Curcumin potentiates cholesterol-lowering effects of phytosterols in hypercholesterolaemic individuals. A randomised controlled trial

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Abbreviations: ACAT, acyl coenzyme A:cholesterol acyltransferase; ATP-binding cassette; CC, curcumin group; CHD, coronary heart disease; CVD, cardiovascular disease; CYP7A, cholesterol 7alpha-hydroxlyase; HDL-C, HDL-cholesterol; HMG-CoA reductase, 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase; HOMA-IR, homeostatic model assessment of insulin resistance; LCn-3PUFA, long-chain omega-3 polyunsaturated fatty acids; LDL-C, LDL-cholesterol; LXR, liver X receptor; NPC1L1, Niemann-Pick C1-Like 1; PL. placebo group; PS, phytosterols; PS-CC, phytosterols and curcumin combination; RCT, randomised controlled trial; SREBP, sterol regulatory element binding protein; TC, total cholesterol; TC:HDL, total cholesterol-to-HDL ratio; TG, triglycerides.

ABSTRACT

Background: Dietary phytosterols (PS) are well-known hypocholesterolaemic agents. Curcumin elicits hypolipidaemic and anti-inflammatory effects in preclinical studies, however, consistent findings in humans are lacking.

Objective: Concurrent PS and curcumin supplementation may exhibit enhanced hypocholesterolaemic and anti-inflammatory effects to optimise cardio-protection. The objective of this trial was to investigate the effects of dietary intervention with PS with or without curcumin on blood lipids (primary outcome) in hypercholesterolaemic individuals.

Methods: A double-blinded, randomised, placebo-controlled, 2x2 factorial trial was conducted in hypercholesterolaemic individuals. Participants received either placebo (PL, no phytosterols or curcumin), phytosterols (PS, 2g/d), curcumin (CC, 200mg/d) or a combination of PS and curcumin (PS-CC, 2g/d-200mg/d respectively) for four weeks. Primary outcomes included fasting total cholesterol (TC), LDL-cholesterol, HDL-cholesterol, triglycerides (TG), TC-to-HDL-C ratio (TC:HDL-C). Secondary outcomes included anthropometrics and fasting blood glucose concentrations.

Results: Seventy participants with a mean (±SEM) fasting TC concentration of 6.57±0.13 mmol/L completed the study (PL, n=18; PS, n=17; CC, n=18; PS-CC, n=17). PS and PS-CC supplementation significantly lowered TC, LDL-cholesterol and TC:HDL-C post-intervention (p<0.05). Reductions from baseline in the PS group were 4.8% and 8.1% for TC and LDL-cholesterol respectively (p<0.05). CC exhibited non-significant reduction (2.3% and 2.6%) in TC and LDL-C respectively, however, the PS-CC resulted in a greater reduction in TC (11.0%) and LDL-cholesterol (14.4%) than either of the treatments alone (p<0.0001). The reduction in the PS-CC treatment was significantly greater compared to those for CC

(p<0.05) or PL (p<0.01) alone. Plasma HDL-cholesterol and TG concentrations remained unchanged across all groups. No adverse side effects were reported.

Conclusions: The addition of curcumin to phytosterol therapy provides a complementary cholesterol-lowering effect that is larger than phytosterol therapy alone. Implications of these findings include the development of a single functional food containing both the active ingredients for enhanced lipid-lowering and compliance in hypercholesterolaemic individuals.

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1. INTRODUCTION

Cardiovascular disease (CVD) remains the number one killer worldwide claiming 31% of all deaths [1]. In Australia one in six people are affected by CVD and 45,392 lives were claimed by the disease in 2015 [2]. These statistics are alarming since every 12 minutes, one Australian dies from CVD [3]. CVD is Australia's most expensive condition to treat, costing $1.8 billion per year for CVD medications [4] and $18.3 billion in total economic costs in 2014 [5]. CVD poses several risk factors and 90% of the Australian population are estimated to have at least one [6]. Dyslipidaemia, as indicated by elevated concentrations of TC, LDL-cholesterol (LDL-C) and TG, as well as low concentrations of HDL-cholesterol (HDL-C), continues to be a major CVD risk factor [7]. In 2011-12, one third of the Australian adult population had abnormal or elevated LDL-C concentrations and 23% had low concentrations of HDL-C [8]. Whilst lifestyle and pharmacological therapies have proven useful for managing dyslipidaemia, it is evident that simple, safe, free of serious side effects, cost-effective and more efficacious strategies are required.

Phytosterols (PS) are non-nutritive compounds naturally found in foods of plant origin that are structurally analogous to cholesterol and are well known for their cholesterol-lowering ability [9]. In Australia, PS have been added to common foods such as vegetable fat spreads and dairy milk in order to assist individuals with achieving therapeutic doses. An average daily dose of 2g phytosterols lowers plasma LDL-C by approximately 0.31-0.34 mmol/L or 8-10% within 3-4 weeks [10, 11]. Their ability to lower LDL-C concentrations through reduced intestinal absorption has been well documented. The most widely accepted mechanism underlying this property is attributed to competitive displacement of dietary and biliary cholesterol in mixed micelles due to higher affinity for PS [12]. Dietary supplementation of PS are safe and measured absorption [13] and plasma concentrations are very small with the majority recovered in the stool [14]. Curcumin is a polyphenol compound

found in the perennial herb *Curcuma Longa* (turmeric) which belongs to the ginger family. Curcumin is well known for its abundance of health benefits, namely anti-inflammatory, anti-proliferative, anti-oxidant and anti-apoptotic effects [15]. In regards to cholesterol homeostasis, preclinical studies have shown that curcumin modulates hepatic gene expression, inhibits cholesterol biosynthesis via down-regulation of major lipogenic factors [16-20], stimulates bile acid secretion, enhances clearance of cholesterol as bile [21, 22] and mobilises and decreases accumulation of lipids from adipose tissue [21, 23]. Curcumin is poorly bioavailable as it rapidly undergoes degradation in the liver [15]. Incorporation of curcumin into liposomes and phospholipids [24], encapsulation as polymer nanoparticles and complexed with piperine [25], a known inhibitor of hepatic and intestinal glucuronidation have been shown to improve bioavailability. Curcumin supplementation is safe in humans with no adverse side effects or events reported, even at higher doses for over 12 months [8, 15, 26].

Since elevated blood lipids and inflammation are the two major CVD risk factors, concurrent PS and curcumin therapy has the potential to provide a safe and efficacious means of protection against the development of CVD. PS and curcumin are well tolerated in humans and do not bear any serious side effects even at high doses [27, 28]. Both nutraceuticals are recognised as lipid-lowering tools in clinical practice; PS for their major role as a cholesterol absorption inhibitor and curcumin as an inhibitor of hepatic cholesterol synthesis [20]. The aim of this study was to examine the effects of dietary intervention with PS with or without concurrent supplementation with curcumin on circulating blood lipids and to investigate whether curcumin complements or acts in synergy with PS to modulate lipid markers.

2. METHODS

2.1 Recruitment

Participants with hypercholesterolaemia were recruited from the Hunter region (NSW, Australia) by radio announcements, newspaper articles and advertisements placed around the local community. Volunteers were assessed for eligibility by a study investigator if they were: healthy adults aged between 18 to 70 years; fasting plasma total cholesterol > 5.5 mmol/L; no CVD, diabetes mellitus, kidney/liver conditions, chronic inflammatory diseases, neurological conditions or untreated hypertension (≥ 140/95 mm Hg); not taking lipid-lowering or anti-inflammatory medication; not consuming PS-enriched products or curcumin/turmeric supplements or any other supplements known to influence study outcomes e.g. fish oil, krill oil, flaxseed oil, coenzyme Q10, fibre; BMI < 40 kg/m^2; not pregnant or lactating; non-smoker; and no strong aversion and/or intolerance/allergy to the foods used in the study. The study protocol was approved by the Human Research Ethics Committee, University of Newcastle (protocol no. H-2015-0162) and was conducted in accordance with the 1975 Declaration of Helsinki as revised in 1983. All participants provided written informed consent prior to inclusion in the study. Participants were de-identified at point of enrolment and identified by alphanumeric codes. The trial was registered with the Australian and New Zealand Clinical Trials Registry at http://www.anzctr.org.au/ as 1261500095650.

2.2 Trial design

Subjects participated in a four-week, double-blinded, randomised, placebo-controlled, 2x2 factorial intervention in four parallel groups. Allocation to treatment groups was conducted by the lead investigator (JF) using a computer-generated block randomisation approach with subjects stratified by gender (Random Allocation Software version 1.0.0). Blocks of size eight were used to achieve the goal sample size in each group. Participants

were de-identified and assigned alphanumeric codes for identification. The treatment allocations were concealed and revealed at the conclusion of the study by the senior investigator (MG). Food product containers were labelled before study commencement with a code to ensure that neither the investigators nor volunteers could determine intervention allocation.

Participants were randomised to one of four treatment combinations: placebo (PL; 25 g/d fat spread plus two placebo tablets), phytosterol-only (PS; 25 g/d PS-enriched fat spread containing 2 g/d PS plus two placebo tablets), curcumin-only (CC; or 25 g/d fat spread plus two tablets containing 200 mg/d curcumin) or a combination of PS plus curcumin (phytosterol + curcumin, PS-CC). Each 1 gram curcumin tablet contained 500 mg curcuma phospholipid (Meriva ® Indena) plus excipients; delivering 100 mg curcumin. The placebo tablet was comparable but devoid of both curcuminoids and soy lecithin, containing more microcrystalline cellulose. Participants were instructed to consume two tablets each day (one with morning meal and one with evening meal). The PS-enriched vegetable fat spread was commercially available (Logicol Original) and predominately based on canola oil and the placebo spread was equivalent in nutritional profile including fats but devoid of PS fortification (MeadowLea Canola), see **Table 1**. The main source of PS esters used in Australian PS-fortified food products is derived from soybean oil or tall (pine) oil [29]. The fat spreads were provided to participants in individually portioned containers (25 g/d) and participants received detailed instruction to displace all habitual margarine and/or butter consumption with the study spread. Compliance was monitored by evaluating the consumption log, weighing returned tubs, tablet count pre- and post-intervention and evaluation of dietary records (analysed with FoodWorks, Xyris ®, Professional Edition Version 8.0.3551). Irregular use of medications or illness experienced were also logged for the duration of study participation.

2.3 Clinical assessments

Participants attended two visits (baseline and post-intervention) at the University of Newcastle, Callaghan, NSW in an overnight fasted state (≥ 10 h) where anthropometric measures, medical history, dietary intake, physical activity patterns and fasting blood samples were collected for primary (plasma TC and LDL-C, HDL-C, TC:HDL ratio, TG) and secondary outcome measures (glucose).

2.4 Anthropometry and body composition

Anthropometrics (height, weight, waist circumference, weight, BMI) and body composition was collected at baseline and post-intervention. Participants were measured wearing light clothing and asked to remove shoes and all metal and/or electronic devices on person 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. Waist circumference was measured using a tensible tape measure positioned midway between the lower rib margin and the iliac crest (approximately belly-button) horizontally. BMI was calculated as weight/height^2 (kg/m^2). Weight, along with other body composition parameters (skeletal muscle mass, fat mass, total body water etc) were measured using bioelectrical impedance (BIA) utilising two different frequencies (InBody230, Biospace Co.). Measurements were taken in the standing position following a ≥10 h fast and participants refrained from vigorous physical activity and alcohol consumption 24 h prior to testing.

2.5 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; prescribed/over-the-counter medication(s); use of supplements and habitual intake of alcohol,

PS-enriched products, curcumin/turmeric supplements and habitual use of fats, oils and added sugars. A three-day food diary and physical activity questionnaire (International Physical Activity Questionnaire; IPAQ Long Last 7 Days Self-Administered Format, October 2002) were used to collect measures of habitual dietary intake and physical activity levels (respectively). Dietary data was entered into a food database system (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.

2.6 Blood sampling and serum lipid analyses

Fasting blood samples (10 h) were collected into tubes pre-coated with EDTA via venepuncture by a trained phlebotomist at baseline and post-intervention. Samples were prepared by centrifuging (Heraeus Biofuge Stratos) for 10 minutes at 3000 x *g* at 4ºC. Plasma, buffy coat and red blood cell sub-fractions were aliquoted and stored at -80 ºC until further analysis. Plasma TC, HDL-C, TG and glucose concentrations were measured on a VP auto analyser using standardized reagents by Hunter Area Pathology Service. LDL-C concentration was determined using the Friedewald equation [30].

2.7 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) unless otherwise specified and the significance level for all statistical tests was set at 0.05. Sample size was calculated based on previous estimates of variance in plasma TC concentration (standard deviation 0.5) yielding 80 participants in total (20 in each group) to provide 80% power at a 0.05 significance level for detection of a 0.50 mmol/L (~10%) reduction in TC and accounting for a 20% dropout rate. At baseline,

comparability of treatment groups for age, height, weight, BMI, body composition, dietary intake, fasting lipid profile and change in dietary parameters were assessed by ANOVA for normally distributed data and Kruskal-Wallis when the assumption of normality was not met. Comparability between treatment groups for ethnicity and gender was evaluated by the chi-square test. Within-treatment comparisons from baseline to post-intervention were performed using the paired samples t-test. Absolute change (mmol/L) was calculated as post-intervention value minus baseline value and the percent change (%) was calculated as the absolute change divided by the baseline value, then multiplied by 100. Therefore, for change data, a negative sign denotes a reduction. The effect of each treatment on the absolute and percent change from baseline to follow up on the dependent variables (lipid profiles) between groups was explored using one-way ANOVA. Two-way ANOVA was used to determine whether there was a significant main effect for each independent variable. An interaction term [PS x curcumin] was tested between the two independent variables to investigate their effect on the dependent variables. For significant effects, Tukey's honestly significant difference was used to perform post hoc comparisons to test for complementarity and/or synergy between PS and curcumin. A separate multiple linear regression model was then considered for each response variable (absolute- and percent change in TC and LDL-C) with treatment group and the corresponding baseline values of the response variables included as explanatory variables. The effect of multiple explanatory variables were also adjusted for in the model by their inclusion as explanatory variables (age, BMI, baseline LDL-C concentrations, baseline dietary total fat, fibre, alcohol and LCn-3PUFA). Correlations between potential baseline explanatory variables were assessed and variables with correlation coefficients above 0.8 were identified as potentially multicollinear and the number of potential predictors to include in the regression was reduced accordingly. The backward stepwise procedure was used for each regression to select the optimal set of predictors for each model.

3. RESULTS

3.1 Baseline characteristics

This study recruited 76 participants during the period from end-October 2015 to end-October 2016. Four participants dropped out of the study due to inability to comply with daily fat spread consumption (n=3) and illness (n=1). A further two participants were excluded from the study (poor compliance with study products, n=1 and unreliable (abnormally high TG) blood test results, n=1). A total of 70 participants (57% females) were included in the final analysis (PL, n=18; PS, n=17; CC, n=18; PS-CC, n=17) with a mean age of 50.70±1.51 y, BMI of 27.16±0.50 kg/m^2, waist circumference of 92.05±1.32 cm, waist-to-hip ratio of 0.93±0.01, skeletal muscle mass of 30.04±0.90 kg and fat percentage of 31.82±1.00. The majority of study participants were north-west European (79%). Participant allocation is presented in the CONSORT diagram (**Figure 1**). At baseline, study participants were hypercholesterolaemic with TC of 6.57±0.13 mmol/L, LDL-C of 4.38±0.11 mmol/L, HDL-C of 1.51±0.05 mmol/L, TC:HDL ratio of 4.61±0.15 and median (IQR) TG of 1.29 (0.78) mmol/L. All participants were comparable on baseline characteristics since there were no statistically significant differences between treatment groups (**Table 2**).

3.2 Dietary intake, physical activity and compliance

All groups were comparable at baseline for dietary intake (**Table 3**). Comparisons between groups showed no significant differences in the mean change of dietary parameters from baseline to post-intervention. Since there was no significant change in dietary fat intake or body weight, it is likely participants replaced habitual fat intake with the intervention fat spread.

Mean compliance with study product intake was excellent for both fat spreads (94.58±1.14 %) and tablets (95.77±0.83 %) in all groups. The intervention was tolerated well,

and no adverse events were reported. Physical activity levels did not significantly change from baseline to post-intervention nor was there any statistically significant differences between groups at both time points (data not shown).

3.3 Effect of phytosterol and curcumin intervention on plasma lipid profile and glucose

After four weeks intervention TC, LDL-C and TC:HDL ratio were all significantly reduced both in terms of absolute- and relative change values: -0.34±0.11 mmol/L (p=0.008) and -4.8±1.7% (p=0.013), -0.38±0.10 mmol/L (p=0.002) and -8.1±2.4% (p=0.004) and -0.36±0.11 (p=0.004) and -7.2±2.2% (p=0.006) respectively in the PS group and -0.74±0.16 mmol/L (p=0.0002) and -11.0±1.9% (p<0.0001), -0.63±0.12 mmol/L (p=0.001) and -14.4±2.3% (p<0.0001) and -0.48±0.14 (p<0.01) and -9.6±2.2% (p=0.0004) respectively in the PS-CC group (**Table 4**, **Figure 2**). Blood cholesterol parameters did not significantly change from baseline in the PL and CC group. Across all four treatment groups, there was a significant difference in absolute and percent change in TC (p<0.01), LDL-C (p< 0.01), TC:HDL ratio (p<0.05) and plasma glucose (p<0.05). Post-hoc analyses showed that for TC, PS-CC had a significantly larger reduction in absolute (-0.62±0.19 mmol/L, p=0.011) and percent change (-8.70±2.80%, p=0.015) compared to CC, and compared to PL (-0.66±0.19 mmol/L, p=0.006 and -9.78±2.80%, p=0.005, respectively). The same trend was evident for LDL-C, whereby the PS-CC group had significantly larger reductions in absolute (-0.56±0.17 mmol/L, p=0.012) and percent change (-11.86±3.96%, p=0.020) compared to CC, and PL (-0.56±0.17 mmol/L, p=0.010 and -13.53±3.96%, p=0.006). The reduction in TC:HDL ratio was significantly larger in the PS-CC group compared to the CC group only (-0.58±0.19 mmol/L, p=0.020 and -11.57%, p=0.019). Absolute and percent change in plasma glucose concentrations significantly reduced in the CC group compared to PL group only (-0.34±0.13 mmol/L, p=0.038 and -6.94±2.51%, p=0.036). There was no statistically significant differences in HDL-C or TG concentrations between groups. When the data were analysed

using two-factor ANOVA, PS x curcumin interactions were not significant. Additionally, there was no significant main effect of curcumin on post-treatment absolute or percent TC and LDL-C values, however, there was a significant (p<0.01) main effect of PS on post-intervention absolute and percent TC and LDL-C values. Baseline data including BMI, LDL-C concentration and dietary intake of total fat, fibre, alcohol and long chain omega-3 polyunsaturated fatty acids (LCn-3PUFA) were investigated as potential confounders using multiple linear regression models. The final reduced models revealed that treatment was the only significant predictor of the change in TC and LDL-C.

4. DISCUSSION

The hypocholesterolaemic property of PS has been well established and recognised as an asset to CVD risk reduction. The potential to heighten their lipid-modulating property has been demonstrated by coupling with LCn-3PUFA [31-33] and statin therapy [34-37]. The results presented in this study demonstrate the efficacy of curcumin to potentiate the cholesterol-lowering effects of PS in hypercholesterolaemic individuals. Our findings provide evidence of a complementary reduction in TC and LDL-C following concomitant supplementation with PS and curcumin. Although statistically insignificant, TC and LDL-C reduction following dual supplementation exceeded the additive effect of the two bioactives.

Reductions in TC and LDL-C following PS supplementation are consistent with previous studies of similar duration [10, 38-40]. A systematic review and meta-analysis of 84 randomised controlled trials (RCT) concluded that LDL-C reduced by -8.8% (vs -8.1% in our study) after a mean dose of 2.15 g/d of PS [10]. Higher baseline plasma LDL-C results in a greater reduction in plasma LDL-C [10, 41], however, in this study, covariate analysis showed that baseline LDL-C was not a predictor of reductions in TC or LDL-C.

In addition to competitive micellar incorporation [9], there are a number of reported mechanisms by which PS lower plasma cholesterol concentrations, some of which may be enhanced when combining PS with curcumin. Preclinical findings report PS may suppress acyl coenzyme A:cholesterol acyltransferase (ACAT) and therefore inhibit cholesterol esterification causing a reduction in cholesterol uptake and/or transport in the intestine [42]. A study in db/db mice showed that curcumin supplementation significantly lowered the activity of hepatic ACAT in addition to observed reductions in plasma concentrations of free fatty acids and cholesterol [43], suggesting curcumin may play a modulatory role in the activity of enzymes involved in lipid homeostasis. PS may also promote cholesterol efflux by initiating

the expression and activity of ATP-binding cassette A1 (ABCA1) transporter which cannot differentiate between cholesterol and PS [44, 45].

Cholesterol transporters ABCA1, ABCG5 and ABCG8 are regulated by liver X receptor alpha (LXRα) and mediate efflux of free sterols from enterocytes. PS have been shown to be a potent activator of LXR in regards to agonising LXR [46, 47]. In this regard, PS metabolites have been identified as natural ligands for LXR [48]. Likewise, curcumin treatment has been shown to upregulate the expression of LXRα in association with reduced TG accumulation in the liver [16]. It is in this way curcumin may potentiate the mechanistic hypocholesterolaemic effects of PS to interact with intracellular cholesterol sensors like LXR to indirectly modulate transporter activity as well as compete for cholesterol transporters [12].

Preclinical studies report several mechanisms by which curcumin acts as a lipid-modulating agent [16, 21, 49, 50]. Curcumin has been shown to down-regulate 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase (HMG-CoA reductase) [16], the rate limiting step in cholesterol biosynthesis. Curcumin therapy has been shown to lower plasma and hepatic cholesterol concentrations [16]. These effects were comparable to that of lovastatin treatment and were caused by transcriptional inhibition of HMG-CoA reductase. Moreover, preclinical studies have demonstrated decreased activity of hepatic ACAT after curcumin treatment in mice fed a high fat diet [43, 51], resulting in reduced cholesterol uptake and transport in the intestine [42]. In the intestinal Caco-2 monolayer, curcumin inhibited Niemann-Pick C1-Like 1 (NPC1L1) expression via the inhibition of sterol regulatory element binding protein-2 (SREBP-2) thus lowering cholesterol esterification [52]. Inhibition of HMG-CoA reductase and NPC1L1, the two key molecular targets of statins and ezetimibe respectively, characterises curcumin as a potentially powerful cholesterol absorption inhibitor, alluding to complementary effects when coupled with PS. Curcumin has also been shown to promote efflux and clearance of cholesterol by stimulating bile secretion via enhancing hepatic gene

expression of cholesterol 7alpha-hydroxlyase (CYP7A) mRNA, the rate-limiting enzyme involved in bile acid biosynthesis from cholesterol [21, 22, 49]. CYP7A is further promoted by curcumin via the activation and upregulation of LXR and ABCA1 in the liver and subcutaneous adipocytes [53]. In addition, curcumin has been shown to decrease cholesterol biosynthesis via down-regulation of SREBP-1 and SREBP-2; key nuclear receptors involved in mediating lipid metabolism [17, 18].

Despite the promising findings from preclinical investigations, current clinical evidence remains scarce and somewhat inconsistent. A recent RCT by Panahi et al reported significant improvements in circulating blood lipids after supplementation with bioavailability-enhanced curcuminoids in metabolic syndrome individuals [54]. Curcuminoid supplementation led to significantly lower concentrations of TC, LDL-C, non-HDL-C, TG and lipoprotein(a) in addition to significant elevations in HDL-C [54]. These clinically significant trends in lipid parameters were not replicated in the CC group of the present study. It is likely that the study duration of the present trial was inadequate for curcumin to elicit significant hypolipidaemic responses. It should be noted that all subjects in Panahi et al [54] trial received standard care including administration of medications for lipid-lowering, hypotension and hyperglycaemia. Therefore, it is difficult to ascertain the precise modulatory effects of curcumin given the concurrent lifestyle intervention, diet and pharmacological modifications. A recent systematic review and meta-analysis of five RCTs concluded curcumin did not elicit any significant effects on blood lipids in humans [55]. The included studies were largely heterogeneous; individuals were not exclusively dyslipidaemic and none of the studies used bioavailability-enhanced formulations of curcumin. These limitations possibly impeded any significant effects on blood lipids, particularly given the poor bioavailability of curcumin in native form. Since the degree of LDL-C-lowering achieved in the PS-CC group was nearly double that of

the well-established reduction following PS therapy [10], complementary lipid-lowering mechanisms may be in play following the combined PS/curcumin supplementation.

Irrespective of the mechanisms, it is evident that curcumin may potentiate cholesterol-lowering effects of PS, since the PS-CC group had the greatest reduction in plasma cholesterol compared to either of the treatments alone. It is possible that in the combined group, bioavailability of curcumin is enhanced to heighten its hypolipidaemic effects as the bioavailability of curcumin has been shown to be enhanced in a fat-rich medium [56]. The short duration of the study remains a limitation, however, it is well documented that PS elicit hypocholesterolaemic effects within 3-4 weeks, less is known about the duration regarding the hypolipidaemic effects of curcumin. It is possible that a longer study duration may provoke more pronounced hypolipidaemic effects, since positive longer-term trials investigating the hypolipidaemic effects of curcumin have been reported [54, 55]. Participants in the current study were not specifically instructed to consume the vegetable fat spreads at the same time point as the tablets. Thus, some participants consumed the two products widely apart across the day. As previously mentioned, curcumin has enhanced bioavailability in a fat-rich medium, therefore, a single food matrix may likely further enhance the synergy between the two bioactive compounds, hence, the development of a single food containing the two compounds, provoking the translational aspect of these research findings. Strengths of this study include the robust study design pertaining 2x2 factorial group treatment to isolate the effects of each treatment arm as well as combined effects, in a double-blinded, placebo-controlled randomised manner in free-living individuals. We used a curcumin complexed with phosphatidylcholine (Meriva®) that ensured a high bioavailability of curcumin. This delivery matrix retains the biological effects and free-radical scavenging activity of curcumin [56]. The tablets and delivery of PS-enriched fat spread was easily compliable and transferable to the

everyday diet (respectively) ensuring adequate intake of both active ingredients with no adverse effects.

The LDL-C reductions reported in the current study hold clinical significance for minimising heart disease risk since coronary heart disease (CHD) all-cause mortality is reduced by 12% for every 1 mmol/L reduction in LDL-C [57]. In the present study, LDL-C was lowered by 0.63 mmol/L representing approximately 7.6% reduction in CHD mortality after only four weeks of PS + curcumin intervention. Considering the known anti-inflammatory, anti-aggregatory and anti-oxidant effects [15] and safety of curcumin [58], the complementary and possibly synergistic cholesterol-lowering effects demonstrated makes the combined treatment a good candidate for a safe, effective alternative or adjunct therapy for CVD risk reduction that potentially bares a multifaceted approach. The combined PS-CC therapy bears significant implications for use as an adjunct therapy to statins and/or ezetimibe and potentially enhance lipid-lowering, reduce drug dependence and possibly reduce the dose required. The concomitant therapy of PS and statins has shown incremental reductions in LDL-C of 10-15%; a superior outcome compared to doubling the statin dose (6%) [59]. The use of PS-CC as an adjunct therapy may provide a safe and effective avenue for high-risk patients who fail to achieve LDL-C targets whilst on statin monotherapy or those who are statin intolerant and/or otherwise seeking an alternative approach to pharmacological interventions due to side effects such as chronic musculoskeletal pains [60].

In conclusion, dietary combination of PS and curcumin for four weeks significantly reduced fasting plasma TC and LDL-C in hypercholesterolaemic individuals. Compared to single supplementation of PS or curcumin, the combined group elicited a greater reduction than either treatment administered alone including a statistically greater reduction compared to PL and CC. Curcumin may potentiate the cholesterol-modulating effects of PS, suggestive of additive effects and potentially synergistic in nature. Further investigations exploring this

combination are warranted to determine the optimum delivery mode and duration to maximise the cardio-protective properties of PS. Our findings initiate the need for future research exploring the potential mechanistic actions of curcumin to enhance our understanding of its role in lipid metabolism in humans and to confirm the cardio-protective benefits it may offer to PS therapy. The reason for a reduction in glucose level in the CC group but no change in the PS-CC group is not clear and should be followed up in further studies. In addition to cholesterol-lowering, concurrent PS and curcumin supplementation may exhibit anti-inflammatory effects, therefore, providing additional cardiometabolic benefits, however, investigation into circulating chronic inflammatory parameters following this combination therapy are required to confirm this hypothesis. Future investigations involve the development of a single food containing PS and curcumin for ease of consumption, improved compliance and bioavailability, and ultimately enhanced lipid-lowering in hypercholesterolaemic individuals at risk of CVD.

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6. CONFLICT OF INTEREST STATEMENT

The authors have no conflicts of interest to declare.

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9. FIGURE LEGENDS

Figure 1

CONSORT flow diagram of participant recruitment, screening and assessment.

Figure 2

Percent change in plasma TC (A), LDL-C (B), HDL-C (C), TC:HDL ratio (D), TG (E) and Glucose (F) from baseline to post-intervention in hypercholesterolaemic individuals who consumed PL, PS, CC and PS-CC for 4 weeks. Data represent mean ± SEM accept for TG where data are median (IQR). Symbols indicate significant changes from baseline as analysed by paired samples t-test: * $p<0.05$, ** $p<0.01$, *** $p<0.001$, **** $p<0.0001$. One-way ANOVA and Tukey's honestly significant difference was used to perform post hoc comparisons of group means. Means with a common letter significantly differ:

[a] PS-CC and PL significantly differ for fasting plasma TC ($p=0.005$) and LDL-C ($p=0.006$).

[b] PS-CC and CC significantly differ for fasting plasma TC ($p=0.015$), LDL-C ($p=0.020$) and TC:HDL ratio ($p=0.019$).

[c] CC and PL significantly differ for fasting plasma glucose ($p=0.036$).

CC, curcumin; HDL, HDL-cholesterol; LDL-C, LDL-cholesterol; PL, placebo; PS, phytosterols; PS-CC, phytosterol-curcumin; TC, total cholesterol; TC:HDL ratio, total cholesterol-to-HDL ratio; TG, triglycerides.

10. TABLES

Table 1: Nutrient composition of phytosterol and control spread.[1]

Dietary component	Control spread	Phytosterol spread
Energy (kJ)	600	600
Total fat (g)	16.25	16.00
Saturated (g)	4	4
Monounsaturated (g)	9.00	7.75
Polyunsaturated (g)	3.25	4.25
Omega-3 (ALA) (g)	1.00	1.05
Phytosterols (g)	0	2
Sodium (mg)	87.5	87.5
Potassium (mg)	12.5	10.0

[1] Nutrient information is given for one serving (25 g). Each participant had to consume one serving of spread per day. Data provided by manufacturer (MeadowLea Foods) and Goodman Fielder.

ALA, alpha-linolenic acid.

Table 2. Participant characteristics at baseline in the placebo (PL), phytosterol (PS), curcumin (CC) and phytosterol + curcumin (PS-CC) groups.[1]

	PL (n = 18)	PS (n = 17)	CC (n = 18)	PS-CC (n = 17)
Sex, *n* (%)				
Male	7 (39)	8 (47)	8 (44)	7 (41)
Female	11 (61)	9 (53)	10 (56)	10 (59)
Ethnicity, *n* (%)				
North-west European	13 (72)	11 (65)	15 (83)	14 (82)
South-east European	0 (0)	4 (24)	2 (11)	0 (0)
Other [2]	5 (28)	2 (11)	1 (6)	3 (18)
Age (y)	50.11 ± 2.96	51.35 ± 3.62	51.00 ± 2.34	50.35 ± 3.36
Height (cm)	168.64 ± 2.77	171.56 ± 2.08	170.60 ± 2.43	169.30 ± 2.21
Waist circumference (cm)	87.79 ± 2.47	92.72 ± 2.16	96.54 ± 2.65	91.14 ± 3.00
Waist-to-hip ratio	0.93 ± 0.01	0.93 ± 0.01	0.95 ± 0.01	0.93 ± 0.02
Weight (kg)	74.36 ± 3.72	80.61 ± 2.19	83.86 ± 3.98	76.64 ± 3.83
BMI (kg/m^2)	25.94 ± 0.86	27.31 ± 0.43	28.79 ± 1.31	26.57 ± 1.12
Skeletal muscle mass (kg)	28.14 ± 1.93	31.21 ± 1.53	31.56 ± 1.95	29.29 ± 1.72
Body fat (%)	31.99 ± 2.33	31.19 ± 1.94	32.95 ± 2.04	31.09 ± 1.78

[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; South-east Asian; North-east Asian; Southern and central Asian; Sub-Saharan Africa; Other (n=1 unsure, n=1 combination of races).

CC, curcumin; PL, placebo; PS, phytosterols; PS-CC, phytosterol-curcumin

Table 3. Reported macronutrient and fatty acid intakes of hypercholesterolaemic adults who consumed placebo (PL), phytosterol (PS), curcumin (CC) and phytosterol + curcumin (PSCC) at baseline and change from baseline to post-intervention.[1]

	PL (n=18)		PS (n=17)		CC (n=18)		PSCC (n=17)	
	BL	Δ	BL	Δ	BL	Δ	BL	Δ
Energy (kJ)	8744.85 ± 548.93	-68.72 ± 386.68	8816.17 ± 558.83	152.30 ± 411.20	9015.90 ± 620.12	-224.29 ± 362.04	8820.07 ± 354.72	734.18 ± 505.65
Protein (g)	99.57 ± 7.80	-6.34 ± 6.31	96.09 ± 8.86	-9.71 ± 8.12	106.07 ± 7.67	-5.60 ± 4.03	96.38 ± 5.64	-2.46 ± 5.32
CHO (g)	224.14 ± 15.51	-14.35 ± 9.48	224.14 ± 15.31	-11.22 ± 12.29	203.78 ± 19.04	-12.72 ± 11.63	207.69 ± 13.64	6.28 ± 16.67
Sugars (g)	99.30 ± 10.56	-16.94 ± 6.68	89.90 ± 7.52	-2.73 ± 6.62	88.32 ± 8.74	-7.05 ± 8.33	89.12 ± 8.20	3.62 ± 7.70
Starch (g)	122.76 ± 8.76	2.21 ± 5.43	132.45 ± 11.71	-9.01 ± 9.34	113.10 ± 11.76	-4.29 ± 8.24	115.83 ± 9.28	2.09 ± 9.91
Total fat (g)	78.29 ± 5.67	4.67 ± 5.01	77.53 ± 6.12	16.51 ± 6.88	86.40 ± 6.58	6.10 ± 3.78	86.34 ± 6.08	18.17 ± 6.11
Saturated (g)	28.16 ± 2.55	0.19 ± 2.71	26.17 ± 2.29	4.36 ± 2.43	31.39 ± 2.43	-0.36 ± 2.25	31.40 ± 3.00	4.79 ± 2.42
Trans (g)	1.28 ± 0.13	0.04 ± 0.20	1.16 ± 0.14	0.18 ± 0.15	1.38 ± 0.17	-0.08 ± 0.16	1.48 ± 0.17	0.11 ± 0.20
MUFAs (g)	29.38 ± 2.03	3.71 ± 2.11	30.75 ± 2.80	7.26 ± 3.04	34.27 ± 2.96	3.94 ± 1.75	33.41 ± 2.80	7.23 ± 3.06
PUFAs (g)	13.45 ± 1.56	1.06 ± 1.01	13.99 ± 1.53	4.12 ± 1.51	13.56 ± 1.35	2.46 ± 0.92	14.42 ± 1.89	5.27 ± 1.43
Cholesterol (mg)	320.82 ± 30.60	-27.20 ± 46.46	260.63 ± 51.36	-11.02 ± 50.02	348.25 ± 30.61	-61.72 ± 26.60	324.69 ± 43.58	-25.21 ± 31.80
Fibre (g)	28.54 ± 2.47	2.52 ± 2.52	31.51 ± 2.65	-3.02 ± 1.86	26.36 ± 2.08	-0.88 ± 1.46	25.88 ± 1.95	1.94 ± 2.27
Alcohol (g)	4.22 ± 2.12	2.74 ± 1.46	9.18 ± 4.02	-2.08 ± 2.90	12.04 ± 2.89	-4.20 ± 2.86	8.81 ± 3.63	-0.34 ± 0.89

[1] Values are reported as means ± SEM.

BL, baseline; CC, curcumin; Δ, change from baseline to post-intervention; CHO, carbohydrates; PL, placebo; PS, phytosterols; PS-CC, phytosterol-curcumin.

Table 4. Change in plasma outcome measures in the placebo (PL), phytosterol (PS), curcumin (CC) and phytosterol + curcumin (PS-CC) groups from baseline to post-intervention.[1]

	PL	PS	CC	PS-CC	*p* [2]
TC					
BL	6.63 ± 0.18	6.39 ± 0.25	6.72 ± 0.36	6.51 ± 0.19	
PI	6.55 ± 0.21	6.05 ± 0.21*	6.60 ± 0.42	5.76 ± 0.16**	
Δ mmol/L[3]	-0.08 ± 0.11†	-0.34 ± 0.11	-0.12 ± 0.16$^{\gamma}$	-0.74 ± 0.16$^{\dagger\gamma}$	0.004
LDL-C					
BL	4.55 ± 0.18	4.23 ± 0.21	4.42 ± 0.31	4.32 ± 0.15	
PI	4.48 ± 0.19	3.85 ± 0.18*	4.35 ± 0.36	3.69 ± 0.16**	
Δ mmol/L [4]	-0.07 ± 0.12†	-0.38 ± 0.10	-0.07 ± 0.14$^{\gamma}$	-0.63 ± 0.12$^{\dagger\gamma}$	0.004
HDL-C					
BL	1.49 ± 0.11	1.43 ± 0.10	1.59 ± 0.10	1.54 ± 0.09	
PI	1.52 ± 0.11	1.46 ± 0.11	1.54 ± 0.12	1.51 ± 0.07	
Δ mmol/L	0.02 ± 0.04	0.04 ± 0.04	-0.05 ± 0.04	-0.03 ± 0.04	0.386
TC:HDL					
BL	4.79 ± 0.34	4.74 ± 0.31	4.47 ± 0.30	4.44 ± 0.28	
PI	4.62 ± 0.30	4.38 ± 0.29*	4.57 ± 0.37	3.96 ± 0.20*	
Δ [5]	-0.17 ± 0.14	-0.36 ± 0.11	0.10 ± 0.16$^{\gamma}$	-0.48 ± 0.14$^{\gamma}$	0.022
TG					
BL	1.27 (0.57)	1.50 (0.71)	1.23 (0.65)	1.24 (0.65)	
PI	1.08 (0.59)	1.41 (0.78)	1.46 (0.93)	0.96 (1.02)	
Δ mmol/L	-0.02 (0.54)	0.01 (0.49)	-0.02 (0.5)	-0.06 (0.3)	0.578
Glucose					
BL	4.93 ± 0.09	5.05 ± 0.10	5.33 ± 0.12	5.09 ± 0.16	
PI	5.14 ± 0.13	5.01 ± 0.07	5.19 ± 0.14	5.04 ± 0.15	

Δ mmol/L [6]	0.21 ± 0.11‡	-0.04 ± 0.08	-0.13 ± 0.07‡	-0.05 ± 0.10	0.046

[1] Values are reported as means ± SEM for all plasma concentrations except triglycerides. Triglycerides are median (IQR) due to lack of normality of the distribution. All baseline and post-intervention data is in mmol/L except for TC:HDL ratio. Significant change from baseline, * $p<0.01$, ** $p<0.001$.

[2] 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 honestly significant difference post-hoc analyses were used to compare differences in mean change between groups when significance was found. Values with matching symbols in each row indicate statistically significant differences between corresponding groups.

[3] TC significantly reduced in the PS-CC group compared to the PL (Δ mmol/L, $p=0.006$) and the CC group (Δ mmol/L, $p=0.011$).

[4] LDL-C significantly reduced in the PS-CC group compared to the PL (Δ mmol/L, $p=0.010$) and CC group (Δ mmol/L, $p=0.012$).

[5] TC:HDL ratio significantly reduced in the PS-CC group compared to the CC group (Δ mmol/L, $p=0.020$)

[6] Glucose significantly reduced in the CC group compared to the PL group (Δ mmol/L, $p=0.038$).

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