of Fuller et al. (1996) and calculated by the method of Ahotupa et al. (1996,1998), and inflammation was measured by serum C-reactive protein (CRP) using human ELISA kit (Alpha Diagnostic, San Antonio, TX, USA). All kits and devices were used following their manufacturer's instructions.

Statistical analyses. Values obtained at visits 1 and 2 were averaged. A two-way analysis of variance (ANOVA) was performed for each variable, comparing the value from visit 3 to the average of visits 1 and 2 for FOS vs. SOS (model 1). A Tukey's post-hoc test was performed to determine if there were any significance differences between treatment and control when a F test in the two-way ANOVA indicated a significant difference using model 1. Next, apolipoprotein E genotypes were added to model 1 to give model 2. Model 2 was used to determine what, if any, impact each of the apolipoprotein E genotypes might have on the cluster of seven. The extent of changes, if any, in the FOS group compared to those in the SOS group allowed a decision on whether FOS had any impact on the cluster of seven (model 1) and if there were any statistically significant differences among the apolipoprotein E genotypes (model 2) in the cluster of seven due to FOS.

Results

Eighteen patients in the FOS group and 14 patients in the SOS group completed the trial. The results are presented in Table 1 and reflect treatment (FOS) effect relative to placebo (SOS). Only bleeding time significantly increased, while LDL-CDs showed some potential (p=0.21) for reduction by FOS. No genotype (E2/E3, E2/E4, E3/E3, E3/E4, E4/E4) identified in the patients showed any FOS-induced statistically significant difference in the cluster of seven.

Discussion

It is important to understand the relationship between genotype or its absence within the cluster of seven in T2D to expand information on the etiology

Table 1
Cluster of seven with flaxseed oil (FOS) (n=18, 10 males, 8 females) vs. safflower oil (SOS) (n=14, 8 males, 6 females) for visit/treatment only and visit/treatment/apo E genotype

Cluster of seven			Model 1 Visits treatment (FOS vs. SOS)	Model 2 Visits treatment (FOS vs. SOS) with genotype
1	Waist (cm)	FOS Average visits 1 and 2	103.8±2.6	p>0.05 for all genotypes
		Visit 3	104.0±2.9	
		SOS Average visits 1 and 2	99.2±2.2	
		Visit 3	98.8±2.2	
2	Serum glucose (mmol/L)	FOS Average visits 1 and 2	8.4±0.8	p>0.05 for all genotypes
		Visit 3	9.0±0.9	
		SOS Average visits 1 and 2	7.7±0.7	
		Visit 3	8.5±1.2	
3a	SBP (mm Hg)	FOS Average visits 1 and 2	140.6±3.8	p>0.05 for all genotypes
		Visit 3	143.9±3.2	
		SOS Average visits 1 and 2	140.0±5.1	
		Visit 3	139.8±5.2	
3b	DBP (mm Hg)	FOS Average visits 1 and 2	82.6±2.0	p>0.05 for all genotypes
		Visit 3	86.8±2.1	
		SOS Average visits 1 and 2	86.6±2.5	
		Visit 3	85.4±1.2	
4a	Serum HDLc (mmol/L)	FOS Average visits 1 and 2	0.43±0.02	p>0.05 for all genotypes
		Visit 3	0.42±0.02	
		SOS Average visits 1 and 2	0.44±0.04	
		Visit 3	0.47±0.05	
4a	Serum TG (mmol/L)	FOS Average visits 1 and 2	2.1±0.4	p>0.05 for all genotypes
		Visit 3	1.9±0.2	
		SOS Average visits 1 and 2	1.7±0.1	
		Visit 3	1.6±0.1	
5	Bleeding time (s)	FOS Average visits 1 and 2	238.2±13.7[a]	p>0.05 for all genotypes
		Visit 3	314.5±17.5[b] (p=0.01)	
		SOS Average visits 1 and 2	271.9±23.5[ab]	
		Visit 3	294.5±19.7[ab]	
6	Serum LDL-CDs (µmol/L)	FOS Average visits 1 and 2	79.2±18.6	p>0.05 for all genotypes
		Visit 3	53.0±15.4 (p=0.21)	
		SOS Average visits 1 and 2	92.1±16.7	
		Visit 3	126.5±43.6	
7	CRP (mg/ dL)	FOS Average visits 1 and 2	9.8±1.5	p>0.05 for all genotypes
		Visit 3	9.5±1.5	
		SOS Average visits 1 and 2	5.7±1.6	
		Visit 3	6.5±2.1	

The values represent mean±SEM. Values not sharing letters are statistically significantly different ($p<0.05$) from each other. Data with no letters are not statistically significantly different ($p>0.05$). Volumes are serum. Abbreviations: CRP – C-reactive protein; DBP – diastolic blood pressure; FOS – flaxseed oil supplementation; HDLc – high-density lipoprotein cholesterol; LDL-CDs – low-density lipoprotein conjugated dienes; SBP – systolic blood pressure; SOS – safflower oil supplementation; TG – triglycerides.

and pathogenesis of the disease based on genotypic analysis and potentially contribute to personalized medicine (Listgarten et al. 2014; Ramos-Lopez 2024).

Some apolipoprotein E genotypes have varying levels of association to certain members of the cluster of seven in T2D (Barre et al. 2024a). However, there have been no studies to our knowledge investigating whether there are statistically significant differences between various apolipoprotein E genotypes in the responsiveness of all members of the cluster of seven to FOS in persons with T2D. The responsiveness to various medications or dietary approaches, when apolipoprotein E genotypic polymorphisms are considered, is inconsistent in terms of which members of the cluster of seven are affected among interventions (Tamasawa et al. 2003; Saito et al. 2004; Tavintharan et al. 2007; Carvalho-Wells et al. 2012; Sapkota et al. 2015; Yassine et al. 2020; Barre et al. 2024b). As well, it is interesting that Han et al. (2018) have shown a flaxseed oil-induced increase in serum HDLc and reduction in serum triglycerides, non-LDL-CDs oxidative stress, and in the CRP-elevating levels of serum tumor necrosis factor-α (TNFα) and interleukin-6 albeit in non-diabetic, apo E knockout mice. However, in this study, even though FOS only significantly increased bleeding time and showed potential for reducing LDL-CDs (Table 1), none of the apolipoprotein E genotypes showed any connection to responsiveness in the cluster of seven, perhaps due to an insufficient number of patients for each genotype. As well, even though LDL oxidation including LDL-CDs drives inflammation (as measured by CRP) (Tan et al. 1999; Lugrin et al. 2014; Dongway et al. 2015; Sproston and Ashworth 2018; Boren et al. 2020), the potential for a decrease in LDL-CDs was not reflected by a significant decrease or potential for a decrease in CRP (Table 1).

Polymorphisms in other genes and other factors contributing to differences in the cluster of seven also need to be examined before reaching any definitive conclusions about the significance of apolipoprotein E alleles in the responsiveness of the cluster to FOS. At the moment, it appears that FOS has no statistically significant impact on abdominal obesity, blood glucose, hypertension, dyslipidemia, oxidation as measured by LDL-CD level, and inflammation as measured by CRP. The absence of change in six of the seven members of the cluster of seven may also reflect that any medications may have exerted an effect that could not be further changed by FOS. Furthermore, a much larger study may reveal one of more apolipoprotein E genotypes that contribute significantly to the responsiveness among the cluster of seven.

This study concluded that there were no statistically significant differences among apolipoprotein E genotypes in responsiveness to FOS of any of the members of the cluster of seven in persons with well-controlled T2D. However, a larger study may show significant differences among apolipoprotein E genotypes in responsiveness to FOS in the cluster of seven.

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