LDL-C Response to Portfolio Foods Containing High Levels of Phytosterols, Whole Food Fiber, and Alpha-Linolenic Acid in Statin Reluctant Patients: Impact of CYP7A1-rs3808607 and APOE Isoforms

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INTRODUCTION

- HMG-CoA reductase inhibitor (statin) therapy is widely utilized as part of primary and secondary prevention efforts for coronary heart disease. However, in clinical practice up to 20% of outpatients receiving statins experience treatment reluctance on the basis of side effects, especially muscle pain.
- Despite the recognition that dietary solutions can ameliorate cardiovascular disease (CVD) risk in statin resistant patients, no studies have been undertaken to explore the use of a portfolio of healthy, appetizing foods with functional food ingredients, in improving cardiovascular health in statin reluctant patients.
- Clinical trials by De Castro-Oros et al (2011), MacKay DS et al (2015) and Wang Y et al (2016), suggests varied cholesterol up-take and metabolizing abilities by CYP7A1-rs3808607 and APO-E variants. These studies demonstrated that CYP7A1-rs3808607 and APOE heterogeneity affects variability in response to phytosterol or fiber intake in low-density lipoprotein cholesterol (LDL-C) or total cholesterol (TC) lowering.
- We hypothesized that a practical food-based approach can be utilized to lower LDL-C in statin reluctant patients and that the lipid response can be predicted based upon CYP7A1-rs3808607 and APOE genetic isoforms.

OBJECTIVES

- To investigate the effect of a range of hedonically acceptable proprietary food products specifically formulated in positively influencing cholesterol levels in statin-reluctant individuals.
- To evaluate the changes in serum LDL-C, triglyceride (TG), high density lipoprotein cholesterol (HDL-C), TC and glucose concentrations over a 4-week regimen using foods rich in fiber, phytosterols, alpha-linolenic acid and antioxidants.
- To correlate heterogeneity in LDL-C response to CYP7A1 and APOE isoforms.

CLINICAL TRIAL DESIGN

- A multicenter, randomized, double-blind, free-living cross-over study was comprised of 2 regimented phases of 4 weeks each, separated by a 4-week washout.
- The participants received an assortment of packaged, ready-to-eat, shelf-stable food products along with printed instructions to ingest two servings of the foods per day, without any other modifications in their diet or lifestyle.
- Treatment products consisted of oatmeal, pancakes, cranberry bars, chocolate bars, and smoothies formulated specifically to provide a minimum of 5 g of fiber, 1000 mg of omega-3 fatty acids, 1000 mg of phytosterols and 1800 µmol antioxidants per serving.
- Control products were calorie-matched like-items drawn from the general grocery marketplace.

METHODS

- Serum lipid profile (total cholesterol, HDL-C, and TG) was measured using a Vitros Chemistry System 350 (Ortho-Clinical Diagnostics, Johnson & Johnson). LDL-C was calculated using the Friedewald equation. Average values of days 1 and 2 were used as baseline, and averages of days 29 and 30 were considered as endpoint values. Ingestion of foods was confirmed by C18:3n3 serum level assessment.
- Genomic DNA was extracted from white blood cells by using a column based DNA extraction kit (DNeasy Blood and Tissue Kit, QIAGEN Sciences) and integrity of the genomic DNA was assessed by micro-volume spectrophotometer (NanoDrop 2000,Thermo Fisher Scientific).
- DNA samples were genotyped by TaqMan SNP genotyping assay (Life Technologies, Burlington, ON) on a StepOnePlus Real-Time PCR System (Applied Biosystems, Life Technologies, Burlington, ON).
- Statistical analyses were performed with statistical software, SAS using a mixed model ANOVA procedure for endpoint-to-endpoint analysis.

Figure 1. Clinical trial design

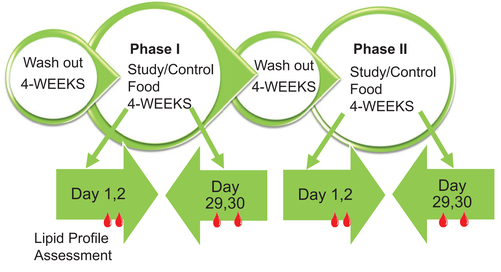
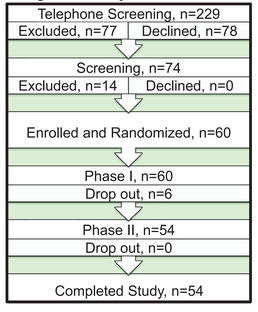


Table 1. Baseline characteristics

Parameter	n=54 (mmol/l, mg/dl)
Glucose	5.28±0.47, 95.04± 8.46
Cholesterol	5.65±0.95, 218.5±36.7
Triglycerides	1.76±0.93, 155.9±82.4
HDL-Cholesterol	1.46±0.36, 56.5±13.9
LDL-Cholesterol	3.38±0.83, 130.7±32.1

Values in Means ± Standard Deviation (Means ± SD)

Figure 2. Study flow



RESULTS

Table 2. Lipid profile and glucose levels in response to study foods: treatment compared to control

Parameter	Control n=54 (mmol/l, mg/dl)	Treatment n=54 (mmol/l, mg/dl)	P value	Average percent difference
Glucose	5.39±0.07 97.0±1.26	5.38±0.07 96.8±1.26	0.8214	-0.17%
Cholesterol	5.55±0.13 214.6±5.03	5.28±0.13 204.2±5.03	<.0001	-5.08%
Triglycerides	1.84±0.13 162.9±11.51	1.85±0.13 163.9±11.51	0.8188	0.32%
HDL-Cholesterol	1.42±0.04 54.9±1.55	1.43±0.04 55.3±1.55	0.5524	0.95%
LDL-Cholesterol	3.28±0.11 126.8±4.25	3.00±0.11 116.0±4.25	<.0001	-8.8%

Values in LS-means ± SE. If p<0.05, significantly different

Figure 3. Serum lipid profile responsiveness to study foods compared to control

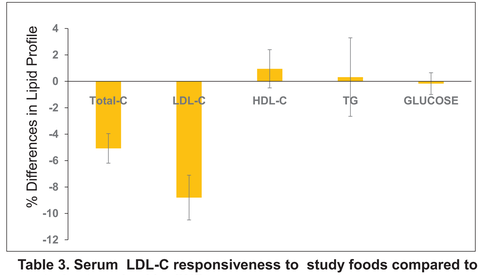


Figure 4. Individual responsiveness in LDL-C to study foods compared to control

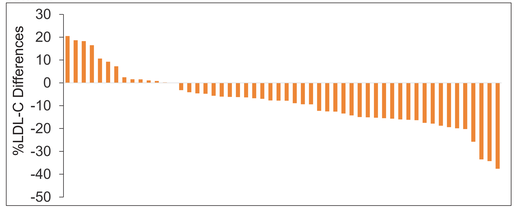


Table 3. Serum LDL-C responsiveness to study foods compared to control categorized by SNPs

SNPs	LDL-C response to treatment compared to control (mmol/l, mg/dl)	Percentage LDL-C response to treatment compared to control (%)
APO_**E3**	-0.25±0.07 -9.55±2.71	-6.27
APO_**E4**	-0.31±0.08 -12.03±3.3	-9.95
CYP7A1-rs3808607 **GG**	- 0.28±0.09 -10.8±3.3	-7.75
CYP7A1-rs3808607 **TG**	-0.28±0.09 -10.8±3.3	-9.17
CYP7A1-rs3808607 **TT**	-0.28±0.08 -10.8±3.2	-6.98

Values in Means ± SE.

Figure 5. LDL-C differences stratified by Apo E isoform

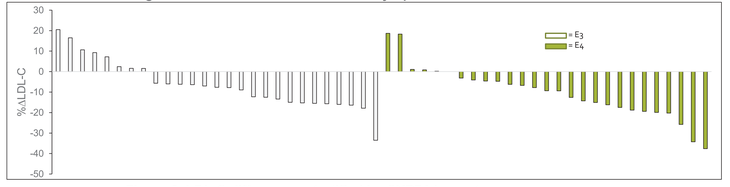
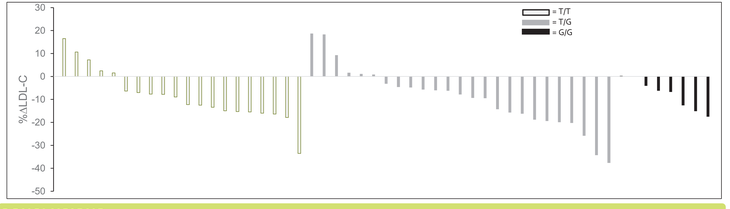


Figure 6. LDL-C differences stratified by CYP7A1 genotype



CONCLUSIONS

- In comparison to control, LDL-C decreased 8.8% in the treatment arm (p<0.0001, range -37.6% to +20.5%) and total cholesterol fell 5.1% (p<0.004).
- No significant change was seen in HDL-C, TG or fasting glucose levels.
- The APOE-4 and CYP7A1-GG carriers may benefit more from this food intervention compared to the other heterogenetic groups.
- Consumption of a portfolio of ready-to-eat bioactive foods significantly improves serum lipid profiles in patients unable or unwilling to take statin drugs, with some participants achieving medication level LDL-C reductions.

SIGNIFICANCE

- The ability of a practical food intervention using novel, hedonically acceptable ready-to-eat portfolio foods to significantly reduce cardiovascular risk has profound implications for public health and practice improvement.

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