The Lipid-Lowering Effect of Once-Daily Soya Drink Fortified With Phytosterols in Normocholesterolaemic Chinese: A Double-Blind Randomized Controlled Trial

Running title: Lipid-lowering with soya drink

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1   **Abstract**

2   **Purpose**

3   Phytosterols reduce intestinal cholesterol absorption and help to lower LDL-cholesterol.

4   Many Chinese adults are lactose-intolerant and cannot tolerate bovine milk enriched with

5   phytosterol. Soya-milk is a common beverage in Asia and it has beneficial effects on general

6   health. We therefore conducted a randomized double-blind controlled trial to assess the

7   effectiveness of a phytosterols-enriched soya drink in lowering serum LDL-cholesterol level

8   (primary outcome) and other cardiovascular parameters (secondary outcomes).

9   **Methods**

10   One hundred and fifty-nine normocholesterolaemic participants (85 men and 74 women; aged

11   19-79) were randomized to daily intake of one serving of phytosterols-enriched soya drink

12   (N=82), equivalent to 2 g of phytosterol per day, or a matched soya drink without

13   phytosterols (N=77) for three weeks. Adverse events, withdrawal and compliance were

14   documented.

15   **Results**

16   Among the treatment group (N=82), phytosterols-enriched soya drink significantly decreased

17   LDL-cholesterol by 5.96 % (SE, 1.48, 95% CI: -8.91%, -3.00%) with a median of 6.74%

18   compared with baseline, resulting in a significant reduction of 4.70% (95% CI: -8.89%, -

19   0.51%; p=0.028) with a median of 5.20% compared with placebo (N=77). In contrast, there

20 were no significant changes in other lipid parameters, blood glucose, blood pressure, body

21 weight or waist circumference. Remarkably, 95% of the participants randomized to the

22 fortified drink reported no adverse events at all.

23 **Conclusions**

24 Daily consumption of a phytosterols-enriched soya drink may be a simple and cost-neutral

25 means of lowering LDL-cholesterol in individuals in China, with massive population and

26 rising incidence of coronary heart disease. (ClinicalTrials.gov identifier: NCT02881658; Date

27 of registration: 14 Aug 2016)

28 **Keywords**

29 Cholesterol, phytosterol, plant sterol, dyslipidemia, LDL, clinical trial

30

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 gram per serving of vegetable oil plant sterol esters, eaten twice a day with meals for a daily total intake of at least 1.3 grams, 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.

50   When the same amount of phytosterols is consumed in low-fat milk, greater reduction of

51   LDL-C was observed compared with that consumed in bread or cereal [7]. Whereas, another

52   study showed a similar effect on lipid reduction in low- and medium-fat soymilk fortified

53   with phytosterols [8].

54

55   Adding phytosterols to dairy products is the most common approach in commercial products

56   and their effectiveness in lowering LDL-C has been shown in clinical trials [9]. Our previous

57   randomized controlled trial (RCT) showed that drinking phytosterols-enriched milk twice a

58   day lowered LDL-C in Hong Kong Chinese [10]. However, it is well documented that lactose

59   intolerance is prevalent in Asians [11]. Thus, soya milk, a popular beverage in Asia [12], may

60   serve as a better food matrix for phytosterols. Soya milk is a protein-rich drink and regular

61   intake is considered as part of a healthy diet. It has potential benefits in relieving hot flushes

62   [13] and improving lipid profiles [14]. Long-term intake of isoflavones from soya milk has

63   bone-sparing effects [12]. However, whether phytosterols-enriched soya milk is useful in

64   lowering LDL-C is largely unknown. In particular, the effects of 2g-phytosterol formulation

65   taken once daily in people with normocholesterolemia has not been previously tested. We

66   therefore conducted a double-blind RCT to evaluate whether taking phytosterols-enriched

67   soya drink once daily would reduce LDL-C in normocholesterolemic Chinese.

68

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 (>40g/day for men; >30g/day for women); intolerance or dislike of soya products; known soy allergy; history of

88   sitosterolaemia; history of other major chronic diseases such as diabetes (type I or type II),

89   thyroid, renal or liver disease or any GI malabsorption syndrome; refusal to stop consuming

90   phytosterols-enriched products other than the product under investigation, if any, during the

91   study; receiving systemic treatment or topical treatment likely to interfere with the evaluation

92   of the study parameters; pregnancy or lactation during the study period.

93

94   Each participant was randomly assigned to either placebo or treatment group, given packs of

95   soya drink containing phytosterols at a daily dose of either 0 g or 2 g respectively, for a

96   period of three weeks in a double-blind manner. The randomization was handled by the Data

97   Management and Medical Statistics Unit of the Clinical Trials Centre, The University of

98   Hong Kong. Prior to the start of the study, the randomized specifications were defined and

99   the randomization codes were generated online using a web-based randomization system,

100   namely a randomization module within Research Electronic Data Capture (REDCap), which

101   is a secure web application developed by Vanderbilt University, for building and managing

102   online surveys and databases. Block randomization was used to balance the study groups in

103   terms of participant numbers, sex and age group (18-40 years old and >40 years old). Block

104   randomization of sizes 2, 4 or 6 with equal probability were used to maintain approximately

105   1:1 allocation of treatment within the four stratum: (i) Male aged 18-40 years; (ii) Male

106   aged >40 years; (iii) Female aged 18-40 years and (iv) Female aged >40 years. Both

107   investigators and participants were completely blinded to treatment allocation throughout the

108   study.

109

110   **Demographic information, medical and medication history**

111   During the baseline visit, the participant's demographic information, medical and medication

112   history were obtained. Medical history included past and current illnesses, alcohol and

113   tobacco use, dietary and exercise habits, and pregnancy status if applicable. Information on

114   prescription, over-the-counter and herbal medications as well as health supplements within

115   three months were recorded.

116

117   **Study product and intervention**

118   The product used was based on a commercially-available soya drink (Vitasoy Calci-Plus Hi-

119   calcium Plant Sterol Soya Milk provided by Vitasoy International Holdings Ltd.).

120   Participants in the treatment and placebo group were given packs of 250mL soya drinks with

121   identical appearances. The 250mL soya drinks for the treatment group contained 2g

122   phytosterols while that for the placebo group were not fortified. The nutrient profiles of two

123   drinks were the same except higher total fat in phytosterols-enriched soya drink

124   (2.8g/100mL) compared with the placebo (1.2g/100mL) (Supplementary Table 1). In

125   preliminary blind tasting, the drink for the placebo group was found to be well-matched and

126 indistinguishable from that for the treatment group. According to EU commission regulation,

127 "a daily intake of 1.5-2.4g/s plant sterols or stanols reduces LDL-C by 7-10% in two to three

128 weeks" [16]. Thus, the current study was designed to evaluate the effectiveness of lipid–

129 lowering effect by a phytosterols-enriched soya drink for three weeks. Participants were

130 asked to consume one pack of 250mL soya drink daily during their main meal for three

131 consecutive weeks. Both groups were advised to maintain their usual dietary and exercise

132 habits during the study period.

133

134 **Study compliance**

135 The study product was given to the subject during their baseline visit. At the end of the study,

136 participants were requested to return unused packs and a card that recorded product

137 consumption so that compliance could be assessed.

138

139 **Physical and laboratory assessments**

140 At the start date and end date of the study, the height, weight, waist and hip circumference,

141 body temperature and blood pressure of study participants were measured. Blood pressure

142 was measured using a GE CARESCAPE V100 (Fairfield, CT) automated blood pressure

143 monitor. Waist and hip circumference were measured at the midpoint between the lowest rib

144 and the iliac crest, and at the widest part of the hip, respectively, in a horizontal plane. Fasting

145 blood samples at the two visits were taken to measure serum LDL-C, HDL-C, total

146 cholesterol, triglycerides, creatinine, and blood glucose by the Ortho-Vitros Fusion 5.1

147 automated analyzer.

148

149 **Statistical analysis**

150 PASS 12 was used to calculate the sample size of this study. ANCOVA was used to estimate

151 the sample size with baseline serum LDL-C level as the independent covariate, assuming a

152 power of 0.8 according to a previous study [17], an attrition rate of 25% and a maximum

153 tolerable false positive rate of 5%.

154

155 Results were expressed as mean and standard deviation for continuous variables, and as

156 number and percentage for categorical variables. The primary objective of the current study

157 was to compare the changes in serum LDL-C level at the end of week 3 between the groups.

158 ANCOVA was used to evaluate the least square (LS) mean differences and % changes with

159 age, sex, baseline BMI and baseline LDL-C value as covariates. Efficacy analysis was

160 performed on both intention-to-treat (ITT) and the per-protocol (PP) population. ITT

161 consisted of all cases that had been randomized. PP included all eligible participants who had

162 80% compliance with the study product, efficacy data collected within the pre-specified

163 allowable visit window, and no significant violation against the protocol.

164

165   The secondary objective of this study was to compare the changes in other cardiometabolic

166 risk factors (including blood pressure, BMI, waist and hip circumferences, serum HDL-C,

167 total cholesterol, triglycerides, and fasting glucose) between the treatment and placebo

168 groups. The secondary efficacy analysis was performed on the ITT population. Subgroup

169 analyses were performed to compare LS mean difference in primary and secondary endpoints

170 between groups in the PP population; and changes in serum lipid profile of subjects in ITT

171 population stratified by baseline LDL-C level between groups. All data management and

172 statistical analysis were performed with SAS®.

173

174 **Results**

175 A total of 201 subjects were recruited (100 in the treatment group and 101 in the placebo

176 group) in July 2016 and all final visits were completed in January 2017 (Fig 1). Seven

177 participants did not return for the final visit, one withdrew from the study and six were lost to

178 follow-up. Sixteen and 19 participants from treatment and placebo groups respectively were

179 removed from further analysis because they met the exclusion criteria or had an

180 undetermined baseline LDL-C level (Supplementary Table 3). The baseline characteristics of

181 participants are shown in Table 1.

182

183   Finally, 159 participants (82 treatment; 77 placebo) were included in the ITT analysis. The

184   mean compliance rate was 97.9% (range 76.2 – 100%) in the treatment group and 97.7%

185   (range 76.2 – 100%) in the placebo group. The treatment group had a reduction in serum

186   LDL-C by LS mean 0.12 mmol/L (SE, 0.058) (Table 2) when compared with the placebo

187   group after adjusted for baseline LDL-C. Among the treatment group, LDL-C decreased from

188   2.80 mmol/L (SE, 0.08) to 2.62 mmol/L (SE, 0.08) over the 3-week study period,

189   representing a significant decrease by 5.96% (SE, 1.48) with a median of 6.74%. Meanwhile,

190   the placebo group had a reduction in LDL-C from 2.69 mmol/L (SE, 0.08) to 2.64 mmol/L

191   (SE, 0.09) across the study period, representing an insignificant decrease by 1.25 % (SE,

192   1.51) with a median of 1.54%. There was a difference of 4.70% (SE, 2.12) with a median of

193   5.20% between treatment and placebo groups. Similar results were found in the PP

194   population (Supplementary Table 4). As there was no significant interaction with sex

195   (p=0.76), the analysis was not stratified by sex. No significant differences were found

196   between groups with respect to changes in blood pressure, weight, BMI, waist circumference,

197   hip circumference, serum creatinine, HDL-C, triglycerides, or fasting glucose levels (Table

198   2).

199

200   Adverse events are shown in Table 3. Only 5 (3.1%) out of all 159 study participants reported

201   adverse events. The adverse events were nearly all gastrointestinal discomfort without any

202   serious adverse events and did not result in withdrawal.

203

204   **Discussion**

205   In this randomized, double-blind controlled trial, a phytosterols-enriched soya drink, with 2g

206   of phytosterols per day, was shown conclusively to lower LDL-C level, with only 3.1%

207   participants reporting adverse events related to GI discomfort.

208

209   This cholesterol-lowering effect of the phytosterols-enriched soya drink is consistent with

210   previous studies, many of which involved Caucasians consuming dairy-type products, with a

211   reported LDL-lowering effects ranging from 6% to 12% [18]. Our previous RCT using

212   phytosterols-enriched milk powder showed similar LDL-C lowering effect among Chinese

213   adults, but more participants (around 30%) experienced adverse events such as diarrhea and

214   flatulence when consuming milk products (9). In contrast, there is a long tradition of taking

215   soya drinks in Asian countries, such as China, Japan, Korea, Thailand, Malaysia and

216   Singapore, because soya milk is well-tolerated. As shown in the current study, the compliance

217   rate was close to 100%. The number of adverse events was strikingly low.

218

219   Components other than phytosterols in soya milk may offer additional benefits. The LDL-C

220   was also lowered in the placebo group, although the reduction did not reach statistical

221 significant (mean change -0.05mmol/L, 95%CI:-0.13, 0.03). Such beneficial effect may be

222 contributed by other nutrients such as soy protein [19] and isoflavones [20]. In addition, soy

223 product has been reported to exert multiple beneficial effects, such as alleviating hot flushes,

224 reducing risk of coronary heart disease, breast and prostate cancer [21]. Notably, the total

225 LDL-C reduction in the treatment group was 5.96% (median, 6.74%), with the mean

226 reduction as compared with the placebo group at 4.70% (median, 5.20%). This demonstrated

227 that consuming phytosterols-enriched soya drink lowered LDL-C by a greater extent than the

228 standard soya drink. Although a greater reduction of LDL-C was shown among people with

229 higher serum cholesterol at baseline in a previous study [21], there were no significant

230 differences in LDL-C, HDL-C, total cholesterol, and triglycerides level in our subgroup

231 analysis stratified by baseline LDL-C level (Supplementary Table 5). Phytosterols are

232 fortified in food with stanol esters, sterol esters, or free (unesterified) plant sterols and

233 stanols. Their particle size and the fatty acid used for esterification affect the cholesterol-

234 lowering property due to the bioavailability and efficacy in competing with intestinal

235 cholesterol absorption [9]. Therefore, the formulation of phytosterols has an important

236 bearing on the effectiveness of cholesterol lowering effect. The phytosterols included in the

237 fortified soya drink are mainly sterol esters (>90%), therefore these components are likely to

238 account for the treatment effect. Meanwhile, the free sterols included are mainly β-sitosterol,

239 campesterol, and stigmasterol (Supplementary Tables 2). Our study might lack the specificity

240   to determine which phytosterols are responsible for the reduction of LDL-C, but evidence

241   demonstrates no conclusive difference in lipid-lowering effect between free and esterified

242   plant sterols [4]. Nevertheless, there are factors affecting lipid profile other than phytosterol

243   and soy intake, such as diet and lifestyle. Participants were therefore advised not to change

244   their diet and lifestyle during the study period. In an intervention period lasting only a few

245   weeks, these factors are unlikely to change significantly.

246

247   Lowering LDL-C may have additional benefits beyond simply reducing cardiovascular risk.

248   There are substantial studies showing that high serum cholesterol and LDL-C levels are

249   correlated with reduced bone mineral density (BMD) and consequent increased risk of

250   osteoporotic fracture [22]. Similarly, it has also been reported that high serum cholesterol is

251   associated with tumour development and progression, including cancers of breast, prostate

252   and colon [23]. Thus, lowering LDL-C may have a great impact on general health. Although

253   statins are the first-line drugs to lower LDL-C level, there are disadvantages in using these

254   pharmacological interventions. Statins are known to cause myositis and liver enzyme

255   elevations, and increase the risk of developing diabetes [24]. Evolocumab, an antibody that

256   binds specifically to proprotein convertase subtilisin kexin 9 (PCSK9), blocks PCSK9-

257   mediated degradation of low-density lipoprotein receptors in the liver and effectively

258   increases the clearance of LDL-C. However, the drug is expensive, exceeds generally

259 accepted cost-effectiveness thresholds, and has potential risk of causing injection site

260 reaction, allergic reaction and muscle-related events [25,26]. In contrast, non-pharmaceutical

261 management such as phytosterols are well-tolerated and serious adverse effects are almost

262 absent [27]. Phytosterols are therefore suitable for those at low cardiovascular risk and have

263 no or only mild elevation in LDL-C. In patients already taking statins, phytosterols still have

264 an additive role in lowering LDL-C [28]. From the public health perspective, fortified food

265 with potential health benefits, such as phytosterols-enriched drinks, are generally more

266 acceptable to the public and hence has greater public health impact.

267

268 This study has several strengths and clinical implications. It is worth noting that we studied

269 normocholesterolaemic adults of both sexes, with a wide age range (from 19 to 79 years).

270 This is the first study to investigate if phytosterol is useful in reducing LDL-C among

271 Chinese individuals with normocholesterolaemia, while previous studies mainly examined

272 the effects of phytosterols in individuals with hyperlipidemia. In reality, people with

273 hypercholesterolemia may be prescribed pharmacological intervention like statins, which is

274 more efficacious in lowering LDL-C level. The importance of phytosterols-enriched food lies

275 in preventing the rise in cholesterol and retarding the development of atherosclerosis in an

276 early stage in asymptomatic individuals. Moreover, our participants are typical of the general

277 population and differ from the high-risk older patients in most lipid lowering trials, thus our

278 findings are generalizable to the whole population. The high tolerability, low toxicity and low

279 antigenicity of phytosterols make them ideal for primary prevention in the general

280 population, except for people with sitosterolaemia. In addition, the parallel design adopted in

281 this study is more advantageous than the cross-over design commonly employed in

282 pharmacokinetic and pharmacodynamics studies. Unlike the cross-over design, participants in

283 the present study do not require a wash-out period between receiving treatment and placebo,

284 resulting in shorter completion time and lowering the chance of dropout. Carry-over effect of

285 phytosterols between treatment and placebo can be avoided using the parallel design. Based

286 on FDA's suggestion, the formulation of consuming one serving a day is more convenient

287 than consuming twice daily [6]. The use of soya milk as a phytosterols-enriched beverage

288 provides an alternative to people with lactose intolerance, which is not only prevalent in Asia,

289 but also present in other regions. Soya milk per se may offer additional health benefits.

290

291 Nevertheless, there are limitations. Firstly, study participants are usually more motivated and

292 have better compliance with regular consumption of the product than the general population.

293 Moreover, compliance may decrease with time in reality. Secondly, as the trial was not

294 conducted in an inpatient setting, the diet of the participants during the trial period could not

295 be strictly controlled, especially when phytosterols are naturally present in many foods.

296 However, the amount of phytosterol in typical food items is extremely low. Thirdly, we did

297 not measure plasma phytosterols or lathosterol levels to document their changes during the

298 trial. Phytosterols work mainly at a local level in the gut and so plasma levels may not

299 directly reflect their efficacy. Moreover, it is not a standard practice to measure participants'

300 plasma levels in clinical trials of efficacy of investigational products.

301

302 In conclusion, daily intake of phytosterols-enriched soya drink with 2g phytosterols is an

303 effective way to reduce LDL-C and is well-tolerated in Chinese. If this highly accepted and

304 inexpensive way of lowering LDL-C level is implemented on a nationwide scale, a

305 consequent slight decrease in LDL-C at the population level would be expected to have a

306 highly positive impact on cardiovascular disease and general health.

307

308 **Author contributions statement**

309 B.M.Y.C. and C.L.C. designed the study, interpreted the data and wrote the first draft. Y.P.C.,

310 Y.C.C., C.W.S., M.F.T., V.K.F.C., G.K.Y.L. were involved in subject enrollment, subject

311 interview and performing physical assessments, B.M.Y.C. and C.L.C. supervised the whole

312 RCT. All authors have read and approved the final version of the manuscript and its

313 conclusions. The corresponding authors had full access to the data and had final

314 responsibility for the decision to submit for publication.

315

References

1. Yusuf S, Hawken S, Ounpuu S, Dans T, Avezum A, Lanas F, McQueen M, Budaj A, Pais P, Varigos J, Lisheng L (2004) Effect of potentially modifiable risk factors associated with myocardial infarction in 52 countries (the INTERHEART study): case-control study. The Lancet 364 (9438):937-952. doi:10.1016/S0140-6736(04)17018-9

2. Cholesterol Treatment Trialists C (2012) The effects of lowering LDL cholesterol with statin therapy in people at low risk of vascular disease: meta-analysis of individual data from 27 randomised trials. The Lancet 380 (9841):581-590. doi:10.1016/S0140-6736(12)60367-5

3. Davies JT, Delfino SF, Feinberg CE, Johnson MF, Nappi VL, Olinger JT, Schwab AP, Swanson HI (2016) Current and Emerging Uses of Statins in Clinical Therapeutics: A Review. Lipid Insights 9:13-29. doi:10.4137/LPI.S37450

4. Jones PJH, Shamloo M, MacKay DS, Rideout TC, Myrie SB, Plat J, Roullet JB, Baer DJ, Calkins KL, Davis HR, Barton Duell P, Ginsberg H, Gylling H, Jenkins D, Lutjohann D, Moghadasian M, Moreau RA, Mymin D, Ostlund RE, Jr., Ras RT, Ochoa Reparaz J, Trautwein EA, Turley S, Vanmierlo T, Weingartner O (2018) Progress and perspectives in plant sterol and plant stanol research. Nutrition Reviews 76 (10):725-746. doi:10.1093/nutrit/nuy032

5. Jew S, Antoine J, Bourlioux P, Milner J, Tapsell LC, Yang Y, Jones PJH (2015) Nutrient essentiality revisited. Journal of Functional Foods 14:203-209. doi:10.1016/j.jff.2015.01.024

6. United States FDA (2018) Health claims: plant sterol/stanol esters and risk of coronary heart disease (CHD), 21 C.F.R. §101.83. https://www.ecfr.gov/cgi-bin/ECFR?SID=9597fbb69b17673c3588a74ae48fd0e2. Accessed 13/7/2018

7. Clifton PM, Noakes M, Sullivan D, Erichsen N, Ross D, Annison G, Fassoulakis A, Cehun M, Nestel P (2004) Cholesterol-lowering effects of plant sterol esters differ in milk, yoghurt, bread and cereal. Eur J Clin Nutr 58 (3):503-509. doi:10.1038/sj.ejcn.1601837

8. Rideout TC, Chan YM, Harding SV, Jones PJ (2009) Low and moderate-fat plant sterol fortified soymilk in modulation of plasma lipids and cholesterol kinetics in subjects with normal to high cholesterol concentrations: report on two randomized crossover studies. Lipids in Health and Disease 8:45. doi:10.1186/1476-511X-8-45

9. Abumweis SS, Barake R, Jones PJ (2008) Plant sterols/stanols as cholesterol lowering agents: A meta-analysis of randomized controlled trials. Food & Nutrition Research 52. doi:10.3402/fnr.v52i0.1811

10. Cheung CL, Ho DK, Sing CW, Tsoi MF, Cheng VK, Lee GK, Ho YN, Cheung BM (2017) Randomized controlled trial of the effect of phytosterols-enriched low-fat milk on lipid profile in Chinese. Scientific Reports 7:41084. doi:10.1038/srep41084

11. Solomons NW (2002) Fermentation, fermented foods and lactose intolerance. Eur J Clin Nutr 56 Suppl 4:S50-55. doi:10.1038/sj.ejcn.1601663

12. Rizzo G, Baroni L (2018) Soy, Soy Foods and Their Role in Vegetarian Diets. Nutrients 10 (1). doi:10.3390/nu10010043

13. Taku K, Melby MK, Kronenberg F, Kurzer MS, Messina M (2012) Extracted or synthesized soybean isoflavones reduce menopausal hot flash frequency and severity: systematic review and meta-analysis of randomized controlled trials. Menopause 19 (7):776-790. doi:10.1097/gme.0b013e3182410159

14. Clarkson TB (2002) Soy, soy phytoestrogens and cardiovascular disease. J Nutr 132 (3):566S-569S. doi:10.1093/jn/132.3.566S

15. ICH (2016) Integrated Addendum to ICH E6(R1): Guideline For Good Clinical Practice E6(E2). https://www.ich.org/products/guidelines/efficacy/efficacy-single/article/integrated-addendum-good-clinical-practice.html. Accessed 01 May 2019

16. Amending Regulations (EC) No 983/2009 and (EU) No 384/2010 as regards the conditions of use of certain health claims related to the lowering effect of plant sterols and plant stanols on blood LDL-cholesterol Text with EEA relevance. (2014). https://eur-

lex.europa.eu/eli/reg/2014/686/oj. Accessed 01 May 2019

17. Thomsen AB, Hansen HB, Christiansen C, Green H, Berger A (2004) Effect of free plant sterols in low-fat milk on serum lipid profile in hypercholesterolemic subjects. Eur J Clin Nutr 58 (6):860-870. doi:10.1038/sj.ejcn.1601887

18. Ras RT, Geleijnse JM, Trautwein EA (2014) LDL-cholesterol-lowering effect of plant sterols and stanols across different dose ranges: a meta-analysis of randomised controlled studies. Br J Nutr 112 (2):214-219. doi:10.1017/S0007114514000750

19. Ramdath DD, Padhi EM, Sarfaraz S, Renwick S, Duncan AM (2017) Beyond the Cholesterol-Lowering Effect of Soy Protein: A Review of the Effects of Dietary Soy and Its Constituents on Risk Factors for Cardiovascular Disease. Nutrients 9 (4). doi:10.3390/nu9040324

20. Taku K, Umegaki K, Sato Y, Taki Y, Endoh K, Watanabe S (2007) Soy isoflavones lower serum total and LDL cholesterol in humans: a meta-analysis of 11 randomized controlled trials. Am J Clin Nutr 85 (4):1148-1156. doi:10.1093/ajcn/85.4.1148

21. Messina M (2016) Soy and Health Update: Evaluation of the Clinical and Epidemiologic Literature. Nutrients 8 (12). doi:10.3390/nu8120754

22. Mandal CC (2015) High Cholesterol Deteriorates Bone Health: New Insights into Molecular Mechanisms. Frontiers in endocrinology 6:165. doi:10.3389/fendo.2015.00165

23. Murai T (2015) Cholesterol lowering: role in cancer prevention and treatment. Biol Chem 396 (1):1-11. doi:10.1515/hsz-2014-0194

24. Sattar N, Preiss D, Murray HM, Welsh P, Buckley BM, de Craen AJ, Seshasai SR, McMurray JJ, Freeman DJ, Jukema JW, Macfarlane PW, Packard CJ, Stott DJ, Westendorp RG, Shepherd J, Davis BR, Pressel SL, Marchioli R, Marfisi RM, Maggioni AP, Tavazzi L, Tognoni G, Kjekshus J, Pedersen TR, Cook TJ, Gotto AM, Clearfield MB, Downs JR, Nakamura H, Ohashi Y, Mizuno K, Ray KK, Ford I (2010) Statins and risk of incident diabetes: a collaborative meta-analysis of randomised statin trials. The Lancet 375

(9716):735-742. doi:10.1016/S0140-6736(09)61965-6

25. Sabatine MS, Giugliano RP, Keech AC, Honarpour N, Wiviott SD, Murphy SA, Kuder JF, Wang H, Liu T, Wasserman SM, Sever PS, Pedersen TR (2017) Evolocumab and Clinical Outcomes in Patients with Cardiovascular Disease. New England Journal of Medicine 376 (18):1713-1722. doi:10.1056/NEJMoa1615664

26. Fonarow GC, Keech AC, Pedersen TR, Giugliano RP, Sever PS, Lindgren P, van Hout B, Villa G, Qian Y, Somaratne R, Sabatine MS (2017) Cost-effectiveness of Evolocumab Therapy for Reducing Cardiovascular Events in Patients With Atherosclerotic Cardiovascular Disease. JAMA cardiology 2 (10):1069-1078. doi:10.1001/jamacardio.2017.2762

27. QuIlez J, GarcIa-Lorda P, Salas-Salvado J (2003) Potential uses and benefits of phytosterols in diet: present situation and future directions. Clinical Nutrition 22 (4):343-351. doi:10.1016/S0261-5614(03)00060-8

28. Han S, Jiao J, Xu J, Zimmermann D, Actis-Goretta L, Guan L, Zhao Y, Qin L (2016) Effects of plant stanol or sterol-enriched diets on lipid profiles in patients treated with statins: systematic review and meta-analysis. Scientific Reports 6:31337. doi:10.1038/srep31337

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

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 three weeks period	1 (1.2%)	0	1 (0.6%)

Data are presented as N (%).

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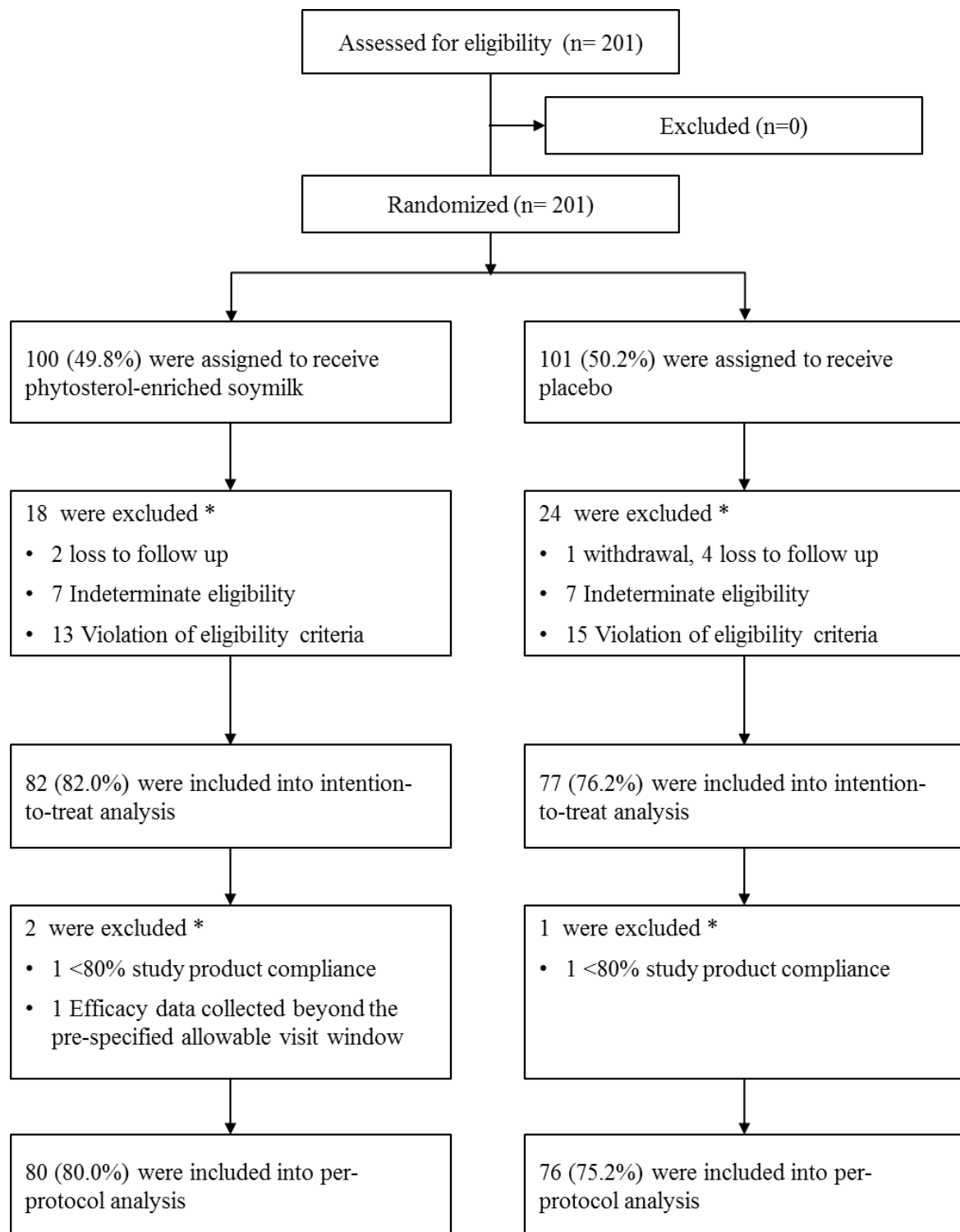
* Some subjects had more than one reasons to be excluded from the PP population.