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ORIGINAL ARTICLE

Chitin-glucan fiber effects on oxidized low-density lipoprotein: a randomized controlled trial

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BACKGROUND/OBJECTIVES: Elevated oxidized low-density lipoprotein (OxLDL) may promote inflammation, and is associated with increased risk of atherosclerotic coronary heart disease and worsening complications of diabetes mellitus. The primary objective of this study was to evaluate the efficacy of chitin-glucan (CG), alone and in combination with a potentially anti-inflammatory olive oil (OO) extract, for reducing OxLDL in subjects with borderline to high LDL cholesterol (LDL-C) levels.
SUBJECTS/METHODS: This 6-week, randomized, double-blind, placebo-controlled study of a novel, insoluble fiber derived from the *Aspergillus niger* mycelium, CG, evaluated 130 subjects free of diabetes mellitus with fasting LDL-C 3.37–4.92 mmol/l and glucose ⩽ 6.94 mmol/l. Participants were randomly assigned to receive CG (4.5 g/day; $n = 33$), CG (1.5 g/day; $n = 32$), CG (1.5 g/day) plus OO extract (135 mg/day; $n = 30$), or matching placebo ($n = 35$).
RESULTS: Administration of 4.5 g/day CG for 6 weeks significantly reduced OxLDL compared with placebo ($P = 0.035$). At the end of study, CG was associated with lower LDL-C levels relative to placebo, although this difference was statistically significant only for the CG 1.5 g/day group ($P = 0.019$). CG did not significantly affect high-density lipoprotein cholesterol, triglycerides, glucose, insulin or F2-isoprostane levels. Adverse events did not substantively differ between treatments and placebo.
CONCLUSIONS: In this 6-week study, CG (4.5 g/day) reduced OxLDL, an effect that might affect the risk for atherosclerosis.

INTRODUCTION

Cardiovascular diseases (CVDs), especially atherosclerotic coronary heart disease (CHD) and stroke, are the leading causes of death globally. Low-density lipoprotein cholesterol (LDL-C) level is the primary lipid treatment target to reduce atherosclerotic CHD risk.[1] LDLs are directly involved in the initiation and progression of atherosclerosis. The atherogenicity of LDL is highly dependent upon its oxidation.[2] Oxidized LDL (OxLDL) is a particle produced in the arterial wall by the oxidative modification of the apoB-100 moiety of LDL mediated by reactive oxygen species.

Results from animal studies suggest that consumption of fungi or a fungal extract lowers circulating cholesterol and reduces aortic atherosclerotic lesions.[3,4] Epidemiological data suggest that increased dietary fiber intake may reduce the risk of CVD.[5,6] β-Glucans are nonstarch polysaccharides consisting of β (1→3, 1→4)-D-linked glucose units. Results from well-controlled clinical trials support the view that consumption of soluble β-glucans reduces total cholesterol (TC) and LDL-C, typically without affecting high-density lipoprotein cholesterol (HDL-C) or triglycerides (TGs).[5,6] In hypercholesterolemic, obese men, yeast-derived β-glucan, a glucose polymer with β-(1→3,1→6) linkages, decreased TC but did not affect LDL-C, HDL-C or TGs.[7] The European Food Safety Authority has authorized a health claim related to the maintenance of normal blood cholesterol concentrations for soluble cereal fibers.[8]

Chitin-glucan (CG) is an insoluble dietary fiber extracted from the cell wall of fungi. The potential health benefits of this novel ingredient have been tested in animal models. In hamsters fed an

atherogenic diet, CG mixed with food lowered plasma TGs and markedly reduced the diet-induced formation of aortic fatty streaks.[9] CG also reduced aortic cholesterol, cardiac superoxide anions and hepatic malondialdehyde, and increased hepatic antioxidant enzyme activities (glutathione peroxidase and superoxide dismutase). In mice with a high-fat diet, supplementation with CG (10% w/w) induced cecal enlargement and restored the number of bacteria from clostricial cluster XIVa, including *Rosburia spp.*, which were decreased because of high-fat feeding. Furthermore, CG treatment significantly decreased high-fat-induced body weight gain, fat mass development, fasting hyperglycemia, glucose intolerance, hepatic TG accumulation and hypercholesterolemia, independent of caloric intake. These findings support the beneficial effects of CG with respect to the development of obesity, associated metabolic diabetes and hepatic steatosis.[10] In a pilot study with healthy subjects having a normal weight or slight overweight and a mean TC of 4.6 mmol/l, consumption of CG at 4.5 g/day for 28 days decreased circulating OxLDL.[11]

Reducing LDL oxidation is one of the mechanisms by which the Mediterranean Diet may have favorable effects on cardiovascular health.[12–16] Increased consumption of olive oil (OO), a major component of the Mediterranean Diet, appears to reduce the risk for developing CVD.[17–21] Results from *in vitro* and *in vivo* studies indicate that OO polyphenolic compounds have potential importance in the prevention of atherosclerotic damage through their inhibition of LDL oxidation.[12,22–28] Thus, both scientific and clinical rationales exist to evaluate CG alone, and in combination

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with olive extract, for their ability to reduce OxLDL. The objective of this study was to investigate the efficacy and safety of CG alone and CG with olive extract in a clinical study in hypercholesterolemic subjects.

SUBJECTS AND METHODS

Study design

This study was a randomized, double-blind, placebo-controlled, multicenter study with 6 weeks of intervention and four parallel intervention arms conducted at Louisville Metabolic and Atherosclerosis Research Center (Louisville, KY, USA), Provident Clinical Research/Biofortis North America (Addison, IL, USA) and KGK Synergize (London, ON, Canada; September 2010–January 2011). Ethical approval was obtained from the Institutional Review Board, Quorum Review Inc. (Seattle, WA, USA; file number: 25358 and 25358CDN). The study was conducted in accordance with all federal, state and local requirements and under the guidelines of Good Clinical Practice/International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use guidelines. The study was registered at clinicaltrials.gov (ID no. NCT01232309). The subjects were recruited in response to advertisements or from study site databases. Written, informed consent was obtained from all participants before any study-related activities.

Subjects

To be eligible for enrollment, subjects were required to: be generally healthy as confirmed by screening laboratory results, medical history and physical examination, be between 21 and 70 years of age, have a body mass index of 18.5–34.9 kg/m^2, have screening fasting serum levels of LDL-C 3.37–4.92 mmol/l and be willing to take a supplement three times daily for 6 weeks and comply with other study procedures. Subjects were excluded if they had: diabetes mellitus, cancer, gastrointestinal disease and cardiovascular disease, were pregnant or lactating, fasting serum TGs > 3.39 mmol/l, been taking lipid-altering drugs or dietary supplements within 4 weeks of screening, experienced a > 5% change in body weight within 4 weeks of screening or had a known hypersensitivity or intolerance to fiber or fiber-containing products.

Randomization and concealment

Eligible subjects were randomized using a block randomization scheme to receive one of the four treatments: 4.5 g/day of CG (n = 34), 1.5 g/day of CG (n = 33), 1.5 g/day of CG plus 135 mg/day olive extract (n = 33) or matching placebo capsules (n = 35). Each study site received a site-specific randomization schedule and unique randomization numbers and subjects were randomized to a treatment group in sequence (block size = 8; 1:1:1:1 ratio). The screening number and treatment code dispensed were recorded on the source document and case report forms for each subject as well as on the treatment dispensing log. Treatment assignments were concealed from the subjects and site personnel involved with the conduct of the study. Blinding was maintained for the duration of the study period and statistical analyses.

Interventions

The CG is a purified insoluble fiber (~80–85% fiber according to AOAC 991.43 modified), composed of chitin (poly-N-acetyl-D-glucosamine) and β (1,3)-D-glucan chains. CG is the main component of the cell walls of the Aspergillus niger mycelium, a biomass that remains after the manufacture of food grade citric acid. CG was formulated and administered in vegetarian capsules containing: 500 mg CG, 167 mg CG, 167 mg CG, 15 mg olive extract and 500 mg placebo (rice flour). All capsules contained similar fill amounts of material with the balance beyond the active components provided as rice flour. Subjects were instructed to take 3 capsules three times a day, with water before each main meal for 6 weeks. CG (ARTINIA) was obtained from KitoZyme (Herstal, Belgium), olive extract (Hydroxytyrosol-20) was from Eisai Food and Chemical Co. (Tokyo, Japan) and rice flour was from PGP International (Woodland, CA, USA).

Outcome measurements

Blood measurements at screening included safety tests (Chem 20; Medpace Reference Laboratories, Cincinnati, OH, USA), thyroid-stimulating hormone, complete blood count, pregnancy test and fasting serum lipids: TGs, LDL-C, TC and HDL-C levels. Subjects were counseled on a body

weight maintenance version of the Therapeutic Lifestyle Change (TLC) diet regimen at the screening visit and asked to follow this diet throughout the study. No dietary adjuncts were employed other than the study products.

After 2 weeks on the TLC diet, 135 eligible participants were randomized to one of the four treatment arms: CG (4.5 g/day; n = 34), CG (1.5 g/day; n = 33), CG (1.5 g/day) plus olive extract (135 mg; n = 33) or matching placebo (n = 35) for 6 weeks. Assessments at baseline (week 0), week 4 and week 6 included clinical measures of weight and blood pressure, fasting blood work for serum lipids (Medpace Reference Laboratories), Chem 20 (Medpace Reference Laboratories), OxLDL (ELISA kit, Mercodia, Uppsala, Sweden) and insulin (Medpace Reference Laboratories), as well as urine sample for F2-isoprostane analysis (ELISA kit, Northwest Life Sciences, Vancouver, WA, USA). At each visit, adverse events (AEs) were assessed, compliance calculated and subjects counseled on adherence to the TLC diet. Intensity of AEs was graded on a three-point scale (mild, moderate and severe) and reported in detail in the study record. The causality relationship of investigational product to the AE was assessed by the investigator as either most probable, probable, possible, unlikely or not related. 'Intervention-related' AEs were defined as probably or most probably because of study treatment.

Sample size estimation

An evaluable sample of 112 subjects (28 per group) was expected to provide 80% power to detect an effect size of ≥ 0.9 s.d. between the placebo and any of the active treatment groups for the primary outcome variable (change from baseline in OxLDL) with a two-sided α of 0.05 after adjustment for three comparisons to the placebo group.[29] Based on an assumed s.d. of 8 U/l for the OxLDL response,[30] the study was projected to have 80% power to detect a difference of ~7.2 U/l in the change from baseline in OxLDL.

Statistical methods

The primary study end point of this study was the difference between the placebo group and each of the active treatment groups in serum OxLDL at the end of the treatment period. Secondary study end points were differences between the placebo and active treatment groups at the end of the treatment period in fasting serum LDL-C, TC, HDL-C, TGs, glucose, insulin, F2-isoprostanes and blood pressure.

Safety data were analyzed for all randomized subjects. Efficacy analyses were performed on a modified intent-to-treat sample that included data from all randomized subjects who received treatment and from whom any efficacy data were captured. Laboratory and vital signs data from unscheduled or repeat visits were not included in the analyses. The data from the last nonbaseline visit completed for early termination subjects were used for end-of-treatment values, regardless of the elapsed days since the randomization visit.

All data summaries and analyses were performed using SAS, Version 9.2 (SAS Institute, Cary, NC, USA). Statistical testing was performed at the P = 0.05 level of significance (two sided). Descriptive statistics are presented for demographic and baseline characteristics.

For efficacy parameters, distributions for some variables were non-normal, particularly OxLDL and insulin. For the primary outcome variable, logarithmic transformation did not fully normalize the residuals, attributable mainly to a very large increase in OxLDL (70.4 U/l) for one subject in the combination CG plus OO extract group. Therefore, rank transformations were applied to the response variables before analysis. Initial analysis of covariance models included terms for treatment group, research site and baseline value as a covariate. Sensitivity analyses were conducted to assess possible treatment by research site interaction. As no material interactions were present, data presented are pooled results. Pairwise comparisons between the placebo and active treatment groups were completed using Dunnett's test.

AEs were coded according to MedDRA system-organ-class classifications. Frequency distributions of the number and percent of subjects with one or more events in each system-organ-class were calculated. The χ^2 or Fisher's exact tests were used to compare the treatment groups with respect to the proportions of subjects who experienced one or more events.

RESULTS

The Consolidated Standards for Reporting of Trials flow diagram shows the progress of subjects during the study (Figure 1). A total

of 286 candidates were screened by the three clinical sites ($N = 118$, KGK Synergize; $N = 107$, Provident; and $N = 61$, L-MARC), and 135 subjects qualified for randomization ($N = 49$, KGK Synergize; $N = 46$, Provident; and $N = 40$, L-MARC). In all, 35 subjects were randomized to receive placebo, 34 to CG 4.5 g/day, 33 to CG 1.5 g/day and 33 to CG combination, of whom 130 provided at least one postrandomization blood sample and were thus eligible for the modified intent-to-treat sample. Table 1 describes the demographic data for the modified intent-to-treat sample.

Baseline values of OxLDL and LDL-C did not differ significantly among treatment groups (Table 2). Regarding the primary end point, administration of 4.5 g/day CG for 6 weeks significantly reduced OxLDL (median change -3.3 U/l) compared with placebo (3.5 U/l, $P = 0.035$; Table 2). The other two CG intervention groups showed no statistically significant differences from placebo in the change from baseline for OxLDL (Table 2).

Regarding secondary end points, Table 2 also shows that at the end of study, the CG 1.5 g/day group showed statistical significance for median change in LDL-C (-0.03 mmol/l), which was significantly different from the change in the placebo group (0.21 mmol/l, $P = 0.019$). However, none of the other treatment groups showed significant changes compared with the placebo group. Additional exploratory analyses were completed to compare responses among all groups; however, none of the comparisons between active treatments reached statistical significance. Supplementation with CG did not significantly affect TC, HDL-C, TGs, glucose, insulin or urinary F2-isoprostanes. Systolic and diastolic blood pressures also showed no significant changes in any of the active treatment groups compared with placebo (data not shown).

Safety and tolerability

Treatment with CG for 6 weeks was generally well tolerated with no significant changes in heart rate or body weight (data not shown). The clinical safety profile (hematology and blood chemistry) for the intervention groups and AEs were not significantly different from placebo (data not shown). Gastrointestinal complaints were the most common AEs reported in this study; however, no significant differences were found in their frequencies between any of the three intervention groups and the placebo group.

Five AEs were judged by investigators to be probably or most probably related to the study intervention. All were in the category of gastrointestinal disorders and classified as either mild or moderate: placebo, 2 subjects, 2 events (abdominal cramping and constipation); CG 4.5 g/day, 1 subject, 1 event (worsening gastroesophageal reflux disease); CG combination, 1 subject, 2 events (increased frequency of bowel movements and excessive gas).

No serious AEs were reported. Four subjects terminated the study early because of AE: CG 4.5 g/day group, one subject (gastrointestinal discomfort); CG 1.5 g/day group, one subject (skin rash); and CG combination group, two subjects (shortness of breath, excessive gas). None of the AEs associated with subject withdrawal were categorized as probably or most probably related to study intervention.

DISCUSSION

Increased OxLDL is found in atherosclerotic lesions and in the circulation, particularly in patients with stable coronary artery disease or acute coronary syndromes (unstable angina; acute myocardial infarction).[31] Oxidation of LDL particles creates unstable oxygen free radicals that can be pathogenic to cells. If the reactive oxygen species production exceeds a biological system's ability to detoxify them, then this 'oxidative stress' can contribute to inflammatory processes, which is associated with metabolic disturbances, including atherosclerosis.[32]

In this study investigating the efficacy of the insoluble fiber CG in reducing OxLDL, CG (4.5 g/day) significantly reduced OxLDL after 6 weeks in comparison with placebo in subjects with mild to high LDL-C (3.37–4.92 mmol/l). Results of this study are consistent with previously reported effects in both animals[9] and humans.[11]

Elevated TC and LDL-C are associated with increased risk of CVD.[33] Results from previous studies suggest that regular intake of dietary fiber favorably affects the serum lipid profile.[5,6] In the current study, CG supplementation did not significantly alter LDL-C except in the 1.5 g/day group. Given the lack of a significant effect in the other two active treatment groups, including a higher intake level of 4.5 g/day, the authors consider this an equivocal finding (possibly a Type I statistical error) and additional research will be required to further assess the ability of dietary CG to lower circulating cholesterol levels. No changes in HDL-C or TG levels were observed in this study and these results are similar to those reported by others on yeast β-glucans.[7]

In an animal model, CG (dose equivalent to 4.5 g/day in humans) reduced aortic cholesterol, cardiac superoxide anion production and hepatic malondialdehyde, and increased hepatic antioxidant enzyme activities (glutathione peroxidase and superoxide dismutase) after 4 weeks of supplementation.[9] In the
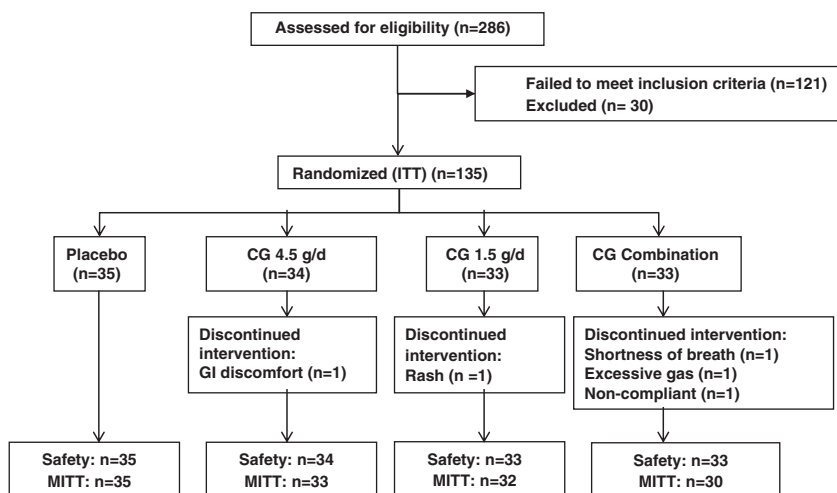

Figure 1. Consolidated Standards for Reporting of Trials diagram. ITT, intent-to-treat; MITT, modified intent-to-treat. Refer to text for details. The dates for the initiation of recruitment through the completion of trial were September 2010 through January 2011, respectively.

Table 1. Baseline demographic characteristics of the modified intent-to-treat analysis sample[a]

	Placebo (n = 35)	CG 4.5 g/day (n = 33)	CG 1.5 g/day (n = 32)	CG combination (n = 30)
Age (years)	52.0 ± 11.3	49.1 ± 11.2	50.9 ± 10.3	50.7 ± 9.8
Gender [n (%)]				
Male	14 (40.0%)	17 (51.5%)	13 (40.6%)	16 (53.3%)
Female	21 (60.0%)	16 (48.5%)	19 (59.4%)	14 (46.7%)
Race/ethnicity [n (%)]				
Caucasian	31 (88.6%)	29 (87.9%)	28 (87.5%)	19 (63.3%)
Black	3 (8.6%)	3 (9.1%)	3 (9.4%)	8 (26.7%)
Asian	0 (0.0%)	1 (3.0%)	1 (3.1%)	3 (10.0%)
Native American	1 (2.9%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Weight (kg)	78.7 ± 12.8	84.2 ± 15.5	79.7 ± 14.0	81.7 ± 14.2
Height (cm)	168.4 ± 7.8	171.9 ± 10.6	169.3 ± 8.0	170.4 ± 8.3
BMI (kg/m^2)	27.7 ± 3.5	28.4 ± 4.3	27.6 ± 3.2	28.0 ± 3.3
SBP (mm Hg)	115.8 ± 10.8	115.2 ± 14.0	119.8 ± 13.9	119.8 ± 14.9
DBP (mm Hg)	73.1 ± 9.4	76.3 ± 12.1	76.3 ± 7.5	77.6 ± 10.4

Abbreviations: BMI, body mass index; CG, chitin-glucan; DBP, diastolic blood pressure; SBP, systolic blood pressure. [a]Data are mean ± s.d. or number (%).

Table 2. Baseline and absolute change values for OxLDL, lipoprotein lipids, triglycerides, glucose, insulin and F2-isoprostanes by treatment group in the modified intent-to-treat analysis sample[a,b]

	Placebo (n = 35)	CG 4.5 g/day (n = 33)	CG 1.5 g/day (n = 32)	CG combination (n = 30)
OxLDL (U/l)				
Baseline	57.40 (33.40 to 87.40)	60.10 (40.70 to 101.80)	56.40 (35.70 to 85.00)	56.55 (32.80 to 89.40)
Change at week 6	3.50 (− 26.10 to 18.30)	− 3.30 (− 21.80 to 12.00)	− 0.40 (− 14.80 to 16.20)	2.15 (− 13.20 to 70.40)
P-value for change[c]	—	0.035	0.412	0.970
Total cholesterol (mmol/l)				
Baseline	6.16 (4.61 to 7.98)	5.91 (5.15 to 7.10)	5.78 (4.97 to 6.73)	5.87 (4.30 to 7.72)
Change at week 6	0.23 (− 1.63 to 1.76)	− 0.10 (− 1.26 to 0.90)	0.00 (− 0.91 to 1.06)	0.16 (− 1.43 to 1.35)
P-value for change[c]	—	0.349	0.096	0.606
LDL-C (mmol/l)				
Baseline	3.76 (2.64 to 5.78)	3.96 (3.00 to 5.15)	3.72 (3.16 to 4.77)	3.73 (2.59 to 5.36)
Change at week 6	0.21 (− 1.63 to 1.74)	0.00 (− 1.11 to 1.01)	− 0.03 (− 0.78 to 1.14)	0.01 (− 1.27 to 2.88)
P-value for change[c]	—	0.298	0.019	0.111
HDL-C (mmol/l)				
Baseline	1.45 (0.73 to 3.11)	1.35 (0.80 to 2.02)	1.35 (0.83 to 2.31)	1.18 (0.65 to 2.75)
Change at week 6	0.00 (− 0.44 to 0.49)	0.00 (− 0.54 to 0.36)	0.04 (− 0.39 to 0.29)	0.00 (− 0.44 to 0.47)
P-value for change[c]	—	0.965	0.961	0.992
Triglycerides (mmol/l)				
Baseline	1.29 (0.59 to 9.54)	1.29 (0.42 to 3.66)	1.57 (0.63 to 3.36)	1.58 (0.71 to 5.56)
Change at week 6	− 0.09 (− 6.59 to 1.79)	0.03 (− 2.05 to 1.29)	− 0.06 (− 1.73 to 1.47)	0.04 (− 3.83 to 3.44)
P-value for change[c]	—	0.587	0.943	0.499
Glucose (mmol/l)				
Baseline	5.3 (4.3 to 6.2)	5.5 (4.4 to 6.2)	5.3 (4.4 to 6.1)	5.3 (4.3 to 6.3)
Change at week 6	0.1 (− 0.8 to 1.0)	0.0 (− 0.7 to 3.3)	0.1 (− 0.7 to 0.7)	0.0 (− 1.1 to 1.4)
P-value for change[c]	—	0.974	0.995	0.496
Insulin (pmol/l)				
Baseline	35.4 (13.2 to 336.6)	42.6 (13.8 to 180.0)	35.7 (11.4 to 80.4)	48.6 (14.4 to 201.6)
Change at week 6	6.6 (− 190.8 to 52.2)	3.0 (− 86.4 to 100.2)	4.8 (− 27.0 to 42.6)	− 1.5 (− 124.2 to 221.4)
P-value for change[c]	—	0.999	0.964	0.930
F2-isoprostanes (ng/ml)				
Baseline	1.6 (0.6 to 5.5)	2.3 (0.2 to 7.0)	2.4 (0.4 to 6.5)	1.9 (0.2 to 9.9)
Change at week 6	0.0 (− 2.4 to 3.0)	0.1 (− 6.0 to 3.0)	− 0.1 (− 2.6 to 3.1)	0.0 (− 4.9 to 27.4)
P-value for change[c]	—	0.999	0.943	0.999

Abbreviations: CG, chitin-glucan; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; OxLDL, oxidized low-density lipoprotein. [a]Values are medians (minimum to maximum). [b]Probability values for tests of differences among treatment groups at baseline from analysis of variance model including treatment group, research site and treatment group × research site showed no significant differences. [c]Probability values for differences between active treatment groups and placebo from analysis of covariance model containing terms for baseline value, treatment group, research site and treatment group × research site. Rank transformations were used before running the models. Dunnett's test was used for pairwise comparisons.

present study, these markers of oxidative stress were not investigated; rather, F2-isoprostane levels were measured. No significant changes in F2-isoprostanes were observed. F2-isoprostanes act as a marker of lipid peroxidation and oxidative stress, and are present in atherosclerotic lesions in people with coronary artery disease.[34] The lack of substantial changes in F2-isoprostanes may be related to general good health of these hypercholesterolemic subjects, who did not have elevated levels of F2-isoprostanes at baseline.

In this study, subjects had baseline fasting glucose levels of ⩽6.94 mmol/l. CG did not significantly affect glucose or insulin levels. No postmeal or glucose load data were obtained; therefore, it was not possible to evaluate potential CG-mediated changes in postprandial glucose or insulin. β-Glucans differ in structure and solubility based on source and manufacturing processes.[35] Insoluble fibers do not affect viscosity whereas some water-soluble β-glucans increase viscosity in the small intestine, delaying digestion and/or absorption of starches and sugars.[36] The reported effects of oat β-glucan on insulin sensitivity may relate to fermentability rather than viscosity.[37,38] Uncooked, high-amylose corn starch, a form of fermentable fiber, markedly improved insulin sensitivity in obese men.[39]

Polyphenol intake has also been associated with lower CVD mortality rates.[40] *In vitro* and *in vivo* data suggest that polyphenolic compounds present in extra virgin OO play an important role in the prevention of atherosclerotic damage through their inhibition of LDL oxidation.[12,22–28] The EUROLIVE study demonstrated that OxLDL levels decreased linearly with increasing phenolic content of OO. In addition, the authors reported an improvement in several CVD risk markers, including TGs, HDL-C and TC/HDL-C ratio.[12]

In the present study, 1.5 g/day CG plus 135 mg/day olive extract for 6 weeks produced no significant difference in OxLDL levels compared with placebo. A previous review of the antioxidant effect of OO polyphenols reported inconsistent results.[19,41] The low dose (135 mg/day) of olive extract used in the present study may not have been sufficient to influence substantial absorption to elicit any change in the OxLDL levels.

The safety and tolerability profile of CG has been documented in previous studies.[11,42] Subchronic oral toxicity studies in rats demonstrated safety levels up to 10% of CG in the diet, corresponding to 6.6 g/kg body weight per day in male rats and 7.0 g/kg body weight per day in female rats.[42] A pilot 4-week study in overweight hypercholesterolemic men demonstrated that 4.5 g/day CG was well tolerated and without AEs.[11] Results of the present study confirm the safety profile of CG. All treatments were well tolerated and AEs comparable to those reported by subjects on placebo. No serious AEs occurred and no clinically significant changes in vital signs, hematology or clinical chemistry were observed. The consumption of olive phenols is considered safe at levels up to 20 mg/kg/day[43] and support the results of the current study.

The lack of marked oxidative stress at baseline and the inability to demonstrate a dose response for CG regarding its influence on OxLDL were the main limitations of this study. Additional research would be helpful to confirm the observe effect and to elucidate the shape of the dose-response curve.

In conclusion, administration of CG at a dose of 4.5 g/day in a 6-week, double-blind, placebo-controlled study of subjects with LDL-C ranging from 3.37 to 4.92 mmol/l significantly reduced OxLDL. Pending further validation of the CHD benefits of reducing OxLDL, these results suggest the potential therapeutic utility of CG in patients at risk for CHD.

CONFLICT OF INTEREST

JLE is an employee of Stratum Nutrition; VM is an employee of KitoZyme; JWA is a consultant of Stratum Nutrition; HEB received research grants from Stratum Nutrition for his site's participation in the study. KCM, ME and RC declare no conflict of interest.

REFERENCES

1 National Cholesterol Education Program Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) final report. *Circulation* 2002; **106**: 3143–3421.

2 Steinberg D, Witztum J. Oxidized low-density lipoprotein and atherosclerosis. *Arterioscler Thromb Vasc Biol* 2010; **30**: 2311–2316.

3 Mori K, Kobayashi C, Tomita T, Inatomi S, Ikeda M. Antiatherosclerotic effect of the edible mushrooms Pleurotus eryngii (Eringi), Grifola frondosa (Maitake), and Hypsizygus marmoreus (Bunashimeji) in apolipoprotein E-deficient mice. *Nutr Res* 2008; **28**: 335–342.

4 Sun JE, Ao ZH, Lu ZM, Xu HY, Zhang XM, Dou WF et al. Antihyperglycemic and antilipidperoxidative effects of dry matter of culture broth of Inonotus obliquus in submerged culture on normal and alloxan-diabetes mice. *J Ethnopharmacol* 2008; **118**: 7–13.

5 Anderson JW, Baird P, Davis Jr RH , Ferreri S, Knudtson M, Koraym A et al. Health benefits of dietary fiber. *Nutr Rev* 2009; **67**: 188–205.

6 Van HL, McCoin M, Kris-Etherton PM, Burke F, Carson JA, Champagne CM et al. The evidence for dietary prevention and treatment of cardiovascular disease. *J Am Diet Assoc* 2008; **108**: 287–331.

7 Nicolosi R, Bell SJ, Bistrian BR, Greenberg I, Forse RA, Blackburn GL. Plasma lipid changes after supplementation with beta-glucan fiber from yeast. *Am J Clin Nutr* 1999; **70**: 208–212.

8 EFSA Panel on Dietetic Products NaAN. Scientific Opinion on the substantiation of health claims related to beta glucans and maintenance of normal blood cholesterol concentrations (ID 754, 755, 757, 801, 1465, 2934) and maintenance or achievement of a normal body weight (ID 820, 823) pursuant to Article 13(1) of Regulation (EC) No 1924/2006. *EFSA J* 2009; **7**: 1254.

9 Berecochea-Lopez A, Decorde K, Ventura E, Godard M, Bornet A, Teissedre PL et al. Fungal chitin-glucan from Aspergillus niger efficiently reduces aortic fatty streak accumulation in the high-fat fed hamster, an animal model of nutritionally induced atherosclerosis. *J Agric Food Chem* 2009; **57**: 1093–1098.

10 Neyrinck A, Possemiers S, Verstraete W, De Backer F, Cani P, Delzenne N. Dietary modulation of clostridial cluster XIVa gut bacteria (Roseburia spp.) by chitin-glucan fiber improves host metabolic alterations induced by high-fat diet in mice. *J Nutr Biochem* 2012; **23**: 51–59.

11 Deschamps A, Nollevaux G, Gautier S, Keller F. Managing oxidative stress with a vegetal ingredient, chitin-glucan. *AgroFood* 2009; **20**: 12–14.

12 Covas MI, Nyyssonen K, Poulsen HE, Kaikkonen J, Zunft HJ, Kiesewetter H et al. The effect of polyphenols in olive oil on heart disease risk factors: a randomized trial. *Ann Intern Med* 2006; **145**: 333–341.

13 Estruch R, Martinez-Gonzalez MA, Corella D, Salas-Salvado J, Ruiz-Gutierrez V, Covas MI et al. Effects of a Mediterranean-style diet on cardiovascular risk factors: a randomized trial. *Ann Intern Med* 2006; **145**: 1–11.

14 Fito M, Guxens M, Corella D, Saez G, Estruch R, De la Torre R et al. Effect of a traditional Mediterranean diet on lipoprotein oxidation: a randomized controlled trial. *Arch Intern Med* 2007; **167**: 1195–1203.

15 Serra-Majem L, Roman B, Estruch R. Scientific evidence of interventions using the Mediterranean diet: a systematic review. *Nutr Rev* 2006; **64**(2 Part 2): S27–S47.

16 Sofi F, Cesari F, Abbate R, Gensini GF, Casini A. Adherence to Mediterranean diet and health status: meta-analysis. *BMJ* 2008; **337**: a1344.

17 Covas MI, Konstantinidou V, Fito M. Olive oil and cardiovascular health. *J Cardiovasc Pharmacol* 2009; **54**: 477–482.

18 Ortega RM. Importance of functional foods in the Mediterranean diet. *Public Health Nutr* 2006; **9**: 1136–1140.

19 Perez-Jimenez F, Alvarez de CG, Badimon L, Barja G, Battino M, Blanco A et al. International conference on the healthy effect of virgin olive oil. *Eur J Clin Invest* 2005; **35**(7): 421–424.

20 Perez-Jimenez F, Ruano J, Perez-Martinez P, Lopez-Segura F, Lopez-Miranda J. The influence of olive oil on human health: not a question of fat alone. *Mol Nutr Food Res* 2007; **51**: 1199–1208.

21 Tripoli E, Giammanco M, Tabacchi G, Di MD, Giammanco S, La GM. The phenolic compounds of olive oil: structure, biological activity and beneficial effects on human health. *Nutr Res Rev* 2005; **18**: 98–112.

22 Cicero AF, Nascetti S, Lopez-Sabater MC, Elosua R, Salonen JT, Nyyssonen K *et al.* Changes in LDL fatty acid composition as a response to olive oil treatment are inversely related to lipid oxidative damage: the EUROLIVE study. *J Am Coll Nutr* 2008; **27**: 314–320.

23 Covas MI, de la Torre K, Farre-Albaladejo M, Kaikkonen J, Fito M, Lopez-Sabater C *et al.* Postprandial LDL phenolic content and LDL oxidation are modulated by olive oil phenolic compounds in humans. *Free Radic Biol Med* 2006; **40**: 608–616.

24 Covas MI. Bioactive effects of olive oil phenolic compounds in humans: reduction of heart disease factors and oxidative damage. *Inflammopharmacology* 2008; **16**: 216–218.

25 Fito M, De la Torre R, Covas MI. Olive oil and oxidative stress. *Mol Nutr Food Res* 2007; **51**: 1215–1224.

26 Gimeno E, Torre-Carbot K, Lanuela-Raventos RM, Castellote AI, Fito M, de la Torre R *et al.* Changes in the phenolic content of low density lipoprotein after olive oil consumption in men. A randomized crossover controlled trial. *Br J Nutr* 2007; **98**: 1243–1250.

27 Marrugat J, Covas MI, Fito M, Schroder H, Miro-Casas E, Gimeno E *et al.* Effects of differing phenolic content in dietary olive oils on lipids and LDL oxidation—a randomized controlled trial. *Eur J Nutr* 2004; **43**: 140–147.

28 Weinbrenner T, Fito M, De la Torre R, Saez GT, Rijken P, Tormos C *et al.* Olive oils high in phenolic compounds modulate oxidative/antioxidative status in men. *J Nutr* 2004; **134**: 2314–2321.

29 Glantz S. *Primer of Biostatistics*. 6th edn. (McGraw-Hill Medical: New York, 2005.

30 Azar RR, Badaoui G, Sarkis A, Azar M, Aydanian H, Harb S *et al.* Effect of ezetimibe/atorvastatin combination on oxidized low density lipoprotein cholesterol in patients with coronary artery disease or coronary artery disease equivalent. *Am J Cardiol* 2010; **106**: 193–197.

31 Holvoet P, Theilmeier G, Shivalkar B, Flameng W, Collen D. LDL hypercholesterolemia is associated with accumulation of oxidized LDL, atherosclerotic plaque growth, and compensatory vessel enlargement in coronary arteries of miniature pigs. *Arterioscler Thromb Vasc Biol* 1998; **18**: 415–422.

32 Bays HE. Adiposopathy: is sick fat a cardiovascular disease? *J Am Coll Cardiol* 2011; **57**: 2461–2473.

33 Heidenreich PA, Trogdon JG, Khavjou OA, Butler J, Dracup K, Ezekowitz MD *et al.* Forecasting the future of cardiovascular disease in the United States: a policy statement from the American Heart Association. *Circulation* 2011; **123**: 933–944.

34 Schwedhelm E, Bartling A, Lenzen H, Tsikas D, Maas R, Brummer J *et al.* Urinary 8-iso-prostaglandin F2alpha as a risk marker in patients with coronary heart disease: a matched case-control study. *Circulation* 2004; **109**: 843–848.

35 Biorklund M, van RA, Mensink RP, Onning G. Changes in serum lipids and post-prandial glucose and insulin concentrations after consumption of beverages with beta-glucans from oats or barley: a randomised dose-controlled trial. *Eur J Clin Nutr* 2005; **59**: 1272–1281.

36 Makelainen H, Anttila H, Sihvonen J, Hietanen RM, Tahvonen R, Salminen E *et al.* The effect of beta-glucan on the glycemic and insulin index. *Eur J Clin Nutr* 2007; **61**: 779–785.

37 Maki KC, Galant R, Samuel P, Tesser J, Witchger MS, Ribaya-Mercado JD *et al.* Effects of consuming foods containing oat b-glucan on blood pressure, carbohydrate metabolism and biomarkers of oxidative stress in men and women with elevated blood pressure. *Eur J Clin Nutr* 2007; **61**: 786–795.

38 Maki KC, Rains TM. Fiber and insulin sensitivity. In: Zimering MB (ed). *Topics in the Prevention, Treatment and Complications of Type 2 Diabetes*. InTech: New York, 2011, pp 177–190. Available at http://www.intechopen.com/contact.html.

39 Maki KC, Pelkman CL, Kelley KM, Lawless AL, Schild A, Rains TM. Effects of type 2 resistant starch consumption on insulin sensitivity in men and women. *FASEB J* 2011; **25**: 587.9.

40 Hertog MG, Kromhout D, Aravanis C, Blackburn H, Buzina R, Fidanza F *et al.* Flavonoid intake and long-term risk of coronary heart disease and cancer in the seven countries study. *Arch Intern Med* 1995; **155**: 381–386.

41 Vissers MN, Zock PL, Katan MB. Bioavailability and antioxidant effects of olive oil phenols in humans: a review. *Eur J Clin Nutr* 2004; **58**: 955–965.

42 Jonker D, Kuper CF, Maquet V, Nollevaux G, Gautier S. Subchronic (13-week) oral toxicity study in rats with fungal chitin-glucan from Aspergillus niger. *Food Chem Toxicol* 2010; **48**: 2695–2701.

43 Soni MG, Burdock GA, Christian MS, Bitler CM, Crea R. Safety assessment of aqueous olive pulp extract as an antioxidant or antimicrobial agent in foods. *Food Chem Toxicol* 2006; **44**: 903–915.