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Various apolipoprotein E genotypes relate to responsiveness to flaxseed lignan complex in older persons with type 2 diabetes mellitus

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Objective. The objective of the study was to determine if there would be statistically significant differences or trends among apolipoprotein E genotypes in the responsiveness of members of a cluster of seven measures in older persons with type 2 diabetes mellitus (T2DM) consuming flaxseed lignan complex (FLC). The cluster of seven are abdominal obesity, hypertension, platelet hyperaggregability, hyperglycemia, dyslipidemia (decreased plasma levels of high-density lipoprotein cholesterol (HDLc), and increased plasma levels of triglycerides), increased low-density lipoprotein (LDL) oxidation and increased inflammation. All cluster members exacerbate T2DM.

Methods. Sixteen patients with well-controlled T2DM participated in this double-blind randomized, placebo-controlled crossover study consisting of four visits. Apolipoprotein E genotyping was done at visit one. The cluster of seven, diet, exercise, smoking and medication use were assessed at each visit.

Results. The 3/4 genotype showed a stronger downward trend in systolic blood pressure compared to the 3/3 genotype with no trend or significant difference in the 2/4 genotype. There was a downward trend in diastolic blood pressure in genotype 3/3 compared genotype 2/4, which showed no significant difference or trend. Only genotype 3/4 showed a significant drop in diastolic pressure compared to genotypes 2/4 and 3/3. HDLc only showed a downward trend in 3/4 relative to genotypes 2/4 and 3/3. LDL apolipoprotein B oxidation (LDL-Box) only showed an upward trend in 3/3 compared to genotypes 2/4 and 3/4. There were no other significant differences or trends by genotype in the cluster of seven.

Conclusions. It appears that those with the 2/4 genotype may not benefit from FLC, those with 3/3 and 3/4 genotypes may benefit only in terms of systolic and diastolic pressures, those with the apo E 3/4 genotype should perhaps avoid FLC to manage HDLc, and those with the 3/3 genotype should perhaps avoid FLC to manage LDL apolipoprotein B oxidation.

Keywords: apolipoprotein E genotype polymorphisms, waist circumference, blood pressure, bleeding time, plasma glucose, high-density lipoprotein cholesterol, triglycerides, low-density lipoprotein conjugated dienes, C-reactive protein, flaxseed lignan complex

Various apolipoprotein E genotypes have been found to contribute to differing plasma lipid and lipoprotein-cholesterol levels (Utermann et al. 1977). However, dyslipidemia in patients with type 2 diabetes mellitus (T2DM) associates with other cluster of seven members (hyperglycemia, hypertension, abdominal obesity, low-density lipoprotein (LDL) oxidation, inflammation, and increased platelet aggregability)

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(Nishigaki et al. 1981; Van Gaal et al. 1989; Tan et al. 1999; Schneider 2009; Tabatabaei-Malazy et al. 2012; Tangvarasittichai 2015; Asghar et al. 2023).

Different apolipoprotein E genotypes have been variously associated with responsiveness to dietary and pharmaceutical interventions in T2DM and hence contribute toward efforts directed at personalized medicine (Tamasawa et al. 2003; Saito et al. 2004; Tavintharan et al. 2007; Barre et al. 2010; Sapkota et al. 2015; Yassine et al. 2020). In that regard, responsiveness to flaxseed lignan complex (FLC) supplementation has never been assessed in terms of apo E genotypes. This paper is a secondary analysis of a previous study using FLC (Barre et al. 2012).

Based on these facts, it was of interest, for the first time, to assess all seven members of the cluster in terms of their responsiveness to FLC supplementation in T2DM patients, collectively, in terms of apolipoprotein E genotypes in those with T2DM. The hypothesis of the current study was that there would be statistically significant differences or trends among apolipoprotein E genotypes for each member of the cluster of seven in older persons with T2DM consuming FLC. The objective of the study was to determine if there would be statistically significant differences or trends among apolipoprotein E genotypes in the responsiveness to FLC of each member of the cluster of seven in older T2DM patients.

Materials and Methods

Patients. Ethics approval was given by the Cape Breton University Human Ethics Committee. Patients with well-controlled T2DM were recruited via a local newspaper advertisement and at their first visit, gave informed consent. Sixteen patients with T2DM (mean age=66 years) completed this double-blind, randomized, cross-over, and placebo-controlled study. Patients consumed four FLC capsules/day (600 mg secoisolariciresinol diglucoside/day) or placebo for 3 months followed by a three-month wash-out period (no exposure to FLC or placebo) followed by three months on that (FLC or placebo) to which they had not been previously exposed in this study.

Blood was taken after a 12–14-h fast at each of the four visits. Patients were asked to keep diet, smoking, medication, and exercise records for three consecutive days every two weeks during the entire trial. Dietary (including alcohol) intake (quantity of each type of food and drink including preparation method per recorded period was analyzed by Food Processor Nutrition and Fitness Analysis Software (ESHA Research, Salem, Oregon, United States)), smoking was assessed by number of each smoking material used per recorded period, medications were assessed by type, dose and frequency per recorded period, and exercise was assessed by intensity and duration for each exercise event and calories expended per recorded period (analyzed using Food Processor Nutrition and Fitness Analysis Software (ESHA Research, Salem, Oregon, United States).

Laboratory methods. Apolipoprotein E genotyping was done by the method of Hixson and Vernier (1990) modified to use 2% agarose gels. Abdominal obesity (as measured by waist circumference) was done by the method of Lemieux et al. (1996), plasma glucose by Wako Glucose C2 kit (Wako USA, Richmond, VA, USA), blood pressures by manual sphygmomanometry, plasma high-density lipoprotein cholesterol (HDLc) by precipitation and enzymatic analysis via the Quantolip HDL kit (Technoclone Vienna, Austria), plasma triglycerides by the L-type triglyceride M kit (Wako USA, Richmond, VA, USA), bleeding time via the Surgicutt kit (ITC, Edison, NJ, USA), plasma LDL conjugated dienes by the method of Ahotupa et al. (1996, 1998), plasma low-density lipoprotein apolipoprotein B oxidation (LDL-Box) using the oxidized LDL ELISA kit (Mercodia, Winston-Salem, North Carolina, USA), and C-reactive protein (CRP) via the human EIA kit (Cayman Chemical, Ann Arbor, MI, USA).

Statistical analyses. A repeated measures analysis of covariance was done for each variable adjusting for age, sex and order of treatment. A Fisher test was done to determine pairwise differences between means for all genotypes.

Results

The results are presented in Table 1 and are discussed in terms of treatment effect relative to placebo. The 3/4 genotype showed only a stronger downward trend in systolic blood pressure compared to the 3/3 genotype with no trend or significant difference in the 2/4 genotype. There was a downward trend in diastolic blood pressure in genotype 3/3 compared genotype 2/4, which showed no significant difference or trend. Only genotype 3/4 showed a significant drop in diastolic pressure compared to genotypes 2/4 and 3/3. HDLc showed only a downward trend in 3/4 relative to genotypes 2/4 and 3/3. LDL-Box showed only an upward trend in 3/3 compared to genotypes 2/4 and 3/4. There were no other significant differences or trends by genotype in the cluster of seven. Diet (including alcohol consumption), physical activity, smoking

and medication use (type and dose) did not change throughout the study.

Discussion

The 3/4 genotype showed only a stronger downward trend in systolic blood pressure compared to the 3/3 genotype with no trend in the 2/4 genotype. This suggests that those with the 3/4 genotype may respond more than the other two genotypes in terms of decreased systolic blood pressure. Compared to non-apolipoprotein E4 carriers, Sapkota et al. (2015) have found that apolipoprotein E4 carriers had significantly lower plasma glucose and systolic blood pressures after certain medications; our results indicated no such associations with FLC.

There was a downward trend in diastolic blood pressure in genotype 3/3 compared to genotype 2/4 which showed no significant difference or trend. Only those with the genotype 3/4 showed a significant drop in diastolic pressure compared to genotypes 2/4 and 3/3. Hence, those with the 3/4 genotype appears to be more sensitive to FLC in terms of diastolic blood pressure lowering. HDLc showed a downward trend for the 3/4 genotype, whereas genotypes 2/4 and 3/3 showed no significant difference or trends suggesting that those with the 3/4 genotype should perhaps avoid FLC in terms of HDLc management. Triglycerides showed no differences or trends by genotype, which is interesting, given the inverse relationships between plasma HDL and triglyceride concentrations (Kontush and Chapman 2008).

However, consistent with our results for triglycerides, Saito et al. (2004) have found no genotypic impact via dietary treatment. Tamasawa et al. (2003) have shown no genotypic distinction on the impact of dietary treatment on HDLc. LDL-Box showed an upward trend in those with genotype 3/3 compared to genotypes 2/4 and 3/4, thus suggesting that those at risk of complications of such oxidation should perhaps avoid FLC. However, this upward trend did not result in an apo E differential genotypic change in CRP; oxidation contributes to increased CRP (Lugrin et al. 2014; Sproston and Ashworth 2018; Boren et al. 2020). However, Carvalho-Wells et al. (2012) have reported 3/4 was best at CRP lowering in response to altered fat intake. There were no other significant differences or trends by genotype in the cluster of seven responsiveness and apparently no other reports of such in the literature.

As diet (including alcohol consumption), physical activity, smoking, and medication use (type and dose) did not change throughout the study, these factors could not have affected the results. It appears that there are no reports regarding the treatment responsiveness distinction among apo E genotypes for LDL-Box, LDL conjugated dienes or bleeding time. To our knowledge, this is the first paper to discuss all members cluster of seven in the context of apolipoprotein E genotypes relative to the responsiveness of the cluster of seven to FLC. It is important to understand relationships between genotype and levels of various factors associated with T2DM so as to better understand the nature of the disease and to suggest possible options for more personalized medicine using FLC.

A larger study would reveal more genotypes and the opportunity for significant differences and/or trends not seen in the current study. As well various

Table 1
Genotypic segregation of treatment effect relative to placebo using Fisher pairwise individual tests for differences of means

Parameter	Genotype 2/4	Genotype 3/3	Genotype 3/4
Glucose	NSD – NT	NSD – NT	NSD – NT
Waist circumference	NSD – NT	NSD – NT	NSD – NT
BP systolic	NSD – NT	NSD – p=0.150 – D	NSD – p=0.084 – D
BP diastolic	NSD – NT	NSD – p=0.167 – D	p=0.009 – D – NT
Triglycerides	NSD – NT	NSD – NT	NSD – NT
HDLc	NSD – NT	NSD – NT	NSD – p=0.180 – D
LDL apolipoprotein B oxidation	NSD – NT	NSD – p=0.112 – I	NSD – NT
LDL conjugated dienes	NSD – NT	NSD – NT	NSD – NT
CRP	NSD – NT	NSD – NT	NSD – NT
Bleeding time	NSD – NT	NSD – NT	NSD – NT

Abbreviations: BP – blood pressure; CRP – C-reactive protein; HDLc – high-density lipoprotein cholesterol; LDL – low-density lipoprotein; D – treatment decrease relative to placebo; I – treatment increase relative to placebo; NSD – no significant difference; NT – no trend; SD – significant difference; T – trend. Significant differences are at $p<0.05$. Trends are at $p=0.05$ to $p<0.20$.

genotypes in other genes need to be examined along with other factors affecting members of the cluster before reaching any definitive conclusions about the significance of apoprotein E alleles in the responsiveness of the cluster to FLC.

In conclusion, it appears that those with the 2/4 genotype may not benefit from FLC, those with the 3/3 and 3/4 genotype may benefit only in terms of systolic and diastolic pressures, those with the apo 3/4 genotype should perhaps avoid FLC to manage HDLc, and those with the 3/3 genotype should perhaps avoid FLC to manage LDL apolipoprotein B oxidation.

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