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ORIGINAL CONTRIBUTION

The lipid-lowering effect of once-daily soya drink fortified with phytosterols in normocholesterolaemic Chinese: a double-blind randomized controlled trial

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Abstract

Purpose Phytosterols reduce intestinal cholesterol absorption and help to lower LDL-cholesterol. Many Chinese adults are lactose-intolerant and cannot tolerate bovine milk enriched with phytosterol. Soya-milk is a common beverage in Asia and it has beneficial effects on general health. We therefore conducted a randomized double-blind controlled trial to assess the effectiveness of a phytosterols-enriched soya drink in lowering serum LDL-cholesterol level (primary outcome) and other cardiovascular parameters (secondary outcomes).

Methods One hundred and fifty-nine normocholesterolaemic participants (85 men and 74 women; aged 19–79) were randomized to daily intake of one serving of phytosterols-enriched soya drink ($N=82$), equivalent to 2 g of phytosterol per day, or a matched soya drink without phytosterols ($N=77$) for 3 weeks. Adverse events, withdrawal and compliance were documented.

Results Among the treatment group ($N=82$), phytosterols-enriched soya drink significantly decreased LDL-cholesterol by 5.96% (SE 1.48, 95% CI −8.91%, −3.00%) with a median of 6.74% compared with baseline, resulting in a significant reduction of 4.70% (95% CI −8.89%, −0.51%; $p=0.028$) with a median of 5.20% compared with placebo ($N=77$). In contrast, there were no significant changes in other lipid parameters, blood glucose, blood pressure, body weight or waist circumference. Remarkably, 95% of the participants randomized to the fortified drink reported no adverse events at all.

Conclusions Daily consumption of a phytosterols-enriched soya drink may be a simple and cost-neutral means of lowering LDL-cholesterol in individuals in China, with massive population and rising incidence of coronary heart disease (ClinicalTrials.gov identifier: NCT02881658; date of registration: 14 Aug 2016).

Keywords Cholesterol · Phytosterol · Plant sterol · Dyslipidemia · LDL · Clinical trial

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Introduction

Elevated serum low-density lipoprotein cholesterol (LDL-C) level is the major modifiable risk factor of cardiovascular diseases [1]. It is well-documented that reducing LDL-C with pharmacological agents, such as statins, prevents cardiovascular events [2]. Although statins are widely used for treating hypercholesterolemia, concerns about their safety and adverse effects have led to discontinuation and non-compliance of the drug [3]. Thus, there is a need to develop non-pharmacological ways of reducing LDL-C.

Phytosterols, or plant sterols, are found naturally in plants and plant-based foods such as soybean, nuts and seeds. They are structurally similar to cholesterol but cannot be absorbed in the human gut; they thus compete with cholesterol for intestinal absorption and enhance cholesterol excretion. Phytosterols may also affect whole-body cholesterol metabolism, and thereby lower blood cholesterol level [4]. The role of phytosterols in reducing serum levels of LDL-C is recognized by regulatory authorities in EU, US, Canada, and Australia/New Zealand [5]. The FDA Health Claims state that "Foods containing at least 0.65 g per serving of vegetable oil plant sterol esters, eaten twice a day with meals for a daily total intake of at least 1.3 g, as part of a diet low in saturated fat and cholesterol, may reduce the risk of heart disease" [6]. Currently, various food products have been fortified with phytosterols. The food matrix and fat content affect the effectiveness of LDL-C reduction. When the same amount of phytosterols is consumed in low-fat milk, greater reduction of LDL-C was observed compared with that consumed in bread or cereal [7]. Whereas, another study showed a similar effect on lipid reduction in low- and medium-fat soymilk fortified with phytosterols [8].

Adding phytosterols to dairy products is the most common approach in commercial products and their effectiveness in lowering LDL-C has been shown in clinical trials [9]. Our previous randomized controlled trial (RCT) showed that drinking phytosterols-enriched milk twice a day lowered LDL-C in Hong Kong Chinese [10]. However, it is well documented that lactose intolerance is prevalent in Asians [11]. Thus, soya milk, a popular beverage in Asia [12], may serve as a better food matrix for phytosterols. Soya milk is a protein-rich drink and regular intake is considered as part of a healthy diet. It has potential benefits in relieving hot flushes [13] and improving lipid profiles [14]. Long-term intake of isoflavones from soya milk has bone-sparing effects [12]. However, whether phytosterols-enriched soya milk is useful in lowering LDL-C is largely unknown. In particular, the effects of 2 g-phytosterol formulation taken once daily in people with normocholesterolemia has not been previously tested. We therefore conducted a double-blind RCT to evaluate whether taking phytosterols-enriched soya drink once daily would reduce LDL-C in normocholesterolemic Chinese.

Methods

The study design was a randomized, double-blind, single-center, two-arm, placebo-controlled trial. The trial was conducted in a University setting, and in accordance with the Good Clinical Practice (GCP) Guideline issued by The International Council for Harmonisation (ICH) [15]. The study protocol was approved by the Institutional Review Board of the University of Hong Kong/Hospital Authority Hong Kong West Cluster (HKU/HA HKW IRB). All participants gave written informed consent. The trial was registered at clinicaltrials.gov on 14 Aug 2016 and was assigned the number NCT02881658.

Participants were healthy Chinese male or female aged 18 or above recruited from the community, with appropriate literacy to understand and ability to comply with the study requirements. After receiving all demographic information, medical and medication history of the participants at their baseline visit, those who met any of the following criteria were excluded: high blood cholesterol, LDL-C and familial hypercholesterolemia; on regular medications of known interference with lipid profiles (e.g. hormone replacement treatment, diuretics, beta-blockers, statins or other lipid-lowering drug treatment) or over-the-counter supplements that claim to reduce blood cholesterol or gastrointestinal (GI) related medications (e.g. antacid); smoking > 1 pack/day; heavy alcohol intake (> 40 g/day for men; > 30 g/day for women); intolerance or dislike of soya products; known soy allergy; history of sitosterolaemia; history of other major chronic diseases such as diabetes (type I or type II), thyroid, renal or liver disease or any GI malabsorption syndrome; refusal to stop consuming phytosterols-enriched products other than the product under investigation, if any, during the study; receiving systemic treatment or topical treatment likely to interfere with the evaluation of the study parameters; pregnancy or lactation during the study period.

Each participant was randomly assigned to either placebo or treatment group, given packs of soya drink containing phytosterols at a daily dose of either 0 g or 2 g, respectively, for a period of 3 weeks in a double-blind manner. The randomization was handled by the Data Management and Medical Statistics Unit of the Clinical Trials Centre, The University of Hong Kong. Prior to the start of the study, the randomized specifications were defined and the randomization codes were generated online using a web-based randomization system, namely a randomization

module within Research Electronic Data Capture (REDCap), which is a secure web application developed by Vanderbilt University, for building and managing online surveys and databases. Block randomization was used to balance the study groups in terms of participant numbers, sex and age group (18–40 years and > 40 years). Block randomization of sizes 2, 4 or 6 with equal probability were used to maintain approximately 1:1 allocation of treatment within the four stratum: (1) male aged 18–40 years; (2) male aged > 40 years; (3) female aged 18–40 years and (4) female aged > 40 years. Both investigators and participants were completely blinded to treatment allocation throughout the study.

Demographic information, medical and medication history

During the baseline visit, the participant's demographic information, medical and medication history were obtained. Medical history included past and current illnesses, alcohol and tobacco use, dietary and exercise habits, and pregnancy status if applicable. Information on prescription, over-the-counter and herbal medications as well as health supplements within 3 months were recorded.

Study product and intervention

The product used was based on a commercially-available soya drink (Vitasoy Calci-Plus Hi-calcium Plant Sterol Soya Milk provided by Vitasoy International Holdings Ltd.). Participants in the treatment and placebo group were given packs of 250 mL soya drinks with identical appearances. The 250 mL soya drinks for the treatment group contained 2 g phytosterols while that for the placebo group were not fortified. The nutrient profiles of two drinks were the same except higher total fat in phytosterols-enriched soya drink (2.8 g/100 mL) compared with the placebo (1.2 g/100 mL) (Supplementary Table 1). In preliminary blind tasting, the drink for the placebo group was found to be well-matched and indistinguishable from that for the treatment group. According to EU commission regulation, "a daily intake of 1.5–2.4 g/s plant sterols or stanols reduces LDL-C by 7–10% in 2–3 weeks" [16]. Thus, the current study was designed to evaluate the effectiveness of lipid–lowering effect by a phytosterols-enriched soya drink for 3 weeks. Participants were asked to consume one pack of 250 mL soya drink daily during their main meal for three consecutive weeks. Both groups were advised to maintain their usual dietary and exercise habits during the study period.

Study compliance

The study product was given to the subject during their baseline visit. At the end of the study, participants were requested to return unused packs and a card that recorded product consumption so that compliance could be assessed.

Physical and laboratory assessments

At the start date and end date of the study, the height, weight, waist and hip circumference, body temperature and blood pressure of study participants were measured. Blood pressure was measured using a GE CARESCAPE V100 (Fairfield, CT) automated blood pressure monitor. Waist and hip circumference were measured at the midpoint between the lowest rib and the iliac crest, and at the widest part of the hip, respectively, in a horizontal plane. Fasting blood samples at the two visits were taken to measure serum LDL-C, HDL-C, total cholesterol, triglycerides, creatinine, and blood glucose by the Ortho-Vitros Fusion 5.1 automated analyzer.

Statistical analysis

PASS 12 was used to calculate the sample size of this study. ANCOVA was used to estimate the sample size with baseline serum LDL-C level as the independent covariate, assuming a power of 0.8 according to a previous study [17], an attrition rate of 25% and a maximum tolerable false-positive rate of 5%.

Results were expressed as mean and standard deviation for continuous variables, and as number and percentage for categorical variables. The primary objective of the current study was to compare the changes in serum LDL-C level at the end of week 3 between the groups. ANCOVA was used to evaluate the least square (LS) mean differences and % changes with age, sex, baseline BMI and baseline LDL-C value as covariates. Efficacy analysis was performed on both intention-to-treat (ITT) and the per-protocol (PP) population. ITT consisted of all cases that had been randomized. PP included all eligible participants who had 80% compliance with the study product, efficacy data collected within the pre-specified allowable visit window, and no significant violation against the protocol.

The secondary objective of this study was to compare the changes in other cardiometabolic risk factors (including blood pressure, BMI, waist and hip circumferences, serum HDL-C, total cholesterol, triglycerides, and fasting glucose) between the treatment and placebo groups. The secondary efficacy analysis was performed on the ITT population. Subgroup analyses were performed to compare LS mean difference in primary and secondary endpoints between groups in the PP population; and changes in serum lipid profile of subjects in ITT population stratified by baseline LDL-C level between groups. All data management and statistical analysis were performed with SAS®.

Results

A total of 201 subjects were recruited (100 in the treatment group and 101 in the placebo group) in July 2016 and all final visits were completed in January 2017 (Fig. 1). Seven participants did not return for the final visit, one withdrew from the study and six were lost to follow-up. Sixteen and 19 participants from treatment and placebo groups respectively were removed from further analysis because they met the exclusion criteria or had an undetermined baseline LDL-C level (Supplementary Table 3). The baseline characteristics of participants are shown in Table 1.

Finally, 159 participants (82 treatment; 77 placebo) were included in the ITT analysis. The mean compliance rate was 97.9% (range 76.2–100%) in the treatment group and 97.7% (range 76.2–100%) in the placebo group. The treatment group had a reduction in serum LDL-C by LS mean 0.12 mmol/L (SE 0.058) (Table 2) when compared with the placebo group after adjusted for baseline LDL-C. Among the treatment group, LDL-C decreased from 2.80 mmol/L (SE 0.08) to 2.62 mmol/L (SE, 0.08) over the 3-week study period, representing a significant decrease by 5.96% (SE 1.48) with a median of 6.74%. Meanwhile, the placebo group had a reduction in LDL-C from 2.69 mmol/L (SE 0.08) to 2.64 mmol/L (SE 0.09) across the study period, representing an insignificant decrease by 1.25% (SE 1.51) with a median

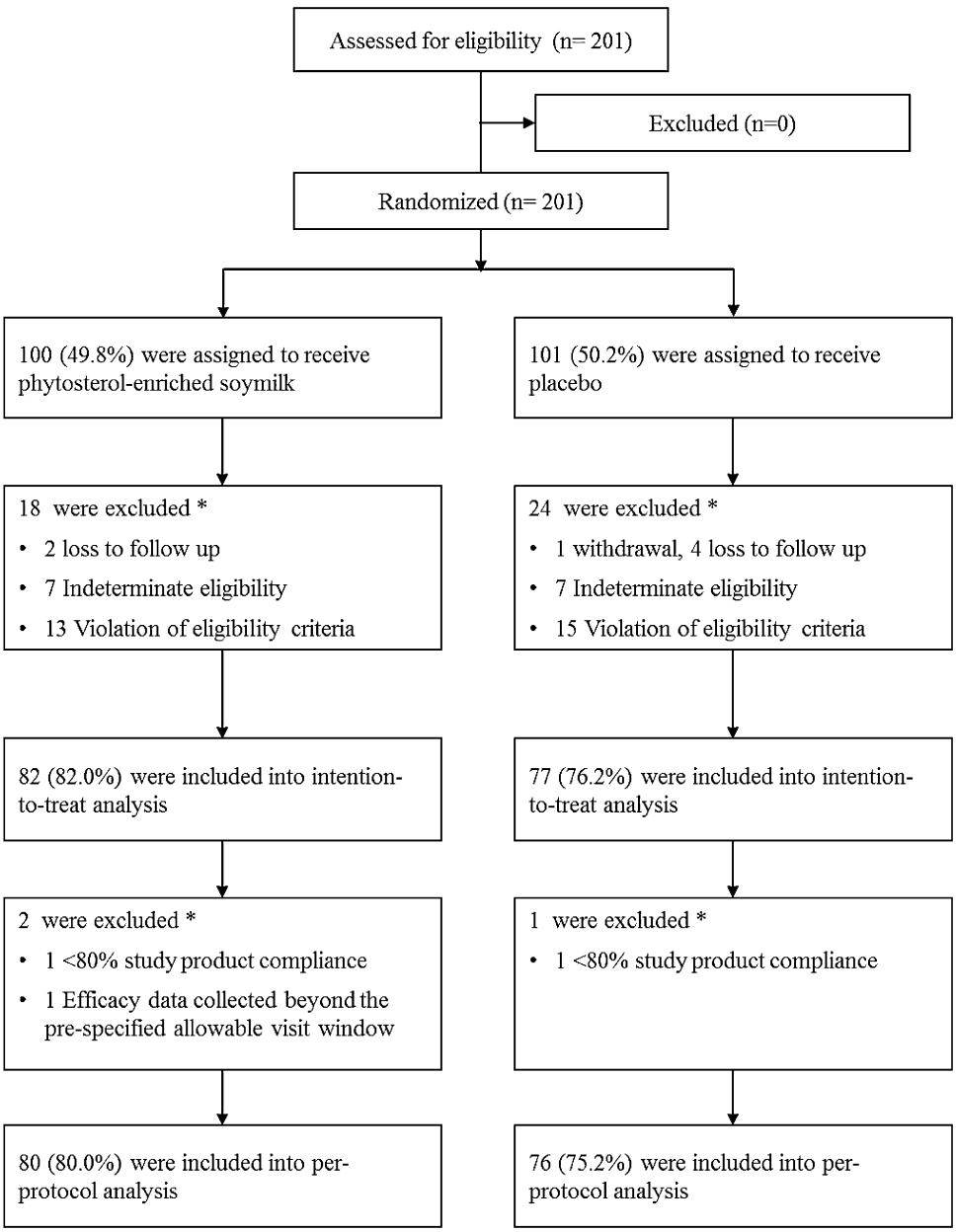

Fig. 1 Patient enrolment, randomization and follow-up. *Some subjects had more than one reasons to be excluded from the PP population

Table 1 Baseline characteristics of the study participants ($N=159$)

	Treatment ($N=82$)	Control ($N=77$)	p value
Age (years)	45 ± 17.1	42 ± 17.0	0.22
Female	36 (43.9%)	38 (49.4%)	0.49
Height (cm)	165.5 ± 7.5	164.8 ± 8.3	0.59
Weight (kg)	62.6 ± 10.8	62.0 ± 12.0	0.72
BMI (kg/m^2)	22.8 ± 3.4	22.7 ± 3.6	0.88
Body temperature (°C)	36.5 ± 0.40	36.5 ± 0.33	0.25
Waist circumference (cm)	81 ± 9.5	80 ± 10.5	0.57
Hip circumference (cm)	93 ± 6.5	93 ± 7.0	0.89
LDL-C (mmol/L)	2.80 ± 0.75	2.69 ± 0.74	0.38
HDL-C (mmol/L)	1.41 ± 0.32	1.48 ± 0.39	0.27
Total cholesterol (mmol/L)	4.69 ± 0.76	4.75 ± 0.76	0.63
Triglycerides (mmol/L)	1.05 ± 0.52	1.04 ± 0.56	0.95
Creatinine (μmol/L)	70.7 ± 14.4	71.9 ± 14.0	0.60
Fasting glucose (mmol/L)	4.9 ± 1.33	4.8 ± 0.50	0.40
Systolic blood pressure (mmHg)	123 ± 18.7	120 ± 17.1	0.32
Diastolic blood pressure (mmHg)	81 ± 11.0	79 ± 11.5	0.34

Values are given as no. (%) or mean ± SD

Table 2 LS mean change of clinical endpoints after the intervention in the ITT population ($N=159$)

Variable	Treatment ($N=82$) Mean change (95% CI)	Control ($N=77$) Mean change (95% CI)	Treatment effect Mean difference (95% CI)	p value
LDL-C (mmol/L)	−0.18 (−0.26, −0.09)	−0.05 (−0.13, 0.03)	−0.12 (−0.23, 0.00)	0.048
HDL-C (mmol/L)	0.01 (−0.02, 0.05)	0.04 (−0.01, 0.09)	−0.04 (−0.10, 0.02)	0.238
Total cholesterol (mmol/L)	−0.12 (−0.22, −0.03)	−0.06 (−0.16, 0.04)	−0.10 (−0.24, 0.03)	0.141
Triglycerides (mmol/L)	0.04 (−0.05, 0.14)	0.00 (−0.06, 0.06)	0.04 (−0.08, 0.15)	0.535
Creatinine (μmol/L)	1.29 (0.19, 2.39)	−0.09 (−1.18, 0.99)	1.07 (−0.47, 2.61)	0.171
Fasting glucose (mmol/L)	0.00 (−0.06, 0.07)	−0.02 (−0.07, 0.04)	0.01 (−0.08, 0.10)	0.831
Systolic blood pressure (mmHg)	−3.13 (−5.34, −0.93)	−1.36 (−2.83, 0.10)	−1.23 (−3.57, 1.11)	0.302
Diastolic blood pressure (mmHg)	−2.73 (−3.90, −1.57)	−1.97 (−3.06, −0.89)	−0.55 (−2.06, 0.96)	0.473
Body temperature (°C)	−0.01 (−0.10, 0.09)	−0.05 (−0.12, 0.02)	0.00 (−0.07, 0.07)	0.974
Weight (kg)	0.04 (−0.14, 0.22)	0.09 (−0.08, 0.27)	−0.06 (−0.31, 0.19)	0.642
BMI (kg/m^2)	0.02 (−0.05, 0.08)	0.04 (−0.03, 0.10)	−0.02 (−0.12, 0.07)	0.610
Waist circumference (cm)	0.24 (−0.51, 1.00)	−0.18 (−1.03, 0.67)	0.38 (−0.64, 1.40)	0.464
Hip circumference (cm)	0.05 (−0.44, 0.54)	−0.30 (−0.75, 0.15)	0.31 (−0.30, 0.93)	0.316

Age, sex, baseline BMI and baseline LDL-C level were adjusted in the model

of 1.54%. There was a difference of 4.70% (SE 2.12) with a median of 5.20% between treatment and placebo groups. Similar results were found in the PP population (Supplementary Table 4). As there was no significant interaction with sex ($p=0.76$), the analysis was not stratified by sex. No significant differences were found between groups with respect to changes in blood pressure, weight, BMI, waist circumference, hip circumference, serum creatinine, HDL-C, triglycerides, or fasting glucose levels (Table 2).

Adverse events are shown in Table 3. Only 5 (3.1%) out of all 159 study participants reported adverse events. The adverse events were nearly all gastrointestinal discomfort without any serious adverse events and did not result in withdrawal.

Discussion

In this randomized, double-blind controlled trial, a phytosterols-enriched soya drink, with 2 g of phytosterols per day, was shown conclusively to lower LDL-C level, with only 3.1% participants reporting adverse events related to GI discomfort.

Table 3 Treatment-related adverse events

	Treatment ($N=82$)	Control ($N=77$)	Overall ($N=159$)
Subjects with at least one adverse event	5 (6.1%)	0 (0.0%)	5 (3.1%)
Most common adverse events (> 1%)			
Diarrhea	3 (3.7%)	0	3 (1.9%)
Flu	1 (1.2%)	0	1 (0.6%)
Stomach bloating	0	0	0 (0.0%)
Slight harden of stool during the 3 weeks period	1 (1.2%)	0	1 (0.6%)

Data are presented as *N* (%)

This cholesterol-lowering effect of the phytosterols-enriched soya drink is consistent with previous studies, many of which involved Caucasians consuming dairy-type products, with a reported LDL-lowering effects ranging from 6 to 12% [18]. Our previous RCT using phytosterols-enriched milk powder showed similar LDL-C lowering effect among Chinese adults, but more participants (around 30%) experienced adverse events such as diarrhea and flatulence when consuming milk products [10]. In contrast, there is a long tradition of taking soya drinks in Asian countries, such as China, Japan, Korea, Thailand, Malaysia and Singapore, because soya milk is well-tolerated. As shown in the current study, the compliance rate was close to 100%. The number of adverse events was strikingly low.

Components other than phytosterols in soya milk may offer additional benefits. The LDL-C was also lowered in the placebo group, although the reduction did not reach statistical significant (mean change −0.05 mmol/L, 95% CI −0.13, 0.03). Such beneficial effect may be contributed by other nutrients such as soy protein [19] and isoflavones [20]. In addition, soy product has been reported to exert multiple beneficial effects, such as alleviating hot flushes, reducing risk of coronary heart disease, breast and prostate cancer [21]. Notably, the total LDL-C reduction in the treatment group was 5.96% (median 6.74%), with the mean reduction as compared with the placebo group at 4.70% (median 5.20%). This demonstrated that consuming phytosterols-enriched soya drink lowered LDL-C by a greater extent than the standard soya drink. Although a greater reduction of LDL-C was shown among people with higher serum cholesterol at baseline in a previous study [21], there were no significant differences in LDL-C, HDL-C, total cholesterol, and triglycerides level in our subgroup analysis stratified by baseline LDL-C level (Supplementary Table 5). Phytosterols are fortified in food with stanol esters, sterol esters, or free (unesterified) plant sterols and stanols. Their particle size and the fatty acid used for esterification affect the cholesterol-lowering property due to the bioavailability and efficacy in competing with intestinal cholesterol absorption [9]. Therefore, the formulation of phytosterols has an important bearing on the effectiveness of cholesterol lowering effect. The phytosterols included in the fortified soya drink are mainly sterol esters (> 90%), therefore these components are likely to account for the treatment effect. Meanwhile, the free sterols included are mainly β-sitosterol, campesterol, and stigmasterol (Supplementary Table 2). Our study might lack the specificity to determine which phytosterols are responsible for the reduction of LDL-C, but evidence demonstrates no conclusive difference in lipid-lowering effect between free and esterified plant sterols [4]. Nevertheless, there are factors affecting lipid profile other than phytosterol and soy intake, such as diet and lifestyle. Participants were therefore advised not to change their diet and lifestyle during the study period. In an intervention period lasting only a few weeks, these factors are unlikely to change significantly.

Lowering LDL-C may have additional benefits beyond simply reducing cardiovascular risk. There are substantial studies showing that high serum cholesterol and LDL-C levels are correlated with reduced bone mineral density (BMD) and consequent increased risk of osteoporotic fracture [22]. Similarly, it has also been reported that high serum cholesterol is associated with tumour development and progression, including cancers of breast, prostate and colon [23]. Thus, lowering LDL-C may have a great impact on general health. Although statins are the first-line drugs to lower LDL-C level, there are disadvantages in using these pharmacological interventions. Statins are known to cause myositis and liver enzyme elevations, and increase the risk of developing diabetes [24]. Evolocumab, an antibody that binds specifically to proprotein convertase subtilisin kexin 9 (PCSK9), blocks PCSK9-mediated degradation of low-density lipoprotein receptors in the liver and effectively increases the clearance of LDL-C. However, the drug is expensive, exceeds generally accepted cost-effectiveness thresholds, and has potential risk of causing injection site reaction, allergic reaction and muscle-related events [25, 26]. In contrast, non-pharmaceutical management such as phytosterols are well-tolerated and serious adverse effects are almost absent [27]. Phytosterols are therefore suitable for those at low cardiovascular risk and have no or only

mild elevation in LDL-C. In patients already taking statins, phytosterols still have an additive role in lowering LDL-C [28]. From the public health perspective, fortified food with potential health benefits, such as phytosterols-enriched drinks, are generally more acceptable to the public and hence has greater public health impact.

This study has several strengths and clinical implications. It is worth noting that we studied normocholesterolaemic adults of both sexes, with a wide age range (from 19 to 79 years). This is the first study to investigate if phytosterol is useful in reducing LDL-C among Chinese individuals with normocholesterolaemia, while previous studies mainly examined the effects of phytosterols in individuals with hyperlipidemia. In reality, people with hypercholesterolemia may be prescribed pharmacological intervention like statins, which is more efficacious in lowering LDL-C level. The importance of phytosterols-enriched food lies in preventing the rise in cholesterol and retarding the development of atherosclerosis in an early stage in asymptomatic individuals. Moreover, our participants are typical of the general population and differ from the high-risk older patients in most lipid lowering trials, thus our findings are generalizable to the whole population. The high tolerability, low toxicity and low antigenicity of phytosterols make them ideal for primary prevention in the general population, except for people with sitosterolaemia. In addition, the parallel design adopted in this study is more advantageous than the cross-over design commonly employed in pharmacokinetic and pharmacodynamics studies. Unlike the cross-over design, participants in the present study do not require a wash-out period between receiving treatment and placebo, resulting in shorter completion time and lowering the chance of dropout. Carry-over effect of phytosterols between treatment and placebo can be avoided using the parallel design. Based on FDA's suggestion, the formulation of consuming one serving a day is more convenient than consuming twice daily [6]. The use of soya milk as a phytosterols-enriched beverage provides an alternative to people with lactose intolerance, which is not only prevalent in Asia, but also present in other regions. Soya milk per se may offer additional health benefits.

Nevertheless, there are limitations. Firstly, study participants are usually more motivated and have better compliance with regular consumption of the product than the general population. Moreover, compliance may decrease with time in reality. Secondly, as the trial was not conducted in an inpatient setting, the diet of the participants during the trial period could not be strictly controlled, especially when phytosterols are naturally present in many foods. However, the amount of phytosterol in typical food items is extremely low. Thirdly, we did not measure plasma phytosterols or lathosterol levels to document their changes during the trial. Phytosterols work mainly at a local level in the gut and so plasma levels may not directly reflect their efficacy. Moreover, it is not a standard practice to measure participants' plasma levels in clinical trials of efficacy of investigational products.

In conclusion, daily intake of phytosterols-enriched soya drink with 2 g phytosterols is an effective way to reduce LDL-C and is well-tolerated in Chinese. If this highly accepted and inexpensive way of lowering LDL-C level is implemented on a nationwide scale, a consequent slight decrease in LDL-C at the population level would be expected to have a highly positive impact on cardiovascular disease and general health.

Author contributions BMYC and CLC designed the study, interpreted the data and wrote the first draft. YPC, YCC, CWS, MFT, VKFC, GKYL were involved in subject enrollment, subject interview and performing physical assessments, BMYC and CLC supervised the whole RCT. All authors have read and approved the final version of the manuscript and its conclusions. The corresponding authors had full access to the data and had final responsibility for the decision to submit for publication.

Compliance with ethical standards

Conflict of interest Yes, there is potential competing interest. All authors declare: grants from The University of Hong Kong, sponsorship from Vitasoy International Holdings Ltd. for the study.

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