Clinical Nutrition ESPEN 10 (2015) e155–e159

Contents lists available at ScienceDirect

Clinical Nutrition ESPEN

journal homepage: http://www.clinicalnutritionespen.com

# A randomized trial of the effects of ezetimibe on the absorption of omega-3 fatty acids in cardiac disease patients: A pilot study

D.P. Blackwood [a, b], R.K. LaVallée [a], A. Al Busaidi [a, b], D.S. Jassal [a, c], G.N. Pierce [a, b, *]

[a] Canadian Centre for Agri-food Research in Health and Medicine, Institute of Cardiovascular Sciences, St. Boniface Hospital, Canada
[b] Department of Physiology, Faculties of Medicine and Pharmacy, University of Manitoba, Winnipeg, Canada
[c] Department of Internal Medicine, Faculties of Medicine and Pharmacy, University of Manitoba, Winnipeg, Canada

ARTICLE INFO

*Article history:*
Received 3 December 2014
Accepted 18 July 2015

*Keywords:*
Polyunsaturated fatty acids
Alpha linolenic acid
Flaxseed
Ezetimibe
Cholesterol
Food–drug interaction

SUMMARY

*Background and aims:* Elevated levels of circulating omega-3 polyunsaturated fatty acids like alpha linolenic acid (ALA) may be beneficial for cardiovascular health. Circulating ALA concentrations are elevated dramatically by a cholesterol supplemented diet which increases ALA bioavailability through enhanced micelle formation in the intestines. Conversely, it is possible that drugs which inhibit cholesterol metabolism in the intestine may also inhibit fatty acid absorption. The purpose of this study is to determine if a cholesterol absorption inhibitor, ezetimibe, will decrease circulating levels of ALA.
*Methods and results:* Cardiac patients (n = 34) between 44 and 80 years old, requiring statin therapy to regulate blood cholesterol levels, were randomly assigned to one of four groups for a 6 week trial: 1) placebo; 2) ezetimibe therapy; 3) a supplement of flaxseed oil (containing 1.0 g ALA in 2.0 g of flaxseed oil); or 4) ezetimibe and flaxseed oil supplementation. Ingestion of flaxseed oil resulted in a significant increase in circulating ALA levels (6 ug/dl) in patients who were not given ezetimibe. However, in the presence of ezetimibe, circulating ALA levels did not increase significantly even in the presence of flax oil supplementation (a decrease of 4 ug/dl). There were no significant differences amongst the groups in terms of circulating total cholesterol, LDL, HDL, triglyceride levels in the blood.
*Conclusion:* Ezetimibe therapy inhibited the absorption of omega-3 fatty acids. Patients receiving ezetimibe therapy may not receive the expected cardiovascular benefits from dietary supplementation with omega-3 fatty acids.
Clinical Trial Registration: NCT00955227.


## 1. Introduction

Cardiovascular disease (CVD) remains the leading cause of death worldwide [1]. While pharmacotherapy has become the leading primary therapy for the treatment of various cardiovascular diseases, there is a recent growing interest in elucidating the effects of natural health products for the prevention and treatment of CVD. One kind of natural health product which has demonstrated desirable effects for the treatment of CVD is omega-3 fatty acids. Alpha linolenic acid (ALA) obtained from flaxseed is an omega-3 fatty acid that possesses several cardioprotective properties in animal studies. This includes anti-atherogenic, anti-inflammatory and anti-arrhythmic capabilities and it is protective against vascular dysfunction [2–4]. Many of these cardioprotective effects have been demonstrated in human populations as well [5,6]. Furthermore, several human trials have shown a correlation of circulating ALA levels with a reduction in both fatal and non-fatal myocardial infarction [7–9]. The Lyon heart study [9], for example, found that a diet rich in ALA was associated with a significantly lower rate of recurrence of the major adverse cardiac events of cardiac death or non-fatal myocardial infarction.

If ALA is important to our cardiovascular health for primary and/or secondary prevention, then understanding factors that influence its bioavailability are critical. Previous studies have demonstrated that 2–4 weeks were required to observe an increase in ALA levels in the blood in response to a dietary supplementation with ALA [10,11]. ALA was best absorbed from the diet when ingested as flaxseed oil in comparison to whole or

* Corresponding author. St. Boniface Hospital Research Centre, 351 Tache Ave., Winnipeg, Manitoba R2H 2A6, Canada. Tel.: +1 (204) 235 3206; fax: +1 (204) 235 0793.
*E-mail address:* gpierce@sbrc.ca (G.N. Pierce).

http://dx.doi.org/10.1016/j.clnesp.2015.07.002
2405-4577/

ground seed [11]. The age of the person does not appear to influence ALA bioavailability [12]. However, in animal studies, an increase in dietary cholesterol facilitated a dramatic increase in circulating ALA concentrations. It has been postulated that this effect may be due to a cholesterol-mediated increase in micelle formation in the gut [2–4,13].

If increasing the levels of cholesterol in the gut will increase omega-3 fatty acid absorption, then it is possible that the opposite is also true: inhibiting dietary cholesterol absorption in the intestine will interfere with ALA absorption. Ezetimibe is a cholesterol-lowering drug that is commonly used to treat patients at risk for CVD. Ezetimibe inhibits cholesterol absorption in the human intestine. It selectively targets and inhibits the Niemann-Pick C1-like 1 (NPC1L1) receptor [13]. The NPC1L1 is located in the jejunal intestinal brush border and is the primary regulator of sterol transport from the gut lumen to within enterocytes [14]. As a result, ezetimibe can lower LDL cholesterol, either as a monotherapy or in conjunction with statin pharmacotherapy. Clinically, ezetimibe may be utilized as an add-on therapy to statin pharmacotherapy when additional lipid lowering or a lower statin dosing is required [15–17]. While ezetimibe can reduce LDL cholesterol, several trials have demonstrated ezetimibe's failure to offer a superior reduction of clinical endpoints such as atherosclerotic lesions or mortality [18–20]. It is possible that this failure is due to other negative side effects that ezetimibe may have on risk factors associated with CVD. Recent emerging data from the IMProved Reduction of Outcomes: Vytorin Efficacy International Trial (IMPROVE-IT) study suggests a combination therapy of simvastatin and ezetimibe may lead to a 6.4% reduction in adverse cardiovascular events. There was no lowering of mortality rates compared to statin therapy alone, however [21]. Thus, the true role of ezetimibe in effective prevention and treatment of cardiovascular disease remains intriguing but unclear.

The purpose of our study was to determine if ezetimibe therapy will alter the bioavailability of the beneficial omega-3 fatty acid, ALA. It is hypothesized that ezetimibe administration to cardiac patients with CVD will attenuate the rise in circulating ALA levels in response to ALA supplementation with flax oil.

## 2. Methods

### 2.1. Study population

This clinical trial was registered at clinicaltrials.gov (NCT00955227). Cardiac patients were recruited from the Bergen Cardiac Centre at St. Boniface Hospital. Male and female subjects aged 18–80 years old were included in the study. Patients were further included/excluded from the study based on several criteria. For inclusion in this study, patients were required to be undergoing statin therapy at the time of initial screening. Furthermore, patients were required to attend two study visits (baseline and six weeks) and donate 10 ml of blood. Patients were also required to adhere to dietary restrictions as described below. The majority of subjects enrolled in the study had experienced a prior acute cardiac or cerebrovascular event and were undergoing aspirin, ACEi/ARB and beta-blocker therapy for the secondary prevention of CVD at time of enrollment. From the 34 subjects, 6 had diabetes (17.65%) (4 took drugs to control the diabetes, 1 controlled glucose levels with diet and 1 was pre-diabetic). All patients were undergoing statin pharmacotherapy (25 were medicated with Lipitor from 10 to 80 mg), 6 took Crestor (from 10 to 40 mg), 2 were taking Zocor (both 40 mg) and 1 person was taking Pravachol (20 mg).

### 2.2. Study design

This was a four arm, parallel group, randomly controlled, open-label clinical trial. The study design was approved by the University of Manitoba Research Ethics Board and the St. Boniface Hospital Research Review Committee. Cardiac patients were randomized by computer program into one of four treatment groups (all patients were maintained on statin therapy and all other regular medications throughout the study): 1) control (statins alone), or 2) plus 10 mg ezetimibe pharmacotherapy, or 3) plus 2 capsules of flax oil (containing a total of 1 g ALA in 2 g of flaxseed oil), or 4) plus ezetimibe and flax oil treatment for a total of 6 weeks. Over the time period, patients receiving flax oil ingested a total of 42 g ALA. Ezetimibe was taken orally (10 mg/day). The timing of the ingestion was not controlled. Each subject provided written informed consent prior to beginning the study. The study schedule included two blood draws, one at baseline and one at follow-up after six weeks of treatment. At each appointment, medical history, height, weight and blood were collected from each patient (Fig. 1).

Subjects were fasted for 12 h prior to their blood draw. During treatment, subjects were not allowed to ingest oils or salad dressings containing oils, as well as seafood. In addition, subjects were required to abstain from any additional source of omega-3 fatty acid supplementation. Subjects who had been ingesting omega-3 supplementation prior to beginning the study were required to undergo a one month period of abstinence from omega-3 ingestion prior to beginning treatment. Subjects were free to withdraw from participation at any time, for any reason, without penalty. This study was conducted on a volunteer basis. No reward (financial or otherwise) was given for participation.

### 2.3. Study procedures

#### 2.3.1. Blood analysis

During each visit, 10 ml of blood was collected by venipuncture in tubes containing 1 mg EDTA/ml from subjects who had fasted for 12 h. Blood samples were centrifuged at 1000× *g* at 4 °C for 10 min, and plasma was then stored at −80 °C until analysis at a later date.

#### 2.3.2. Preparation of plasma fatty acid Methyl esters

Plasma fatty acids methyl esters were measured by gas chromatography coupled with flame ionization detection as described in detail elsewhere [2–4]. In summary, plasma samples were derivatized directly to fatty acid methyl esters using 4:1 methanol:toluene as described by Lepage and Roy [22]. A Varian CP-3800 GC w/flame ionization detector and CP-Sil 88 capillary column 60 m × 0.25 mm × 0.20 μm GC apparatus was used for analysis. The oven temperature was maintained at 111 °C for 1 min, increased by 20 °C/min to 170 °C, raised again by 5 °C/min to 190 °C and finally increased by 3 °C/min to 225 °C. It was maintained at that temperature for 10 min, for a total run time of 29.62 min. Equipment was standardized using GLC 462 (Nu-Chek Prep, Inc.). The internal standard used was C19:0 (Nu-Check Prep, Inc.).

#### 2.3.3. Cholesterol and triglyceride assays

Commercial assay kits (Thermo Electron Corporation, Waltham, MA) were used to determine total cholesterol and triglyceride levels in plasma. A separate assay kit (Biovision Inc., Mountain View, CA) was used to analyze plasma HDL levels. The remaining fraction of LDL/VLDL was calculated from total cholesterol and HDL measured values, as per assay protocol.

#### 2.3.4. Adverse effects monitoring

During the six week follow-up visit, secondary effects associated with the treatments were monitored via questionnaire.

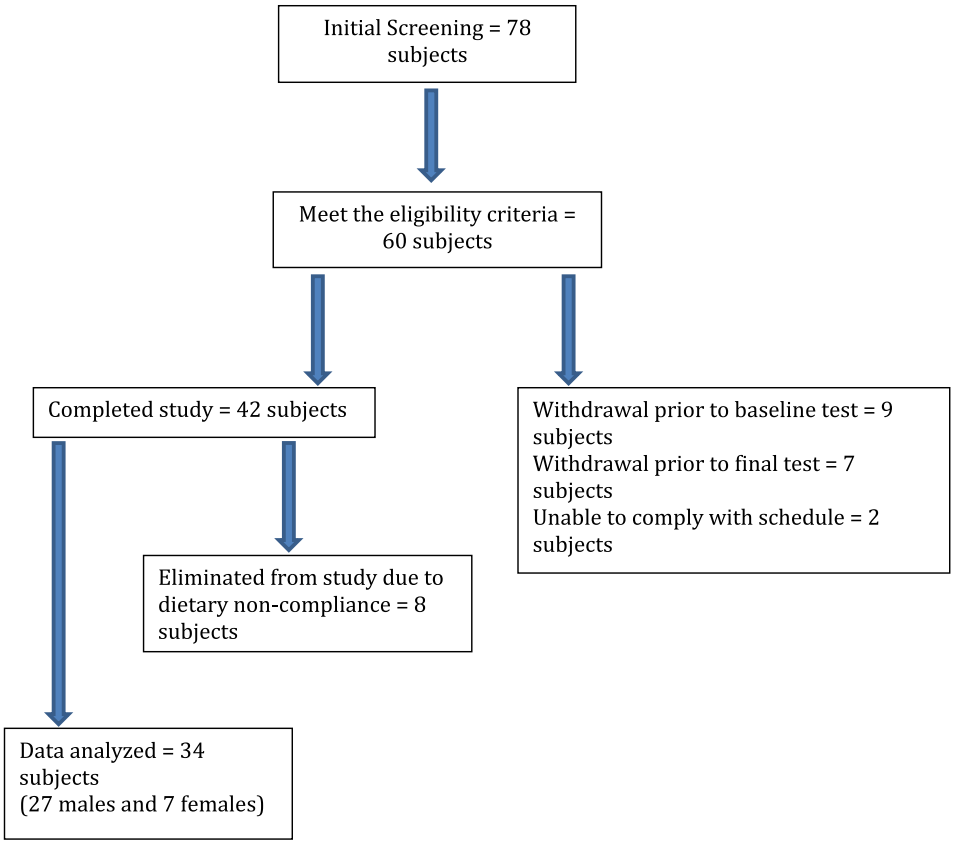


**Fig. 1.** Subject treatment schedule.

### 2.4. Statistical analysis

Statistical significance was determined with a two-way ANOVA using SAS® software (Sigma Stat, SPSS Science Inc., Chicago, IL, USA). Subjects were included in the statistical analysis for efficacy if they had donated two blood draw samples and were compliant with the dietary restrictions as instructed.

## 3. Results

In total, 78 subjects were screened for this trial. Those that did not meet the eligibility criteria or were unable to commit to the study schedule or dietary restrictions were excluded from analysis. The study was completed successfully by 42 of 44 subjects. Two patients were unable to comply with the study schedule and eight additional subjects were removed from the study at the time of data analysis due to non-compliance to dietary restrictions as determined by pill count and/or lipid profile. In total, data was analyzed from 34 patients. No major adverse effects were reported during the course of this study.

### 3.1. Subject cohort characteristics: gender, age and body mass index

Out of the 34 subjects who successfully completed the study, 28 were male and 6 were female. Patients enrolled in the study ranged from 44 to 78 years old and the mean age of the overall subject cohort was 61 ± 11 years old. There were no statistically significant differences between the mean age or body mass index amongst any of the four treatment groups over the six week treatment period (Table 1).

### 3.2. Cholesterol levels

No statistically significant changes in triglycerides, plasma total cholesterol, low-density lipoprotein cholesterol or high-density lipoprotein cholesterol values between 0 and 6 weeks, in either individuals or between groups, were detected during the study duration (Table 2). There was a clear trend to a decrease in total and LDL cholesterol at 6 weeks. However, this was not significant.

### 3.3. Plasma fatty acid levels

After six weeks of treatment, subjects who received flaxseed oil supplementation alone showed a significant increase in plasma ALA concentrations (Fig. 2). However, the administration of ezetimibe blocked this increase. Circulating ALA levels were not significantly different between control and ezetimibe, or between the control and the ezetimibe plus flax oil treatment. The presence of ezetimibe significantly attenuated the amount of circulating ALA compared to flax supplementation alone. This effect was selective for ALA and did not induce changes in other fatty acids (Table 3).

However, there was a trending decrease in all fatty acid levels in the presence of ezetimibe (as has been shown in animal work [4] which varied intestinal cholesterol levels). A larger sample size in future trials may reveal statistically significant differences.

## 4. Discussion

As expected, supplementing the subjects' diet with flaxseed oil alone provided a significant increase in circulating ALA levels. Previous trials in healthy volunteers have shown this dosage of flax oil and the duration of the intervention used here would result in

**Table 1**
Age, gender and BMI characteristics of subjects enrolled in this study.

| Group | Control (n = 8) | Ezetimibe (n = 9) | Flax (n = 9) | Ezetimibe + Flax (n = 8) |
|---|---|---|---|---|
| Male/Female | 5/3 | 9/0 | 6/3 | 6/2 |
| Age (years) | 61 ± 13 | 64 ± 11 | 65 ± 10 | 58 ± 11 |
| Baseline BMI (kg/m$^2$) | 26 ± 7 | 30 ± 6 | 28 ± 4 | 30 ± 6 |
| 6 Week BMI (kg/m$^2$) | 27 ± 7 | 31 ± 6 | 29 ± 4 | 30 ± 6 |

Values represented as means ± SD. No statistically significant differences.

**Table 2**
Total serum cholesterol, HDL, LDL/VLDL fractional concentrations of each treatment group over six weeks of intervention.

| Group | Control | | Ezetimibe | | Flax | | Ezetimibe + Flax | |
|---|---|---|---|---|---|---|---|---|
| | Baseline | 6 Weeks | Baseline | 6 Weeks | Baseline | 6 Weeks | Baseline | 6 Weeks |
| Total cholesterol | 149 ± 23 | 117 ± 10 | 142 ± 17 | 123 ± 19 | 159 ± 21 | 152 ± 19 | 122 ± 18 | 117 ± 13 |
| HDL fraction | 25 ± 8 | 29 ± 5 | 31 ± 4 | 35 ± 6 | 39 ± 6 | 36 ± 6 | 35 ± 9 | 38 ± 8 |
| LDL/VLDL fraction | 124 ± 20 | 89 ± 11 | 114 ± 12 | 79 ± 13 | 131 ± 16 | 119 ± 16 | 88 ± 11 | 80 ± 8 |

Values represented as mean ± SE in mg/dL. No statistically significant differences.

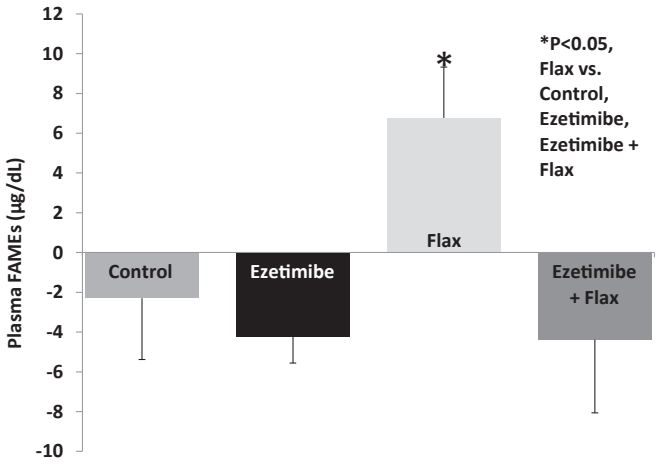


**Fig. 2.** Change in subjects' plasma concentration of ALA following six weeks of treatment. Values represent mean ± SEM. *P < 0.05 vs all other groups.

this increase in ALA bioavailability [10–12]. This information, however, is now extended to a patient population with clinical symptoms of heart disease, and requiring statin pharmacotherapy for either primary or secondary prevention.

Supplementation of the diet with cholesterol simultaneously with flaxseed can result in a marked 120 fold increase in ALA levels in the blood [4,23]. These studies have suggested that the enhanced micelle formation in the intestine induced by the additional dietary cholesterol can facilitate ALA transport across the intestinal wall and can result in elevated ALA levels in the blood [24]. If the intestinal cholesterol load is important in stimulating ALA entry, then it is possible that the corollary is true: disturbing intestinal cholesterol will inhibit ALA absorption.

Ezetimibe blocks the NPC1L1 transporter protein on the intestinal brush border preventing sterol transport from the gut lumen into circulation [14,23]. Although fatty acid absorption does not occur directly via the NPC1L1 transporter, there is a possibility that a decrease in cholesterol absorption/micelle interaction with the intestinal brush border may lead to an overall decrease in ALA being absorbed from the gut. As hypothesized, ezetimibe therapy significantly lowered the absorption of ALA compared to flax oil supplementation alone. Flax oil supplementation was unable to significantly raise circulating ALA levels in the presence of ezetimibe. This drug–food interaction represents the primary finding of this study. Little is currently known about the effects of pharmaceuticals or other naturally occurring products on the absorption of ALA. This study provides the first clinical evidence of the interactions between ALA supplementation and ezetimibe. This finding further questions ezetimibe's overall efficacy for the prevention of cardiovascular disease. Not only may ezetimibe lack pleiotropic effects as compared to statins [25], but it may also elicit deleterious effects on the absorption of other cardioprotective substances like lipid soluble vitamins whose absorption is facilitated by cholesterol uptake [26].

The conclusions obtained from this study are to be interpreted with caution due to the relatively small sample size. Understandably, the small study population number renders the study unsuitable to extrapolate end points such as lipid lowering effects or overall cardiovascular risk of each treatment group to a larger

**Table 3**
Plasma fatty acid concentrations of groups over six weeks of intervention.

| Fatty acid | Control | | Ezetimibe | | Flax | | Ezetimibe + Flax | |
|---|---|---|---|---|---|---|---|---|
| | Baseline | 6 Weeks | Baseline | 6 Weeks | Baseline | 6 Weeks | Baseline | 6 Weeks |
| 16:0 | 634.1 ± 103.4 | 644.7 ± 88.7 | 543.4 ± 52.5 | 480.0 ± 49.4 | 596.2 ± 88.5 | 644.3 ± 69.2 | 620.7 ± 52.3 | 577.0 ± 68.5 |
| 18:0 | 200.3 ± 23.3 | 199.2 ± 18.2 | 182.1 ± 18.3 | 169.3 ± 14.5 | 199.3 ± 28.0 | 213.9 ± 23.6 | 200.9 ± 16.1 | 188.8 ± 18.9 |
| 16:1 | 62.4 ± 22.5 | 54.1 ± 16.0 | 46.5 ± 10.5 | 43.1 ± 12.8 | 51.6 ± 15.8 | 55.8 ± 14.7 | 56.8 ± 9.6 | 61.3 ± 15.0 |
| 18:1 | 798.0 ± 116.5 | 743.2 ± 95.5 | 642.4 ± 63.2 | 598.4 ± 68.3 | 751.9 ± 88.4 | 812.0 ± 85.7 | 783.1 ± 70.5 | 706.1 ± 84.6 |
| 18:2*n*-6 | 607.7 ± 72.7 | 606.4 ± 41.3 | 682.2 ± 47.8 | 545.1 ± 32.6 | 647.8 ± 59.1 | 705.0 ± 60.5 | 759.1 ± 80.0 | 571.2 ± 70.8 |
| 20:4*n*-6 | 220.0 ± 27.4 | 216 ± 31.0 | 207.9 ± 19.8 | 198.7 ± 15.8 | 211.9 ± 26.7 | 232.8 ± 26.5 | 231.4 ± 28.3 | 236.7 ± 26.6 |
| 20:5*n*-3 | 15.6 ± 3.9 | 14.3 ± 4.1 | 17.6 ± 3.0 | 13.7 ± 2.3 | 18.4 ± 4.0 | 20.5 ± 4.6 | 14.5 ± 4.1 | 23.0 ± 4.3 |
| 22:5 | 11.2 ± 1.9 | 18.6 ± 7.3 | 9.9 ± 1.5 | 8.1 ± 1.5 | 12.2 ± 1.9 | 13.7 ± 2.3 | 13.0 ± 2.0 | 14.6 ± 2.5 |
| 22:6 | 38.7 ± 4.5 | 39.4 ± 3.9 | 41.8 ± 3.9 | 33.6 ± 2.7 | 45.4 ± 4.7 | 42.5 ± 4.6 | 43.4 ± 5.6 | 36.9 ± 5.3 |

Values represented as means ± SE in µg/dL. No statistically significant differences.

population. There were no significant effects observed in the lipid profile or CVD biomarkers. Though there is a decreasing trend, no significant change in triglyceride or cholesterol levels were observed. This may be attributed to the relatively normal values obtained in these parameters from the patient cohort, indicating good control of circulating cholesterol with statin treatment. Indeed, the lack of fluctuation in these levels over the six week treatment span provides additionally robust evidence for the hypothesis that changes in gut cholesterol absorption are responsible for the observed changes in circulating ALA levels and they are not due to fluctuating levels of circulating cholesterol or triglycerides.

The present results have implications for patients that are administered any agent that inhibits cholesterol levels in the gut. It is also unknown if other drugs (i.e. cholestyramine, colestipol, etc) or foods (i.e. phytosterols, dietary fiber, etc) that inhibit cholesterol absorption from the intestinal tract may also inhibit ALA absorption. With the growing use of plant sterols in our diet, this is an important consideration. It is also unclear if the effects observed in the present study extend to other forms of fatty acids supplements like fish oil or to their polyunsaturated fatty acid components like DHA and EPA. Based on the foregoing discussion of the mechanism of action involved in the inhibition of ALA bioavailability, it is entirely likely that the bioavailability of these fatty acids will also be inhibited by drugs that interfere with intestinal cholesterol.

The findings of this study, therefore, although preliminary in nature, should encourage larger investigations. Further research elucidating the effects of ezetimibe (and other compounds that affect intestinal cholesterol) on the absorption of other healthy nutrients and, in general, the interaction between sterol and fatty acid intestinal absorption in humans is warranted. These effects are of importance to the CVD patient who may be spending money unnecessarily on these dietary supplements with the assumption that they will receive some cardiovascular benefit.

## Sources of support

This trial is funded by the CIHR and St. Boniface Hospital Research Foundation.

## Acknowledgments

The authors would like to acknowledge A. Austria for his technical assistance during this study. No author reports any conflict of interest regarding this study. DS Jassal and GN Pierce were responsible for the research design. A Al Busaidi, RK LaVallee and DP Blackwood were responsible for the conduct of research and data analysis. DP Blackwood also wrote the manuscript and GN Pierce holds primary responsibility for this study.

## References

[1] Genest J, McPherson R, Frohlich J, Anderson T, Campbell N, Carpentier A, et al. 2009 Canadian Cardiovascular Society/Canadian guidelines for the diagnosis and treatment of dyslipidemia and prevention of cardiovascular disease in the adult – 2009 recommendations. Can J Cardiol 2009;25:567–79.

[2] Rodriguez-Leyva D, Dupasquier CM, McCullough R, Pierce GN. The cardiovascular effects of flaxseed and its omega-3 fatty acid, alpha-linolenic acid. Can J Cardiol 2010;26:489–96.

[3] Ander BP, Weber AR, Rampersad PP, Gilchrist JS, Pierce GN, Lukas A. Dietary flaxseed protects against ventricular fibrillation induced by ischemia-reperfusion in normal and hypercholesterolemic rabbits. J Nutr 2004;134:3250–6.

[4] Dupasquier CM, Weber AM, Ander BP, Rampersad PP, Steigerwald S, Wigle JT, et al. Effects of dietary flaxseed on vascular contractile function and atherosclerosis during prolonged hypercholesterolemia in rabbits. Am J Physiol Heart Circ Physiol 2006;291:H2987–96.

[5] Paschos GK, Magkos F, Panagiotakos DB, Votteas V, Zampelas A. Dietary supplementation with flaxseed oil lowers blood pressure in dyslipidaemic patients. Eur J Clin Nutr 2007;61:1201–6.

[6] Patade A, Devareddy L, Lucas EA, Korlagunta K, Daggy BP, Arjmandi BH. Flaxseed reduces total and LDL cholesterol concentrations in native American postmenopausal women. J Womens Health Larchmt 2008;17:355–66.

[7] Burr ML, Fehily AM, Gilbert JF, Rogers S, Holliday RM, Sweetnam PM, et al. Effects of changes in fat, fish, and fibre intakes on death and myocardial reinfarction: diet and reinfarction trial (DART). Lancet 1989;2:757–61.

[8] Albert CM, Oh K, Whang W, Manson JE, Chae CU, Stampfer MJ, et al. Dietary alpha-linolenic acid intake and risk of sudden cardiac death and coronary heart disease. Circulation 2005;112:3232–8.

[9] de Lorgeril M, Salen P, Martin JL, Monjaud I, Delaye J, Mamelle N. Mediterranean diet, traditional risk factors, and the rate of cardiovascular complications after myocardial infarction: final report of the Lyon Diet Heart Study. Circulation 1999;99:779–85.

[10] Kaul N, Kreml R, Austria JA, Landry MN, Edel AL, Dibrov E, et al. A comparison of fish oil, flaxseed oil and hempseed oil supplementation on selected parameters of cardiovascular health in healthy volunteers. J Am Coll Nutr 2008;27:51–8.

[11] Austria JA, Richard MN, Chahine MN, Edel AL, Malcolmson LJ, Dupasquier CMC, et al. Bioavailability of alpha-linolenic acid in subjects after ingestion of three different forms of flaxseed. J Am Coll Nutr 2008;27:214–21.

[12] Patenaude A, Rodriguez-Leyva D, Edel AL, Dibrov E, Dupasquier CMC, Austria JA, et al. Bioavailability of alpha-linolenic acid from flaxseed diets as a function of the age of the subject. Eur J Clin Nutr 2009;63:1123–9.

[13] Pharma MF-S. Ezetrol (ezitimibe ) product Monograph. Kirkland, QC. 2009.

[14] Davis Jr HR, Basso F, Hoos LM, Tetzloff G, Lally SM, Altmann SW. Cholesterol homeostasis by the intestine: lessons from Niemann-Pick C1 like 1 [NPC1L1. Atheroscler Suppl 2008;9:77–81.

[15] Kishimoto M, Sugiyama T, Osame K, Takarabe D, Okamoto M, Noda M. Efficacy of ezetimibe as monotherapy or combination therapy in hypercholesterolemic patients with and without diabetes. J Med Invest 2011;58:86–94.

[16] Hing Ling PK, Civeira F, Dan AG, Hanson ME, Massaad R, De Tilleghem Cle B, et al. Ezetimibe/simvastatin 10/40 mg versus atorvastatin 40 mg in high cardiovascular risk patients with primary hypercholesterolemia: a randomized, double-blind, active-controlled, multicenter study. Lipids Health Dis 2012;11:18.

[17] Mauro VF, Tuckerman CE. Ezetimibe for management of hypercholesterolemia. Ann Pharmacother 2003;37:839–48.

[18] Villines TC, Stanek EJ, Devine PJ, Turco M, Miller M, Weissman NJ, et al. The ARBITER 6-HALTS trial (arterial biology for the investigation of the treatment effects of reducing cholesterol 6-HDL and LDL treatment strategies in atherosclerosis): final results and the impact of medication adherence, dose, and treatment duration. J Am Coll Cardiol 2010;55:2721–6.

[19] Bang CN, Greve AM, Boman K, Egstrup K, Gohlke-Baerwolf C, Køber L, et al. Effect of lipid lowering on new-onset atrial fibrillation in patients with asymptomatic aortic stenosis: the simvastatin and ezetimibe in aortic stenosis (SEAS) study. Am Heart J 2012;163:690–6.

[20] Rizzo M, Battista Rini G. Ezetimibe, cardiovascular risk and atherogenic dyslipidaemia. Arch Med Sci 2011;7:5–7.

[21] AHA/ASA Newsroom. Cholesterol-lowering drug with different action adds to statin's reduction of cardiovascular risk. 2014. http://newsroom.heart.org/news/cholesterol-lowering-drug-with-differnt-actions-to-statins-reduction-of-cardiovascular-risk?ct=t%28IAS_e_Newsletter_and_e_literature_June_2014_issue_%29.

[22] Lepage G, Roy CC. Direct transesterification of all classes of lipids in a one-step reaction. J Lipid Res 1986;27:114–20.

[23] Dupasquier CM, Dibrov E, Kneesh AL, Cheung PKM, Lee KGY, Alexander HK, et al. Dietary flaxseed inhibits atherosclerosis in the LDL receptor-deficient mouse in part through antiproliferative and anti-inflammatory actions. Am J Physiol Heart Circ Physiol 2007;293:H2394–402.

[24] Thomson AB. Influence of dietary modifications on uptake of cholesterol, glucose, fatty acids, and alcohols into rabbit intestine. Am J Clin Nutr 1982;35:556–65.

[25] Kalogirou M, Tsimihodimos V, Elisaf M. Pleiotropic effects of ezetimibe: do they really exist? Eur J Pharmacol 2010;633:62–70.

[26] Reboul E, Borel P. Proteins involved in uptake, intracellular transport and basolateral secretion of fat-soluble vitamins and carotenoids by mammalian enterocytes. Prog Lipid Res 2011;50:388–402.