High molecular weight oat β-glucan enhances lipid-lowering effects of phytosterols. A randomised controlled trial

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Abbreviations: CVD, cardiovascular disease; HDL-C, HDL-cholesterol; LDL-C, LDL-cholesterol; MW, molecular weight; OBG, oat β-glucan; PL. placebo group; PS, phytosterols; PS-OBG, phytosterols and oat β-glucan combination; RCT, randomised controlled trial; TC, total cholesterol; TC:HDL, total cholesterol-to-HDL ratio; TG, triglycerides, very low-density lipoprotein, VLDL.

The trial was registered with the Australian New Zealand Clinical Trials Registry at http://www.anzctr.org.au/ (ACTRN12618001455257).

ABSTRACT

Background & Aims: Oat β-glucan (OBG) and phytosterols (PS) are known to lower blood cholesterol levels via different mechanisms. Combination of high molecular weight (MW) OBG and PS in a single functional food could have complementary and/or synergistic effects for optimising heart health. The aim of this study was to investigate the effects of dietary supplementation with high-MW OBG with or without PS on plasma lipids in hypercholesterolaemic individuals.

Methods: In a double-blinded, placebo-controlled, 2x2 factorial trial, participants were randomised to receive biscuits fortified with either no PS or OBG (PL, n=18) or 2g PS (PS, n=18), 3g OBG (OBG, n=18), or combination of 2g PS and 3g OBG (PS-OBG, n=18) per day for 6 weeks. Primary outcome was fasting plasma total cholesterol (TC) and secondary outcomes were LDL-cholesterol, LDL-C; HDL-cholesterol, HDL-C; triglycerides, TG and TC to HDL-cholesterol (TC:HDL) ratio.

Results: TC and LDL-C were significantly lowered following PS (-4.6% and -7.6% respectively; $p<0.05$), OBG (-5.7% and -8.6%; $p<0.01$) and PS-OBG (-11.5% and -13.9%; $p<0.0001$) administration. The reduction in TC in the PS-OBG group was significantly greater compared to PL ($p<0.001$) and PS ($p<0.05$). PS-OBG group had a significantly greater reduction in LDL-C compared to PL ($p<0.01$) but not in comparison to PS or OBG groups. TC:HDL ratio was significantly reduced following PS-OBG (-8.9%; $p<0.01$) only, and there was no significant difference found between groups. Plasma TG reduced by 8.4% following PS-OBG, however, this was statistically non-significant. Plasma HDL-C remained unchanged across all groups.

Conclusions: Dietary supplementation with high-MW OBG and PS in a single functional food enhances their lipid-lowering potential. Blood cholesterol lowering by PS and OBG is

additive. Delivery of these two bioactive nutrients in a single food allows optimisation of their lipid-lowering effects and may provide added heart health benefits with enhanced compliance.

Keywords: total cholesterol, LDL cholesterol, triglyceride, phytosterols, β-glucan

INTRODUCTION

Dyslipidaemia is prevalent in over 63% of the Australian adult population and remains a key modifiable risk factor for cardiovascular disease (CVD) [1]. One or more lipid abnormalities such as elevated total cholesterol (TC) or LDL-cholesterol (LDL-C) or triglycerides (TG) or reduced HDL-cholesterol (HDL-C) or a combination of two or more of these lipid profiles comprise dyslipidaemia [1]. A suite of diet and lifestyle changes are used for the management of dyslipidaemia and have been shown to modestly lower LDL-C. Long-term adherence, complexity of adopted diet/lifestyle changes, poor patient motivation, lack of clinical follow-up and food aversions can impede the ability to achieve and sustain target blood lipid levels [2]. Subsequently, pharmacological interventions are often indicated, however, cost, adverse effects and intolerance, lack of effectiveness, patient perceived concern of long-term side effects and complex drug regimens are barriers for long-term compliance [3]. Consequently, nutraceuticals such as phytosterols (PS) and soluble fibres (along with other bioactives) have been recognised as adjunct and/or alternative lipid-modulating therapies for optimising dyslipidaemia control as they are safe, effective and easily compliable for individuals with dyslipidaemia [4-6].

On average, 25-30g of dietary fibre is recommended for adults for optimal heart and gut health [7]. The LDL-C lowering effects of dietary fibre are primarily attributed by the viscous (soluble) fibres such as those found in oat products. The ATP III recommends a multifaceted lifestyle approach including regular consumption of dietary fibres involving 10-25g/d derived from soluble fibres [5]. Oat β-glucan (OBG) is the main soluble fibre found in oats comprising linear glucose polymers with mixed β(1→4) and β(1→3) linkages [8]. Consumption of ≥3g/d OBG has been shown to lower TC and LDL-C concentrations with no significant changes in HDL-C or TG [9]. The key physiological property of OBG is viscosity and solubility which is determined by its molecular weight (MW) [8, 10]. High MW OBG

77 has greater ability to increase viscosity of upper digestive tract contents and therefore
78 influence bile acid metabolism; reduce intestinal bile acid reabsorption [11, 12] and increase
79 bile acid synthesis [13], resulting in lower circulating cholesterol concentrations. There are
80 discrepancies in the literature around the cholesterol-lowering ability of oat products
81 pertaining to OBG, as many studies do not report the MW of the β-glucan used and external
82 factors such as processing and storage can also alter this property [14].

83 PS are naturally found in a variety of plant-based foods such as fruit, nuts, seeds,
84 vegetables and vegetable/nut oils. It has been established that 2g/d of PS from enriched
85 products lowers circulating LDL-C by 8-10% in four weeks with no effects on HDL-C and
86 no or modest TG lowering effects [15-18]. The most widely accepted mechanism of action by
87 PS for cholesterol-lowering is micellar displacement of dietary and biliary cholesterol in the
88 gut, leading to reduced cholesterol absorption and subsequent excretion [19].

89 Since the mechanism by which PS and OBG lower non-HDL cholesterol are different,
90 their combination in an enriched food has the potential to produce complementary and/or
91 synergistic reductions in LDL-C concentrations. Therefore, the primary aim of this study is to
92 investigate the effects of concurrent consumption of PS and high-MW OBG delivered as a
93 snack on fasting blood lipid profile in hypercholesterolaemic individuals.

MATERIALS AND METHODS

Recruitment

Participants with hypercholesterolaemia were recruited from the Hunter region (NSW, Australia) via notice board flyers placed in the local community, word of mouth, newspaper articles and subjects who participated in earlier studies at our department were also invited to participate. Volunteers were assessed for eligibility by the lead investigator (JF) over the phone or in person and were deemed eligible if they were: healthy adults aged 18 to 70 years old; fasting plasma TC ≥ 5.5 mmol/L; not taking lipid- or glucose-lowering medications; no chronic disease such as CVD, diabetes mellitus, kidney/liver conditions, neurological conditions or untreated hypertension (≥ 140/95 mm Hg); not consuming PS- or OBG-enriched products or any other supplements known to influence the primary outcome (e.g. fish/krill/flaxseed oils, coenzyme Q10, fibre supplements); BMI < 40 kg/m^2; not pregnant or lactating; non-smoker and no strong food aversion and/or intolerance or allergy to gluten or wheat. Eligible volunteers were provided with a detailed description of the study and written informed consent was mandatory for enrolment in the study. Participants were de-identified and assigned numeric codes. The study protocol was approved by the Human Research Ethics Committee, University of Newcastle (H-2017-0091) and all procedures were conducted in accordance with the 1975 Declaration of Helsinki as revised in 1983. The trial was registered with the Australian New Zealand Clinical Trials Registry at http://www.anzctr.org.au/ (ACTRN12618001455257).

Study design

This study was a six-week, double-blinded, randomised, placebo-controlled trial with a 2x2 factorial design in four parallel groups. The senior investigator (MG) allocated treatment groups using a computer-generated block randomisation method and participants were stratified by gender (Random Allocation Software version 1.0.0). Participants were de-

identified and assigned numeric codes. Food product packets and storage boxes were labelled with colour-coded stickers upon packaging by the manufacturer and therefore the treatment allocation could not be ascertained by the investigators nor the participants.

Participants were randomly allocated to one of four treatments for six weeks: eight small sweet biscuits/d containing either placebo (PL; no PS or OBG), phytosterol (PS; 2g/d PS), OBG (OBG; 3g/d OBG) or a combination of PS and OBG (PS-OBG; 2g/d PS + 3g/d OBG). All treatment biscuits were identical in sensory characteristics. Manufacturing and packaging of the biscuits were conducted by Sweethings Pasticceria Wholesale Bakery under GMP conditions. The biscuit dough was prepared and mixed in an automated planetary mixer for 5 minutes. Combined dough was placed in an automatic dropping machine (Maxidrop Plus, W&P Reedy) with customised dropping speed, conveyor height and wire-cutting speed to shape dough into uniform biscuits of identical size, shape and weight. The biscuits were baked in a commercial double rack fan-forced oven (Revent 620) at 170ºC for 15 minutes. Each daily serve of biscuits contained 30g of fat derived from a vegetable fat spread. For the PS and PS-OBG biscuits, a commercially available PS-enriched vegetable fat spread (Logicol Original) was used and a vegetable fat spread equivalent in nutritional profile but devoid of PS (MeadowLea Gold'n Canola) was used for the PL and OBG biscuits. The primary source of PS esters used in Australian PS-enriched products is derived from soybean oil or tall (pine) oil obtained from trees during pulping [6]. The OBG and PS-OBG biscuits were enriched with 14 g of high-MW (2000-2500 kDa) [20] oat bran derived from Scandinavian oats (SWEOAT, Prorsum Healthcare AB, Sweden), providing 3g OBG per daily serve of biscuits. The energy content, carbohydrate, sugar, total fat and polyunsaturated, monounsaturated, and saturated fat content of the four experimental products were comparable (**Table 1**). Overall the biscuits were low in sugar (~10g per serve) and in addition to the test and placebo ingredients, they also contained wholemeal plain flour; vanilla extract and pastrycooks

chocolate paste (flavourings); icing sugar and corn flour. The PL and PS biscuits contained 100% whey protein isolate powder as a source of protein, since protein in the OBG and PS-OBG biscuits was derived from the oat bran powder. Participants were instructed to replace one in-between meal (snack) with the study biscuits, all to be consumed at one time. To enhance viscosity of β-glucan, participants were encouraged to consume the biscuits with at least 250 mL fluid. To avoid weight gain, participants were instructed to consume the study biscuits instead of other foods at one snack time each day. Compliance was monitored by evaluation of the biscuit consumption log, biscuit packet count and analysis of habitual dietary intake pre- and post-intervention (analysed with FoodWorks, Xyris ®, Professional Edition Version 8.0.3551).

Clinical assessments

Participants attended clinical trial facility of the Nutraceuticals Research Program, University of Newcastle, Callaghan, NSW Australia following an overnight fast (10 hours) at baseline and post-intervention. Anthropometric measures, blood pressure, medical history, habitual dietary intake, physical activity patterns and fasting blood samples were collected for plasma TC, LDL-C, HDL-C, TC:HDL ratio and TG.

Anthropometry and body composition

Anthropometrics (height, weight, waist circumference, BMI) and body composition in all participants were measured wearing light clothing and participants were asked to remove shoes and all metal and/or electronic devices on their body for all measurements. Height (cm), waist circumference (cm) and weight (kg) were collected to the nearest 0.1 units. Height was measured using a wall-mounted stadiometer with a movable head piece (Seca 206 Bodymeter Wall Height Measure Ruler). Waist circumference was measured using a tensible tape measure positioned midway between the lower rib margin and the iliac crest (approximately in line with the belly-button) horizontally. BMI was calculated as

weight/height^2 (kg/m^2). Weight and other body composition parameters (skeletal muscle mass, fat mass, total body water etc) were measured using bioelectrical impedance utilising two different frequencies (InBody230, Biospace Co.). Body composition was taken in the standing position following a ~10 hour fast and participants refrained from vigorous physical activity and alcohol consumption 24 hours prior to their appointments.

Medical history, dietary intake and physical activity

A self-administered medical history questionnaire was completed by all participants at baseline to collect information regarding past and present medical conditions; history of blood lipid profile, prescribed or over-the-counter medication(s), habitual supplement use and habitual consumption of alcohol, PS-enriched products, fibre, added sugars, fats and oils. Habitual diet and physical activity patterns at baseline and post-intervention were assessed by a 3-day food diary and physical activity questionnaire (International Physical Activity Questionnaire; IPAQ Long Last 7 Days Self-Administered Format, October 2002), respectively. Dietary data was evaluated using FoodWorks, Xyris. Professional Edition Version 8.0.3551. Physical activity data was interpreted as metabolic equivalent of task minutes per week (MET/week) to measure the energy cost of physical activities.

Blood sampling and serum lipid analyses

Fasted blood samples were collected at baseline and post-intervention via venepuncture into tubes pre-coated with EDTA by an experienced phlebotomist. Samples were centrifuged (Heraeus Biofuge Stratos) for 10 minutes at 3000 x *g* at 4ºC. Plasma and red blood cell fractions were aliquoted and stored at -80ºC until further analysis. Blood parameters were measured on a VP auto analyser using standardized reagents by the Hunter Area Pathology Service. LDL-C concentration was determined using the Friedewald equation [21].

Statistical analysis

Statistical analysis was conducted using StataCorp 2015 (*Stata Statistical Software: Release 14.* College Station, TX: StataCorp LP). All data are presented as means ± SEM (standard error of the mean) except for baseline and post-intervention TG values which are presented as median (IQR). The significance level for all statistical tests was set at 0.05. Sample size calculation yielded 80 participants in total (20 per group) based on previous estimates of variance in plasma TC concentration (standard deviation of 0.5) to elicit 80% power at a 0.05 significance level for detection of a 0.50 mmol/L (~10%) reduction in TC whilst accounting for a 20% dropout rate. The Bonferroni correction was applied to account for the fact that there were multiple secondary outcome variables tested (LDL-C, HDL-C, TG, TC:HDL ratio) and the significance level was reduced accordingly. Normality was assessed via the Shapiro Wilk test and visual plots including histograms. Comparison of baseline characteristics across treatment groups for age, height, weight, BMI, body composition, dietary intake, physical activity levels and blood parameters was assessed by ANOVA for normally distributed data and Kruskal-Wallis for non-normally distributed data. The chi-square test was used to compare gender and ethnicity between groups at baseline. Depending on the data being normally distributed, paired samples t-test or Wilcoxon Signed Rank test were performed for change from baseline to post-intervention within-treatment groups. One-way ANOVA was used to investigate the effect of each treatment on the absolute and percent change from baseline to post-intervention on the dependent variables between treatment groups. Two-way ANOVA was used to investigate whether there was a significant main effect for each treatment group (PS, OBG). An interaction effect between the two treatments [PS x OBG] was also tested to investigate their effect on the dependent variables. For any significant effects, Tukey's Honestly Significant Difference (HSD) was used to perform post hoc comparisons to test for complementarity and/or synergy between PS and OBG. Analysis of covariance was also used for each outcome variable (absolute and

percent change in TC and LDL-C) with the inclusion of treatment group as a factor and the corresponding baseline values of the outcome variable as a covariate. Any explanatory variables which were statistically significantly related with the outcome variables from bivariate analyses were included in the model as additional covariates. The covariates considered included age and baseline data for: BMI, waist-to-hip ratio, fat mass, systolic blood pressure, exercise levels, dietary intake (energy, carbohydrates, trans fat, omega-6 polyunsaturated fatty acids, monounsaturated fatty acids, fibre, alcohol). Correlations were used to assess the relationship between explanatory variables and variables with correlation coefficients greater than 0.8 were assessed more closely for multicollinearity and the number of potential predictors to include in the analyses was reduced accordingly. A backward stepwise regression model selection procedure was employed for each model to select the optimal set of predictors for each outcome variable.

RESULTS

Baseline characteristics

Eighty participants were recruited during the period end-September 2017 to mid-August 2018. Five participants dropped out of the trial due to inability to comply with biscuit consumption (n=1), illness (n=2) and personal reasons (n=2). A further three participants were excluded from the trial due to poor compliance (n=1) and significant outliers to the data set (data for change in LDL-C greater than two standard deviations from the mean) (n=2). A total of 72 participants were included in the final analysis, resulting in eighteen participants in each group (**Figure 1**). Most participants were females (63%) and north-west European (78%) with a mean age of 55.07±1.41 y, waist circumference of 92.42±1.32 cm, waist-to-hip-ratio of 0.94±0.01, weight of 76.49±1.79 kg, BMI of 26.79±0.46 kg/m^2, skeletal muscle mass of 28.90±0.94 kg and fat percentage of 32.20±1.03 %. Participants were hypercholesterolaemic at baseline with TC 6.57±0.11 mmol/L, LDL-C 4.39±0.09 mmol/L, HDL-C 1.54±0.04 mmol/L, TC:HDL ratio 4.47±0.12 mmol/L and median (IQR) TG of 1.22 (0.82) mmol/L. Following randomisation, anthropometric measures (weight, waist circumference, BMI, fat mass) were significantly higher in PL group compared to PS-OBG group ($p<0.05$). The effects of these baseline variables were explored further by adjusting for them by their inclusion in the multiple regression analyses. Groups were otherwise comparable for all other baseline characteristics with no other significant differences detected between treatment groups at baseline (**Table 2 and Table 3**). Weight significantly increased from baseline in the PL (0.63±0.26 kg), PS (0.57±0.24 kg) and PS-OBG (0.53±0.14 kg) groups and BMI significantly increased in the PL (0.24±0.08 kg/m^2) and PS-OBG (0.19±0.05 kg/m^2) groups ($p<0.05$). All other anthropometric measures and blood pressure did not significantly change within groups. Changes in anthropometric measures and blood pressure were not statistically significant between groups (data not shown).

Dietary intake, physical activity and compliance

There were no statistically significant differences in dietary intake at baseline across groups (**Table 4**) and nor were there statistically significant differences in the mean change of dietary parameters from baseline to post-intervention across groups. The study biscuits were well tolerated by participants with excellent compliance overall (98.24±0.42%). All groups were comparable for compliance except for the PS group which had significantly greater compliance than the PS-OBG group (3.73±1.13%, $p<0.01$) (**Table 1)**. There was no statistically significant change in physical activity levels from baseline to post-intervention within- and between groups.

Effect of phytosterol and oat β-glucan intervention on plasma lipid profile

After 6 weeks of supplementation, absolute- and relative change in TC were significantly lower: -0.33±0.11 mmol/L ($p=0.010$) and -4.60±1.66% ($p=0.013$) in the PS group; -0.41±0.12 mmol/L ($p=0.003$) and -5.69±1.70% ($p=0.004$) in the OBG group and -0.80±0.13 mmol/L ($p<0.0001$) and -11.48±1.79% ($p<0.0001$) in the PS-OBG group (**Table 3, Figure 2)**. Moreover, absolute- and relative change in LDL-C was significantly lower: -0.37±0.12 mmol/L ($p=0.006$) and -7.58±2.50% ($p = 0.008$) in the PS group; -0.42±0.11 mmol/L ($p=0.001$) and -8.63±2.43 % ($p=0.002$) in the OBG group and -0.66±0.13 mmol/L ($p=0.0001$) and -13.88±2.39% ($p<0.0001$) in the PS-OBG group post-intervention. Absolute- and relative change in TC:HDL ratio was significantly lower in the PS-OBG group (-0.42±0.10 mmol/L, $p=0.0008$; -8.85±2.41%, $p=0.002$) only. Absolute- and relative change in TG reduced by -0.24±0.14 mmol/L ($p=0.022$) and -8.44±5.62 % ($p=0.048$) in the PS-OBG group only which was not statistically significant. HDL-C did not significantly change within any treatment group.

As a result of the unexpected reduction in TG, the effect of baseline values was explored further by including them as covariates in the backward stepwise multiple regression. The

associated model revealed baseline TG concentration as the only significant predictor (p=0.043) of the relative change in TG. To further explore the effect of baseline TG concentrations, TG data was dichotomised for baseline: TG < 1.7 mmol/L and TG ≥ 1.7 mmol/L per treatment group. Those with the higher baseline TG concentrations had larger reductions in TG (p=0.017), however, this was only evident in the PS-OBG group and was not statistically significant due to the adjusted significance level of 0.0125 due to the Bonferroni correction (**Figure 3).** These additional analyses confirmed treatment to not be a statistically significant predictor of TG change after adjusting for baseline TG concentrations.

Blood lipid parameters did not significantly change from baseline in the PL group (**Table 3)**. Comparison across treatment groups demonstrated a significant difference in absolute- and percent change in TC (p<0.001) and LDL-C (p<0.01) across groups. Post hoc analyses showed that PS-OBG had a significantly larger reduction in absolute- and relative change in TC compared to PL (-0.82±0.18 mmol/L, p<0.001; -11.82±2.50%, p<0.001) and PS (-0.47±0.18 mmol/L, p=0.044; -6.88±2.50%, p=0.037) only. As for LDL-C, the PS-OBG group had a significantly greater reduction in absolute (-0.61±0.17 mmol/L, p=0.003) and percent change (-13.06±3.54%, p=0.003) compared to PL only. There were no statistically significant differences in TC:HDL ratio, HDL-C or TG concentrations between treatment groups.

Two-way ANOVA analysis revealed a significant main effect of PS and OBG for absolute- (p=0.004, p<0.001 respectively) and percent change (p=0.003, p<0.001 respectively) in TC. The main effect of PS did not reach statistical significance whereas OBG did for absolute- (p=0.022, p=0.006 respectively) and percent change (p=0.019, p=0.006 respectively) in LDL-C. There was no interaction between PS and OBG on any lipid measures.

The effects of baseline data on change in blood lipid profile

Baseline data including age, BMI, fat mass, waist-to-hip-ratio, systolic blood pressure, LDL-C concentration, exercise levels and dietary intake of energy, carbohydrates, trans fats, monounsaturated fatty acids, omega-6 polyunsaturated fatty acids, fibre and alcohol were assessed as potential confounders in a multiple regression using the backward stepwise regression procedure by including them as predictor variables in the original model. The final reduced models revealed that treatment remained a statistically significant predictor of the change in TC and LDL-C.

DISCUSSION

The cholesterol-lowering ability of specific soluble fibres and PS have been recognised as key adjunct and alternative therapies that can be coupled with dietary changes and medications to enhance the efficacy of reaching blood cholesterol targets. Our findings show that daily dietary supplementation with high-MW OBG combined with PS for six weeks in a functional food, significantly lowered TC (~11.5%) and LDL-C (~14%), in free-living adults with hypercholesterolaemia. In addition, blood lipid profile was further improved as a marked reduction in TG concentration (8.4%) was also apparent in the PS-OBG group.

Consumption of ≥3g/d OBG has been shown to lower TC and LDL-C concentrations by 0.30 mmol/L and 0.25 mmol/L respectively, with no significant changes in HDL-C or TG [9]. An RCT reported that a high-MW (2210 kDa) and medium-MW (530 kDa) OBG induced a greater LDL-C reduction compared to low-MW (210 kDa) and control [8]. Regardless of the MW, OBG has been shown to lower plasma cholesterol by reducing intestinal bile acid reabsorption [22, 23]. Once bile has facilitated the digestion and absorption of dietary fats, it is normally recovered in the distal end of the small intestine and then recycled [22]. OBG has a linear polymer structure to which the adjacent chains form cross-links, enabling it to swell as it absorbs water while passing through the length of the small intestine [23]. The OBG becomes highly viscous which traps bile, rendering it unable to be reabsorbed and thus excreted via stool [22]. This stimulates bile acid synthesis from cholesterol in the liver, which in turn is supplied by increased cholesterol synthesis, evidenced by raised concentrations of lathosterol and 7α-hydroxy-4-cholesten-3-one (a marker of bile acid synthesis) [13, 24, 25] as well as upregulation of LDL receptor expression thus enhancing clearance of LDL-C from the blood to synthesise more bile acid [8, 22]. The degree of viscosity of OBG is determined by its MW [8, 23]. Native unprocessed oat kernels have a naturally high MW (~1730-2800 kDa) [9, 20], however, this can be reduced to as low as ≤ 100 kDa during food processing,

extrusion and storage [20]. The efficacy of LDL-C lowering is reduced by 50% when the MW is 210 kDa, even when the same dose (3g/d) of OBG is administered [8].

Reductions in TC and LDL-C following OBG supplementation in the present study surpass that of previous studies administering the same dose [9], namely one that used the same high-MW (~2210 kDa) OBG [8]. Wolever et al [8] reported a significant 0.15 mmol/L or 5% (vs 0.42 mmol/L, 8.6% in our study) reduction in LDL-C following consumption of 3g/d high-MW OBG delivered via two servings of cereal per day. The lower baseline LDL-C (3.74 vs 4.67 mmol/L in our study) in the previous study's participants could account for the smaller reduction reported [8]. It is also possible that the degree of viscosity in the gut is limited or reduced when the daily dose of OBG is divided into two servings per day, thus minimising the efficacy for LDL-C-lowering. Given the mechanistic action of OBG, it has been suggested that optimal consumption is with meals to coincide with bile release [23], however, in the present study OBG was administered at snack times yet larger reductions in LDL-C were observed compared to previous findings. Further research into divided vs single dose and timing along with other potential confounders in study design or delivery are warranted to better understand how therapies can protect and capitalise on the physiochemical properties and thus hypocholesterolaemic effects of OBG.

The degree of cholesterol-lowering following supplementation with PS reported in this study is similar to previous studies of similar dose and duration, whereby an 8-9% (~0.32-0.35 mmol/L) reduction in LDL-C following 2g/d PS for at least four weeks is well documented [15, 26]. The magnitude of LDL-C lowering has been shown to be influenced by higher baseline LDL-C [15, 26]. In this study, baseline LDL-C and waist-to-hip ratio were predictors of the change in LDL-C, however, treatment remained the most significant predictor. There are several reported mechanisms by which PS lower plasma cholesterol levels, but the most widely accepted mechanism is displacement of cholesterol in the micelle

[19]. This results in an overall net reduction of cholesterol absorption and only minimal uptake of PS into circulation, as the majority are selectively pumped back into the intestinal lumen via ATP-binding cassettes ABCG5 and ABCG8 where their fate is excretion [19].

The reduction in fasting TG observed in the PS-OBG group was unexpected as findings are inconsistent regarding the TG-lowering effects of PS. A meta-analysis [27] and pooled analysis of 12 RCTs [18] by Demonty et al concluded that 2g/d PS induced a modest but statistically significant reduction in TG (0.08-0.12 mmol/L or 4-6%) in hypercholesterolaemic individuals, and larger reductions were associated with higher baseline TG (~1.37-2.0 mmol/L). A more pronounced reduction (0.23 mmol/L or 27.5%) has been demonstrated in metabolic syndrome patients with baseline TG > 2.0 mmol/L [28]. In contrast to our study, Demonty et al [18] found this relationship to be statistically significant for absolute (mmol/L)- but not relative (%) change in TG. It is likely that this discrepancy of high inter-individual variation in TG concentrations and lack of statistical significance is because individual studies are primarily powered to assess effects on LDL-C, thus poorly powered to detect a meaningful change in TG [18]. In this study, baseline TG concentrations in the PS group were lower than the PS-OBG group (1.05 mmol/L vs 1.29 mmol/L) and had an increasing non-significant trend post-intervention (+0.05 vs -0.24 mmol/L, respectively). Further investigation from our additional dichotomous analysis revealed individuals with higher baseline TG ($\geq$ 1.7 mmol/L) had greater reductions in TG post-intervention following PS-OBG supplementation. Since baseline TG concentrations appear to be explaining most of the variation in relative change in TG, it is acknowledged that this observation could possibly be attributed to the regression to the mean effect. The majority of human studies have reported slight but non-significant lowering of TG concentrations following OBG [9, 29] with few demonstrating significant reductions [30, 31]. A study in rats reported significant reductions in serum and hepatic TG concentrations and greater faecal bile acid excretion

following partially hydrolysed OBG (MW 730 kDa) + cholesterol-rich diet [32]. The exact mechanism is unknown but since OBG increases the viscosity of the small intestine, it reduces efficiency of emulsification, which is likely to interfere with lipid digestion by reducing accessibility of fats by digestive enzymes. The potential TG-lowering effect by PS is likely due to lowered hepatic synthesis of large TG-rich very low-density lipoproteins (VLDL)-1 particles, since significant reductions in large and medium size VLDL particles have been reported in dyslipidaemic metabolic syndrome patients after plant stanol supplementation [28]. Some limitations of our findings for the secondary measure, TG, are the small number of participants in each group after the measure was dichotomized as a secondary analysis and hence the limited associated statistical power to detect differences in TG as statistically significant. This study was not powered to detect changes in TG concentrations, and we were not aiming to recruit hypertriglyceridaemic individuals. These findings are exploratory, although prompts interest to investigate the individual action and interaction between PS and OBG in a larger study that is powered to assess change in TG in participants with elevated TG concentrations.

The combination of PS and OBG has been previously investigated in a muesli [13] and a series of food items (cereal, snack bar and beverage) [33], however, in the former study the saturated form of PS was used (plant stanols) at only 1.5g/d dose with 5g/d OBG for four weeks at main meal times via a moderate-fat food item (~15g). In the latter study, 1.8g/d PS with 2.8g/d OBG was delivered via a low-fat food (≤3g per serve) across three separate timepoints including main meal and snack times. Neither of the trials specified MW of the OBG and this along with timing of consumption, multiple time points for consumption and low-moderate fat content of experimental food may contribute to the efficacy of OBG for modulating cholesterol concentrations [9]. Given the two distinct mechanistic roles of PS and OBG regarding cholesterol metabolism, they effectively complement each other for an

amplified reduction in plasma LDL-C. The combination of PS and OBG proves to be efficacious by tackling cholesterol metabolism at the gut level from two angles: increased clearance of cholesterol from circulation via the inhibition of bile acid reabsorption and reduction in cholesterol absorption at the micellar level. Moreover, the addition of OBG to PS therapy potentially extends its health benefits beyond cholesterol-lowering as OBG has been shown to: significantly lower postprandial blood glucose concentrations by suppressing glucose uptake [34] and delaying gastric emptying [35]; increase postprandial fullness and satiety in healthy [36] and overweight/obese [37] individuals and promotes colonic fermentation by gut microbiota to produce short-chain fatty acids [38-40], which play various roles such as mediating calorie intake [41, 42] and inhibiting endogenous cholesterol synthesis [38, 43].

Findings from this study demonstrate a complementary action between high-MW OBG and PS for cholesterol-lowering in hypercholesterolaemic adults. These findings have the potential to provide a safe, efficacious and compliable adjunct therapy that is more efficacious than administering either bioactive alone for the management of dyslipidaemia. This combination may support the development of novel foods that could serve as a solution or adjunct therapy for individuals who are statin intolerant and/or have suffered adverse effects from pharmacological interventions. Further research into the additional health benefits of this dietary combination relating to glycaemic parameters, short-chain fatty acids, calorie intake, weight management and long-term effects are warranted to establish optimal dose, duration and food delivery.

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STATEMENT OF AUTHORSHIP

JJAF and MLG conceptualized and designed the research. JJAF conducted research. JJAF analysed the data. ES provided statistical support. JJAF and MLG wrote the paper; JJAF had primary responsibility for final content. All authors read and approved the final manuscript.

CONFLICT OF INTEREST STATEMENT

The authors have no conflicts of interest to declare.

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578

FIGURE LEGENDS

Figure 1

CONSORT schematic of participant recruitment, screening and assessment.

* Value for primary outcome lies outside two standard deviations from the mean

Figure 2

Percent change in TC, LDL-C, HDL-C, TC:HDL ratio and TG from baseline to post-intervention in hypercholesterolaemic individuals who consumed PL, PS, OBG or PS-OBG for 6 weeks. Data represent mean ± SEM. Note TG relative change is mean ± SEM as this data is normally distributed. Symbols indicate significant changes from baseline as analysed by paired samples t-test: * $p<0.05$, ** $p<0.01$, *** $p<0.001$, **** $p<0.00001$. One-way ANOVA and Tukey's HSD was used to perform post hoc comparisons of group means. Means with a common letter significantly differ to each other.

[a] PL and PS-OBG significantly differ from each other for TC ($p<0.0001$) and LDL-C ($p=0.003$).

[b] PS and PS-OBG significantly differ from each other for TC ($p=0.037$)

OBG, oat β-glucan; PL, placebo; PS, phytosterols; PS-OBG, phytosterol + oat β-glucan

Figure 3

Absolute change in TG (mmol/L) when baseline TG <1.7 mmol/L or ≥1.7 mmol/L in hypercholesterolaemic individuals who consumed PL, PS, OBG or PS-OBG for 6 weeks. A cut off of 1.7 mmol/L in TG was used as this is classified as normal under the ATP III

601 guidelines [5]. Data represent mean ± SEM. Changes from baseline was analysed by
602 Wilcoxon Signed Rank test and One-way ANOVA was used to compare group means.

603 TG <1.7 mmol/L: PL, n= 14; PS, n=16; OBG, n=12; PS-OBG, n=12

604 TG ≥1.7 mmol/L: PL, n= 4; PS, n=2; OBG, n=6; PS-OBG, n=6

605 OBG, oat β-glucan; PL, placebo; PS, phytosterols; PS-OBG, phytosterol + oat β-glucan; TG,
606 triglycerides.

607 **TABLES**

Table 1. Nutrient composition of study biscuits and compliance.[1]

Dietary component	PL	PS	OBG	PS-OBG
Energy (kJ)	1490.6	1567.1	1454.8	1531.3
Protein (g)	7.1	7	4.9	4.8
CHO (g)	33.1	33.1	30.0	30.0
Sugars (g)	9.9	9.9	9.7	9.7
Starch (g)	22.8	22.8	19.8	19.8
Total fat (g)	21.4	23.5	21.7	23.8
Saturated (g)	4.9	5.1	4.9	5.1
MUFAs (g)	10.3	11.1	10.1	11.0
PUFAs (g)	5.1	5.9	5.0	5.8
Phytosterols (g)	0	2.0	0	2.0
Oat β-glucan (g)	0	0	3.0	3.0
Dietary fibre (g)	2.9	2.9	8.0	8.0
Sodium (mg)	118.8	115.8	109.2	106.2
Compliance (%)	98.81 ± 0.58	99.87 ± 0.13[a]	98.15 ± 0.65	96.14 ± 1.33[a]

[1] Nutrient information is given for one serve (8 biscuits, ~80 g). Each participant had to consume one serving of biscuits per day. One-way ANOVA was used to compare compliance. Post-hoc analyses were used to compare differences between groups when significance was found. Values with common superscript in each row indicate statistically significant differences between corresponding groups, $p<0.01$.

OBG, oat β-glucan; PL, placebo; PS, phytosterols; PS-OBG, phytosterol + oat β-glucan

608

609 Table 2. Participant characteristics at baseline in the placebo (PL), phytosterol (PS), oat β-

610 glucan (OBG) and phytosterol + oat β-glucan (PS-OBG) groups.[1]

	PL (n = 18)	PS (n = 18)	OBG (n = 18)	PS-OBG (n = 18)
Sex, *n* (%)				
Male	8 (44)	6 (33)	8 (44)	5 (28)
Female	10 (56)	12 (67)	10 (56)	13 (72)
Ethnicity, *n* (%)				
North-west European	14 (78)	15 (83)	15 (83)	12 (67)
South-east European	1 (6)	1 (6)	0 (0)	0 (0)
Asian	0 (0)	0 (0)	2 (11)	4 (22)
Other [2]	3 (17)	2 (11)	1 (6)	2 (11)
Age (y)	54.78 ± 2.81	54.67 ± 2.77	56.39 ± 2.88	54.44 ± 2.99
Height (cm)	171.28 ± 2.03	166.92 ± 2.49	168.59 ± 2.65	166.85 ± 2.70
Waist circumference (cm)	98.48 ± 2.81[a]	89.32 ± 2.45	94.86 ± 2.06	87.00 ± 2.45[a]
Waist-to-hip ratio	0.95 ± 0.01	0.94 ± 0.01	0.95 ± 0.01	0.93 ± 0.02
Weight (kg)	83.94 ± 3.66[a]	73.11 ± 3.62	79.48 ± 3.02	69.44 ± 3.28[a]
BMI (kg/m^2)	28.49 ± 1.04[a]	26.01 ± 0.76	27.81 ± 0.67	24.84 ± 0.93[a]
Skeletal muscle mass (kg)	30.68 ± 1.87	28.28 ± 1.95	29.93 ± 1.89	26.71 ± 1.78
Body fat mass (g)	28.91 ± 2.33[a]	22.22 ± 1.49	25.86 ± 1.62	21.12 ± 2.01[a]
Body fat (%)	34.49 ± 2.19	30.90 ± 1.90	33.02 ± 2.00	30.42 ± 2.15
SBP (mmHg)	124.97 ± 3.56	119.58 ± 3.23	121.69 ± 3.24	121.61 ± 3.28
DBP (mmHg)	80.56 ± 2.48	74.33 ± 2.08	75.83 ± 2.14	75.39 ± 1.52
MET (mins/wk)	5188 ± 1538	3851 ± 740	3260 ± 712	3109 ± 717

[1] Values are reported as means ± SEM. for continuous measures and as n (%) for categorical measures.

[2] Other races include Oceanian; North African and Middle Eastern; Other (combination of races).

One-way ANOVA was used to compare baseline data for normally distributed data and Kruskal-Wallis for non-normally distributed data. Post-hoc analyses were used to compare differences in baseline data between groups when significance was found. Values with common superscript in each row indicate statistically significant differences between corresponding groups, $p<0.05$.

DBP, diastolic blood pressure; MET, metabolic equivalent; OBG, oat β-glucan; PL, placebo; PS, phytosterols; PS-OBG, phytosterol + oat β-glucan; SBP, systolic blood pressure

Table 3. Change in plasma outcome measures in the placebo (PL), phytosterol (PS), oat β-glucan (OBG) and phytosterol + oat β-glucan (PS-OBG) groups from baseline to post-intervention.[1]

	PL	PS	OBG	PS-OBG	*p* ^
TC					
BL	6.34 ± 0.19	6.31 ± 0.22	6.91 ± 0.24	6.71 ± 0.20	
PI	6.36 ± 0.23	5.98 ± 0.16**	6.49 ± 0.23**	5.91 ± 0.16**	
Δ **mmol/L**[2]	0.02 ± 0.13[a]	-0.33 ± 0.11[b]	-0.41 ± 0.12	-0.80 ± 0.13[ab]	<0.001
LDL-C					
BL	4.22 ± 0.16	4.25 ± 0.16	4.67 ± 0.21	4.42 ± 0.21	
PI	4.17 ± 0.19	3.89 ± 0.11**	4.24 ± 0.20**	3.76 ± 0.14**	
Δ **mmol/L**[3]	-0.05 ± 0.12[a]	-0.37 ± 0.12	-0.42 ± 0.11	-0.66 ± 0.13[a]	0.006
HDL-C					
BL	1.56 ± 0.09	1.54 ± 0.11	1.48 ± 0.06	1.57 ± 0.09	
PI	1.60 ± 0.11	1.55 ± 0.13	1.46 ± 0.06	1.53 ± 0.10	
Δ **mmol/L**	0.04 ± 0.04	0.01 ± 0.03	-0.03 ± 0.03	-0.03 ± 0.03	0.319
TC:HDL					
BL	4.27 ± 0.24	4.29 ± 0.20	4.84 ± 0.29	4.47 ± 0.24	
PI	4.16 ± 0.22	4.16 ± 0.23	4.63 ± 0.30	4.05 ± 0.22**	
Δ	-0.11 ± 0.12	-0.13 ± 0.11	-0.21 ± 0.09	-0.42 ± 0.10	0.140
TG					
BL	1.13 (0.73)	1.05 (0.58)	1.38 (1.1)	1.29 (0.79)	
PI	1.15 (0.93)	1.16 (0.44)	1.36 (1.23)	1.19 (0.86)	
Δ **mmol/L**	0.05 ± 0.06	0.05 ± 0.05	0.08 ± 0.17	-0.24 ± 0.14	0.098

[1] Values are reported as means ± SEM for all plasma concentrations except triglycerides. Baseline and post-intervention triglyceride values are median (IQR) due to lack of normality of the distribution. All baseline and post-intervention data are in mmol/L except for TC:HDL ratio. Significant change from baseline, * $p<0.05$, **

$p<0.01$.

[^] One-way ANOVA was used to compare change in outcome parameters across treatment groups. $P < 0.05$ indicates statistically significant difference between groups. Tukey's HSD post-hoc analyses were used to compare differences in mean change between groups when significance was found. Kruskal-Wallis test was conducted for triglycerides. Values with common superscripts in each row indicate statistically significant differences between corresponding groups.

[2] TC significantly reduced in the PS-OBG group compared to the PL (Δ mmol/L, $p<0.001$) and the PS group (Δ mmol/L, $p=0.044$).

[3] LDL-C significantly reduced in the PS-OBG group compared to the PL group (Δ mmol/L, $p=0.003$) only.

BL, baseline; CC, curcumin; HDL, HDL-cholesterol; LDL-C, LDL-cholesterol; PI, post-intervention; OBG, oat β-glucan; PL, placebo; PS, phytosterols; PS-OBG, phytosterol + oat β-glucan; TC, total cholesterol; TC:HDL ratio, total cholesterol-to-HDL ratio; TG, triglycerides.

611

Table 4. Reported dietary intake of hypercholesterolaemic adults who consumed placebo (PL), phytosterol (PS), oat β-glucan (OBG) and phytosterol + oat β-glucan (PS-OBG) at baseline and mean change from baseline to post-intervention (Δ).[1]

	PL (n=18)		PS (n=18)		OBG (n=18)		PS-OBG (n=18)	
	BL	Δ	BL	Δ	BL	Δ	BL	Δ
Energy (kJ)	9379 ± 636	1259 ± 538	9178 ± 499	-46 ± 433	8756 ± 519	118 ± 375	8398 ± 456	1118 ± 380
Protein (g)	94.49 ± 6.71	8.14 ± 4.78	105.62 ± 5.16	-0.95 ± 6.03	94.81 ± 6.41	-1.97 ± 5.46	94.68 ± 5.93	4.23 ± 6.14
CHO (g)	221.35 ± 14.66	22.18 ± 12.77	218.02 ± 15.69	-2.02 ± 16.09	210.60 ± 18.23	-0.27 ± 13.91	208.54 ± 16.13	15.64 ± 12.90
Sugars (g)	101.75 ± 9.29	7.28 ± 10.52	105.01 ± 9.04	-11.27 ± 9.24	90.22 ± 11.60	-4.43 ± 7.90	85.44 ± 6.51	-3.33 ± 7.79
Starch (g)	117.35 ± 8.95	13.94 ± 8.88	109.48 ± 8.67	10.59 ± 8.82	117.81 ± 10.83	3.25 ± 10.54	121.12 ± 12.92	18.60 ± 10.76
Total fat (g)	91.08 ± 8.01	20.83 ± 8.11	81.23 ± 5.42	5.33 ± 5.73	79.87 ± 5.50	3.99 ± 5.63	71.41 ± 5.30	22.90 ± 4.64
Saturated (g)	32.25 ± 3.20	5.52 ± 2.89	30.96 ± 2.43	-1.24 ± 3.26	30.08 ± 2.88	-1.14 ± 2.72	25.59 ± 2.01	5.06 ± 1.82
Trans (g)	1.48 ± 0.17	0.30 ± 0.17	1.53 ± 0.13	-0.14 ± 0.21	1.57 ± 0.19	-0.07 ± 0.19	1.11 ± 0.13	0.16 ± 0.08
MUFAs (g)	35.69 ± 3.37	7.96 ± 3.59	30.67 ± 2.35	3.66 ± 1.96	30.41 ± 2.18	2.77 ± 2.42	27.89 ± 2.56	10.53 ± 2.03
PUFAs (g)	15.96 ± 1.56	5.90 ± 1.82	12.45 ± 1.27	2.74 ± 1.30	12.16 ± 1.18	1.96 ± 0.93	11.38 ± 0.98	5.83 ± 1.03
Cholesterol (mg)	284 ± 27	-23 ± 29	316 ± 32	37 ± 41	337 ± 59	-82 ± 54	317 ± 47	-11 ± 42

Fibre (g)	26.05 ± 1.84	1.19 ± 1.54	30.90 ± 2.35	-4.90 ± 3.28	26.93 ± 2.26	3.42 ± 2.61	30.35 ± 2.44	4.08 ± 1.86
Alcohol (g)	15.95 ± 5.92	-0.79 ± 2.84	14.79 ± 6.16	-5.01 ± 3.77	13.98 ± 3.80	-0.59 ± 2.32	12.22 ± 4.39	-3.39 ± 2.45

[1] Values are reported as means ± SEM.

BL, baseline; Δ, change from baseline to post-intervention; CHO, carbohydrates; OBG, oat β-glucan; PL, placebo; PS, phytosterols; PS-OBG, phytosterol + oat β-glucan.

612

FIGURE 1

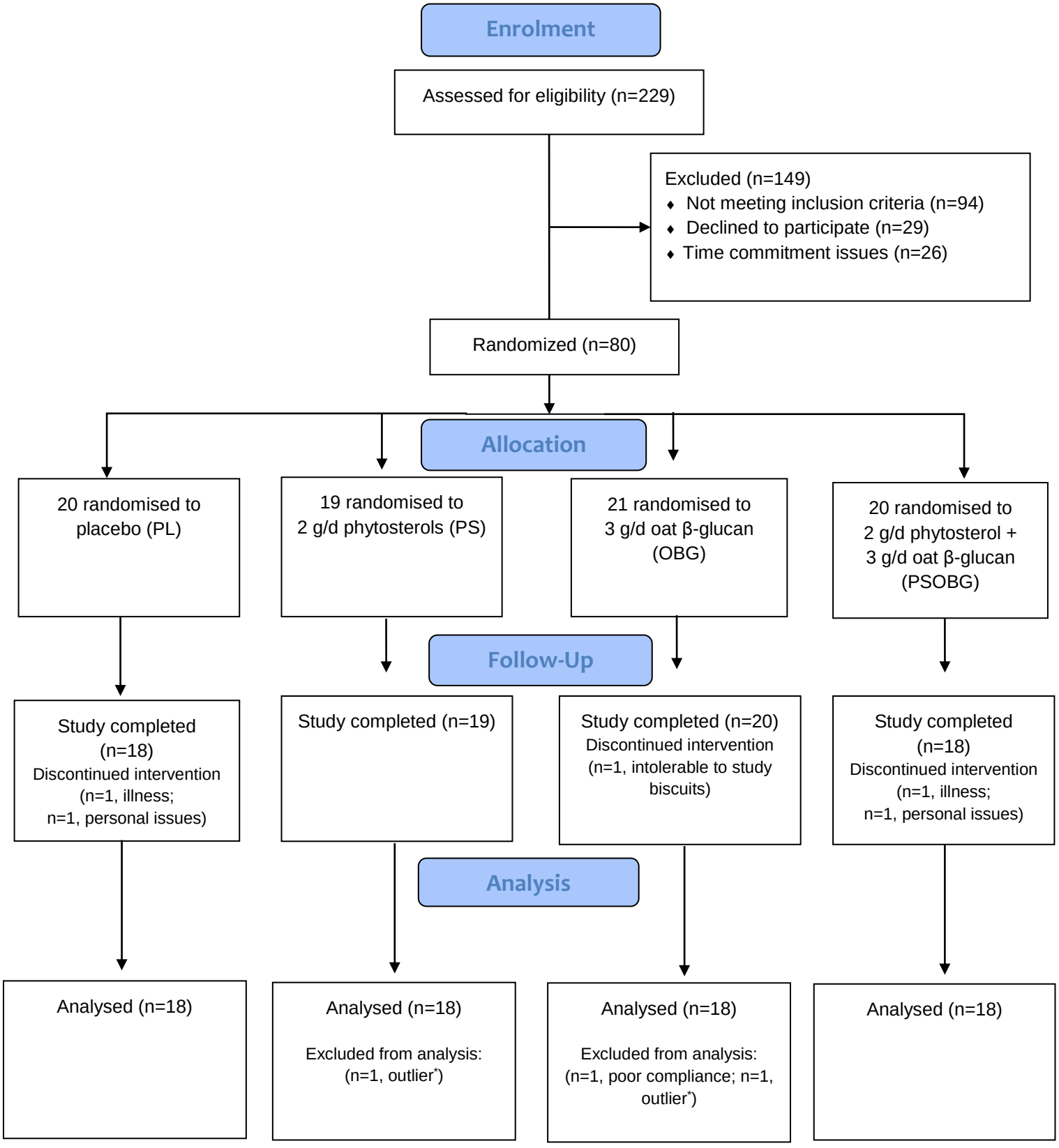
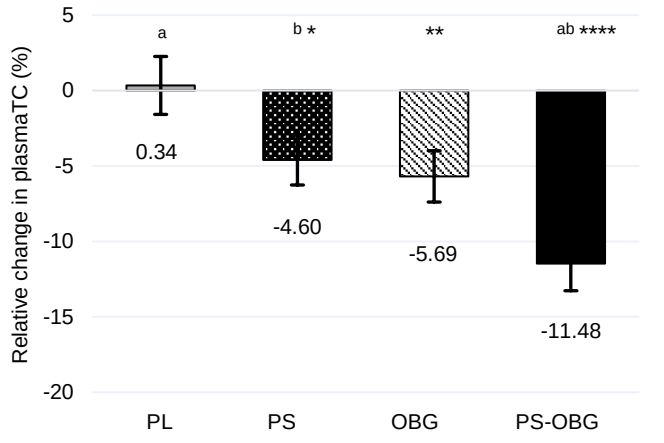
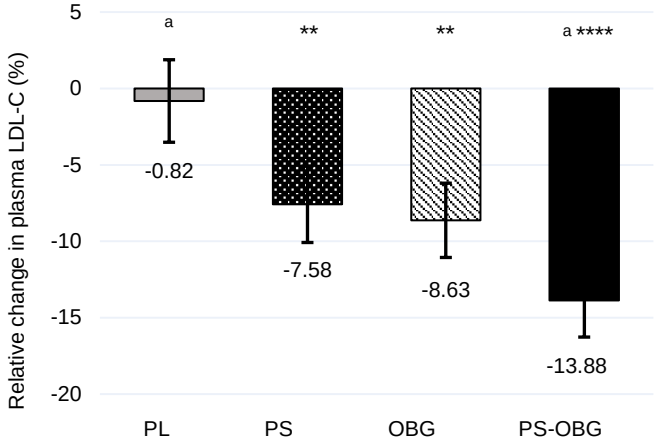
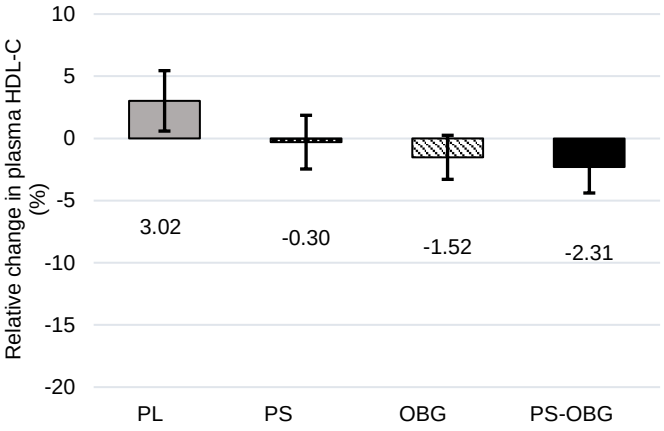
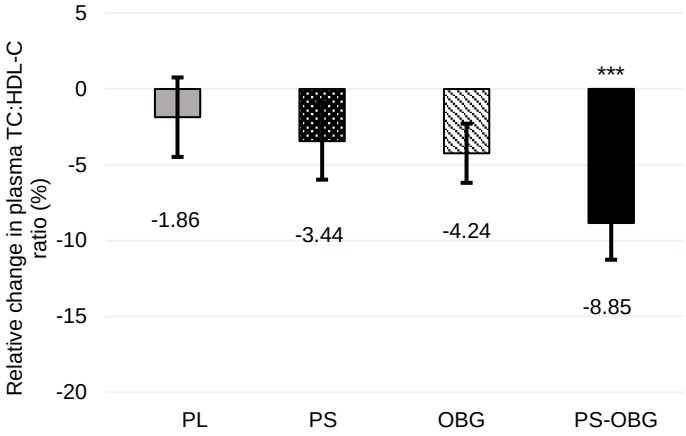

Enrolment
Assessed for eligibility (n=229)
Excluded (n=149)
♦ Not meeting inclusion criteria (n=94)
♦ Declined to participate (n=29)
♦ Time commitment issues (n=26)
Randomized (n=80)
Allocation
20 randomised to placebo (PL)
19 randomised to 2 g/d phytosterols (PS)
21 randomised to 3 g/d oat β-glucan (OBG)
20 randomised to 2 g/d phytosterol + 3 g/d oat β-glucan (PSOBG)
Follow-Up
Study completed (n=18) Discontinued intervention (n=1, illness; n=1, personal issues)
Study completed (n=19)
Study completed (n=20) Discontinued intervention (n=1, intolerable to study biscuits)
Study completed (n=18) Discontinued intervention (n=1, illness; n=1, personal issues)
Analysis
Analysed (n=18)
Analysed (n=18) Excluded from analysis: (n=1, outlier*)
Analysed (n=18) Excluded from analysis: (n=1, poor compliance; n=1, outlier*)
Analysed (n=18)

FIGURE 2


Relative change in plasmaTC (%)
5
0
-5
-10
-15
-20
a
b *
**
ab ****
0.34
-4.60
-5.69
-11.48
PL
PS
OBG
PS-OBG


Relative change in plasma LDL-C (%)
5
0
-5
-10
-15
-20
a
**
**
a ****
-0.82
-7.58
-8.63
-13.88
PL
PS
OBG
PS-OBG


Relative change in plasma HDL-C (%)
10
5
0
-5
-10
-15
-20
3.02
-0.30
-1.52
-2.31
PL
PS
OBG
PS-OBG


Relative change in plasma TC:HDL-C ratio (%)
5
0
-5
-10
-15
-20
***
-1.86
-3.44
-4.24
-8.85
PL
PS
OBG
PS-OBG

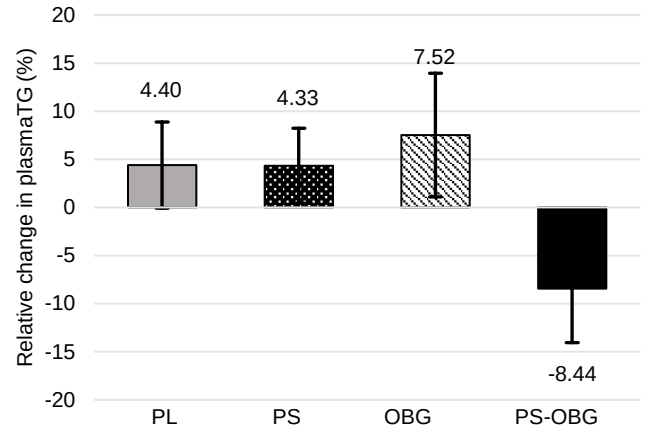
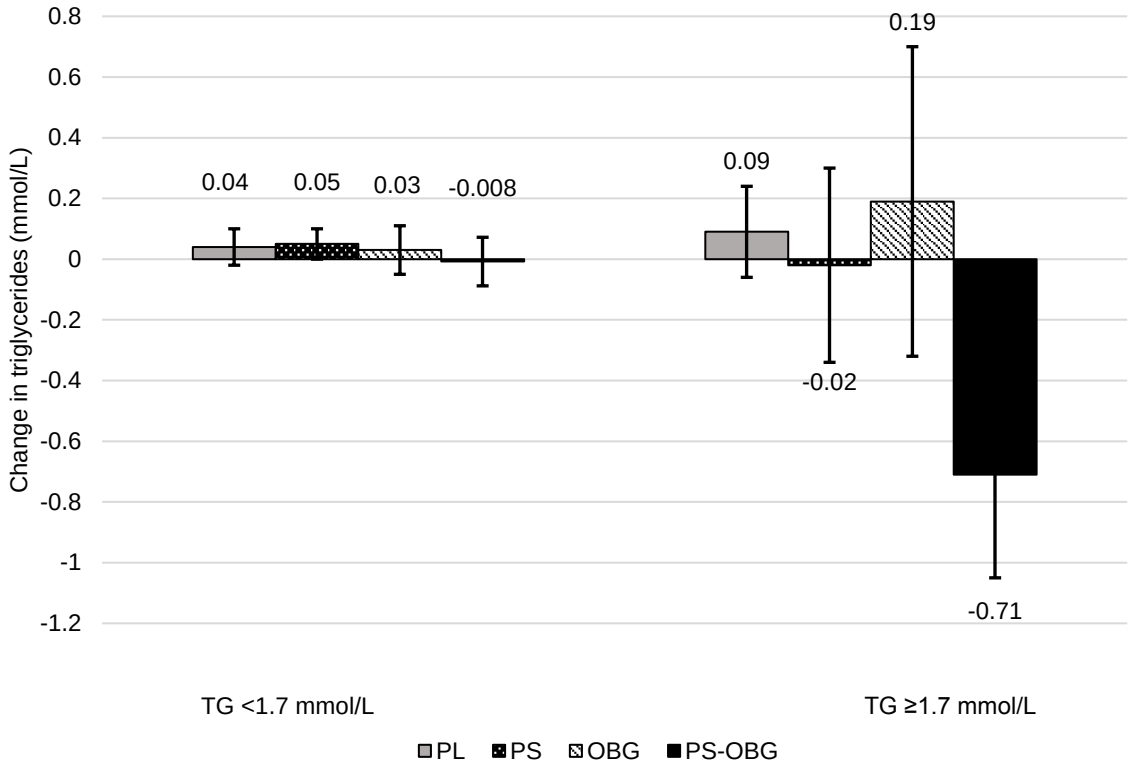

Relative change in plasmaTG (%)
20
15
10
5
0
-5
-10
-15
-20
4.40
4.33
7.52
-8.44
PL
PS
OBG
PS-OBG

FIGURE 3


0.8
0.6
0.4
0.2
0
-0.2
-0.4
-0.6
-0.8
-1
-1.2
Change in triglycerides (mmol/L)
0.04
0.05
0.03
-0.008
0.09
-0.02
0.19
-0.71
TG <1.7 mmol/L
TG ≥1.7 mmol/L
PL
PS
OBG
PS-OBG