Combining Plant Sterols With Walking Lowers Postprandial Triacylglycerol More Than Walking Only in Chinese Men With Elevated Body Mass Index

Ching T. Lye, Swarup Mukherjee, and Stephen F. Burns
Nanyang Technological University

This study examined if plant sterols and walking reduce postprandial triacylglycerol (TAG) concentrations in Chinese men with elevated body mass index (≥23.5 kg/m²). Fifteen Chinese men (mean [SD]: age = 25 [3] years and body mass index = 26.2 [1.5] kg/m²) completed four 10-day trials in random order with a 7- to 10-day washout between trials: (a) daily consumption of a control margarine while sedentary (C-S), (b) daily consumption of margarine containing 2 g/day of plant sterols while sedentary (PS-S), (c) daily consumption of a control margarine with 30-min daily walking (C-W), and (d) daily consumption of margarine containing 2 g/day of plant sterols with 30-min daily walking (PS-W). On Day 11 of each trial, postprandial TAG was measured after a high-fat milkshake. The 5-hr total area under the TAG curve was 22%, 25%, and 12% lower on PS-W (mean [SD]: 8.9 [4.3] mmol·5 hr/L) than C-S (11.4 [4.5] mmol·5 hr/L; \( p = .005; d = 0.56 \)), PS-S (11.9 [4.9] mmol·5 hr/L; \( p = .004; d = 0.67 \)), and C-W (10.1 [4.4] mmol·5 hr/L; \( p = .044; d = 0.27 \)) trials, respectively. Similarly, 5-hr incremental area for PS-W (4.5 [2.7] mmol·5 hr/L) was 31%, 32%, and 18% lower than C-S (6.6 [3.3] mmol·5 hr/L; \( p = .005; d = 0.62 \)), PS-S (6.6 [3.4] mmol·5 hr/L; \( p = .004; d = 0.64 \)), and C-W (5.5 [2.8] mmol·5 hr/L; \( p = .032; d = 0.29 \)). Ten days of daily plant sterol intake combined with walking presents an intervention strategy to lower postprandial TAG in Chinese men with elevated body mass index.

Keywords: exercise, lipids, phytosterols

Plant sterols (PS) can reduce low-density lipoprotein cholesterol (LDL-c) at intakes of ∼2 g/day (Catapano et al., 2016; Gylling et al., 2014), but accumulating evidence also implicates them in the reduction of fasting triacylglycerol (TAG), with greater reductions seen in individuals with higher TAG (Demonty et al., 2013; Gylling et al., 2014). However, circulating TAG is highest after eating with peak concentrations approximately 4 hr after food intake (Kolovou et al., 2011). Thus, any effect of PS on TAG may be most prominent during the postprandial period. Prospective epidemiological studies associate high nonfasting TAG with cardiovascular events and mortality, and for many individuals it represents the usual metabolic state (Nordestgaard et al., 2007).

Aerobic exercise also reduces fasting (Kraus et al., 2002) and postprandial TAG (Maraki & Sidossis, 2013). Given both exercise and PS modify TAG, surprisingly only one study has examined their combined benefits noting reductions in fasting TAG along with LDL-c, but the effects were not additive or synergistic compared with the treatments in isolation (Varady et al., 2004).

We are unaware of any studies examining how PS and exercise combined impact postprandial TAG. However, it is hypothesized that aerobic exercise administered after PS intake may synergistically blunt chylomicron entry to the circulation and reduce hepatic lipid transport resulting in lower very-low-density lipoprotein (VLDL) release (Marinangeli et al., 2006).

Finally, postprandial TAG is exaggerated in overweight men, even when fasting TAG is normal (Halkes et al., 2001). Moreover, cardiovascular risk factors often present before a body mass index (BMI) of 25 kg/m² in Chinese populations (WHO Expert Consultation, 2004), and some recommendations consider PS use even in individuals at intermediate or low cardiovascular disease risk (Catapano et al., 2016; Gylling et al., 2014). However, the efficacy of PS on blood lipids in Chinese is not as strong as for Western populations (Li et al., 2007), and no examination has been made of their impact on postprandial TAG in Chinese. Thus, we hypothesized that combining dietary PS supplementation with daily brisk walking could reduce postprandial TAG concentrations in healthy Chinese men with elevated BMI.

Methods

Participants

This study received Nanyang Technological University ethical approval (IRB-2013-05-027), and written informed consent was obtained from participants before testing. Recruitment took place through advertisement, and 19 individuals completed screening and were randomized. Inclusion criteria were as follows: male, 21–35 years, Chinese for three generations (participants, parents, and
grandparents), self-reported stable body mass (±1.5 kg) in the past 2 months (Wing et al., 2006), BMI of 23.5 kg/m² to less than 35 kg/m² (WHO Expert Consultation, 2004), reported engaging in structured physical activity ≤2 times per week for <20 min per week, fasting TAG of <1.7 mmol/L; free of cardiovascular disease and diabetes, not dieting, nonsmoker, consuming alcohol ≤3 times per week with ≤3 drinks per time by self-report, not using medication affecting lipid or carbohydrate metabolism, and no injuries restricting walking. The upper fasting TAG level reflected that other than elevated BMI, these men were at intermediate or low cardiovascular risk and improved group homogeneity.

Sample Size Justification
A priori analyses using G*Power (version 3.1; Universität Kiel, Kiel, Germany) were powered to detect an effect size (f) of 0.40 (η² = .14) on the 5-hr total postprandial TAG response using a repeated-measures analysis of variance (ANOVA) and within-subject design. For one group under four conditions with an alpha level of .05 and correlation among repeated measures of .5, 13 participants would provide 92% power to detect differences among conditions.

Anthropometric Measurements
Stature was measured using an electronic wall-mounted stadiometer (Seca 242; Seca, Hamburg, Germany) to the nearest 0.1 cm, body mass was recorded on a digital scale (Mettler-Toledo ID1Plus; Mettler-Toledo SEA Pte Ltd, Singapore) to the nearest 0.1 kg, and waist circumference was measured at the umbilicus using a flexible measuring tape. A 30-μl fingertip blood sample was taken for TAG measurement on a dry chemistry analyzer (Reflotron Plus; Roche Diagnostics, Mannheim, Germany; minimum detection level of 0.8 mmol/L) after a 10-hr overnight fast.

Preliminary Exercise Testing
Peak oxygen consumption was determined using a modified continuous walking ramp protocol (Balke & Ware, 1959) set at 6.0–6.5 km/hr. Initial treadmill gradient was 1% and increased by 1% per minute until voluntary exhaustion.Expired air was measured using a metabolic cart (Parvomedics MMS-2400; Parvomedics, Sandy, UT), and heart rate was monitored continuously using short-range telemetry (Polar RS400; Polar, Oulu, Finland). Rating of perceived exertion (RPE; Borg, 1973) was assessed periodically.

Main Trials
Participants completed four 10-day trials: (a) daily consumption of a control margarine while sedentary (C-S); (b) daily consumption of a margarine containing 2 g/day of PS while sedentary (PS-S); (c) daily consumption of a control margarine with 30-min daily walking (C-W); and (d) daily consumption of a margarine containing 2 g/day of PS with 30-min daily walking (PS-W). Randomization was counterbalanced for the first trials with a random integer set generator used to formulate sequences for the remaining trials. On Day 11, participants came to the laboratory for a measurement on a dry chemistry analyzer (Re

**Food Intake, Physical Activity, Alcohol, and Body Mass**
Food intake was self-recorded over 2 weekdays and 1 weekend day prior to the first trial and during trials on Days 3, 6, and 10 using a diary. Participants curtailed physical activities, beyond those of daily living, for each day and refrained from alcohol. Body mass was measured fasted on Days 1 and 11 of each trial.

**Days 1–10**
On Day 1, of all trials, participants reported to the laboratory at 08:00. A fasting blood sample was taken into a 6-ml potassium EDTA vacutainers™ (Becton Dickinson and Co., Franklin Lakes, NJ). Participants were given their margarines to take away for consumption over 10 days. On C-W and PS-W, participants completed a 30-min treadmill walk each day. On Day 10, participants standardized their food consumption and ate one of two standardized evening meals (McChicken®, French Fries, and Coca-Cola®: 4.1 MJ, protein: 20 g, fat: 37 g, and carbohydrate: 138 g or Filet-O-Fish®, French Fries, and Coca-Cola®: 3.9 MJ, protein: 20 g, fat: 33 g, and carbohydrate: 138 g by 21:00. Purchases were repeated on subsequent trials, validated, and reimbursed. Participants then fasted overnight, consuming only water ad libitum.

**Margarines**
Margarines were prepared, formulated, and donated by a local industrial supplier (Lam Soon Group, Singapore). Participants consumed 25 g/day of control or PS (Vegapure® 95 FF; free sterol composition by area: β-sitosterol = 49%, campesterol = 25%, stigmasterol = 18%) margarine on Days 1–10 (Table 1). The PS margarine had a PS ester content of 14%, estimated to provide ~2 g PS per day assuming a coefficient of conversion from PS ester to PS of 0.6. Supplementation of 25 g of soybean oil-based spread providing 1.6 g of PS with β-sitosterol (46.1%) and campesterol (26.6%) can increase serum concentrations of these sterols within 7 days (Clifton et al., 2008). Participants were blind to the supplementation order. Margarines were aliquoted into containers, each with 25 g, by a dieter. Participants were free to consume the margarines within their normal daily food intake pattern and not restricted to a time. They were advised on how to incorporate them into their diet, including spreading or mixing with food items but not frying or cooking, by the dietitian. Consumption of margarines was verified to a research team member daily.

**Walking**
On C-W and PS-W trials, participants completed a 30-min supervised daily treadmill walk at a speed and gradient equivalent to

**Table 1 Energy and Fat Content of the Control and Plant Sterol Margarines**

|                     | Control per 100 g | Plant sterol per 100 g |
|---------------------|------------------|------------------------|
| Energy (MJ)         | 2.03             | 1.96                   |
| Energy from fat (MJ)| 2.01             | 1.94                   |
| Saturated fat (g)   | 13.1             | 12.0                   |
| Monounsaturated fat (g) | 25.1         | 22.7                   |
| Polyunsaturated fat (g) | 14.8         | 16.6                   |
| Trans fat (g)       | 0.2              | 0.2                    |
50% peak oxygen consumption. Heart rate and RPE were recorded during walking. Time of day for walking was catered to fit around each participant’s daily life.

Day 11
Participants reported to the laboratory at 08:00, and a cannula was inserted into a forearm vein. A fasting blood sample was collected 10 min later. Participants then consumed a high-fat milkshake (ice-cream, milk, and cream) providing 1.21 g of fat, 0.62 g of carbohydrate, 0.29 g of protein, and 61 kJ of energy per kilogram of body mass within 10 min. Further blood samples were collected 1–5 hr postconsumption. While in the laboratory, participants read, worked, listened to music, or rested.

Analytical Methods
Blood samples were collected into precooled 6-ml potassium EDTA vacutainers™ (Becton Dickinson and Co.) and centrifuged at 1,000×g for 15 min. Plasma was analyzed for TAG, total cholesterol, and high-density lipoprotein cholesterol (HDL-c) on an automated analyzer (Abbott Architect c4000; Abbott Diagnostics, Chicago, IL). Within- and between-batch coefficients of variation were <2%. The Friedewald formula was used to calculate LDL-c (Friedewald et al., 1972).

Statistics
Data were analyzed using SPSS version 23.0 (IBM, Chicago, IL). TAG and body mass data were non-normally distributed and log transformed. Paired t tests were used to compare mean heart rate and RPE during walking trials. Preintervention data and summary postprandial TAG measures on Day 11 were compared among trials using one-way ANOVA. Two-way ANOVA was used to examine changes among trials and over time. ANOVA was followed by planned contrasts to examine differences between interventions. These are reported along with 95% confidence intervals (CIs) of the differences and effect sizes (Cohen’s d). Significance was set at \( p < .05 \). Data are presented as mean (SD).

Results

Participants
Of 19 individuals randomized, three withdrew because of time commitments or unsuccessful cannulation and one was unable to comply with instructions related to fasting and excluded. Thus, 15 participants were included in the analysis (Table 2).

| Table 2 Physical Characteristics of the Participants \( n=15 \) | Range |
|---|---|
| Age (years) | 25 (3) | 21–33 |
| Height (m) | 1.74 (0.07) | 1.66–1.89 |
| Body mass (kg) | 79.8 (9.1) | 69.6–100.7 |
| BMI (kg/m²) | 26.2 (1.5) | 23.5–29.0 |
| Umbilical waist circumference (cm) | 88.9 (6.4) | 80.8–100.0 |
| Fasting TAG (mmol/L) | 1.1 (0.3) | <0.8–1.7 |
| Maximal oxygen uptake (mL/kg/min) | 36.1 (4.9) | 29.3–48.6 |

Note. Values are presented as mean (SD). BMI=body mass index; TAG=triacylglycerol.

Body Mass and Dietary Intake
Body mass did not differ among trials on Day 1 (\( p = .221 \)) or Day 11 (\( p = .154 \)) or change during trials (interaction, \( p = .520 \)). Preintervention energy intake (Day 1: 7.7 [2.4] MJ; Day 2: 7.5 [2.7] MJ; Day 3: 7.8 [2.3] MJ, \( p = .858 \)), intake of protein (Day 1: 78 [31] g; Day 2: 85 [25] g; Day 3: 75 [18] g, \( p = .442 \), carbohydrate (Day 1: 234 [65] g; Day 2: 211 [64] g; Day 3: 246 [84] g, \( p = .240 \)), and fat (Day 1: 66 [28] g; Day 2: 67 [41] g; Day 3: 66 [28] g, \( p = .958 \)) were similar over 3 days. The preintervention energy and macronutrient intake were similar to the intakes reported during trials (all \( ps > .05 \)) with no differences among trials (data not shown; all \( ps > .05 \)).

Walking
Average heart rate (C-W: 139 [12] beats per min vs. PS-W: 136 [12] beats per min; \( p = .304 \)) and RPE (C-W: 10 [2] vs. PS-W: 10 [2]; \( p = .954 \)) during walking over 10 days were similar between exercise trials.

Fasting Lipid Concentrations on Day 1 and Day 11
Fasting lipids (Table 3) did not differ among trials on Day 1 (all \( ps > .05 \)). Changes in fasting TAG differed among trials and over time (interaction, \( p = .006 \)) increasing on PS-S compared with reductions on C-W (\( p = .019 \)) and PS-W (\( p = .031 \)). This pattern of response was similar for TAG:HDL ratio between Days 1 and 11 (interaction, \( p = .023 \), but contrasts could not identify where the difference occurred. No changes in other lipids were noted (all \( ps > .05 \)). One-way ANOVA based on trial order showed no differences in fasting TAG on Day 1 (\( p = .810 \)) or Day 11 (\( p = .902 \)).

Summary TAG Responses on Day 11
The 5-hr total (main effect of trial, \( p = .001 \); Figure 1a) and incremental (main effect of trial, \( p = .001 \); Figure 1b) areas under the curve differed among trials. For total area, PS-W was 22%, 25%, and 12% lower than C-S (\( d = 0.56, 95\% CI [−0.189, −0.441] \)), PS-S (\( d = 0.67, 95\% CI [−0.213, −0.049] \)), and C-W (\( d = 0.27, 95\% CI [−0.114, 0.009] \)), respectively. Similarly, the 5-hr incremental area with PS-W was 31%, 32%, and 18% lower than C-S (\( d = 0.62, 95\% CI [−0.258, −0.086] \)), PS-S (\( d = 0.64, 95\% CI [−0.301, −0.055] \)), and C-W (\( d = 0.29, 95\% CI [−0.168, 0.005] \)) trials, respectively.

The 5-hr total and incremental area were also lower after C-W than C-S by 11% and 17%, respectively (both \( ps < .05 \)). Peak TAG (main effect of trial, \( p = .002 \)) was 26%, 29%, and 17% lower on PS-W than C-S (\( d = 0.60, 95\% CI [−0.158, −0.033] \)), PS-S (\( d = 0.70, 95\% CI [−0.178, −0.037] \)), and C-W (\( d = 0.36, 95\% CI [−0.099, −0.002] \)) trials, respectively (Figure 1c). One-way ANOVA for trial order showed no differences in TAG total (\( p = .676 \)) or incremental (\( p = .463 \)) area or peak TAG (\( p = .696 \)).

To understand the independent effects of the walking and PS, a two-way ANOVA was conducted for all TAG summary measures (Varady et al., 2004). A main effect of exercise was observed for all summary measures (\( p < .05 \)) but no independent effect of sterols (all \( ps > .05 \)).

Postprandial TAG concentrations on Day 11
There were significant main effects for trial (\( p = .001 \)), time (\( p < .001 \)), and an interaction (\( p = .032 \)) for 5-hr postprandial TAG on Day 11 (Figure 2). Circulating TAG was lower across the postprandial period on the PS-W than the C-S and PS-S trials and...
from 3 to 5 hr than the C-W trial. For the C-W trial, TAG was lower than C-S 2–3 hr after the milkshake.

**Discussion**

Aerobic exercise and PS are hypothesized to synergistically blunt postprandial TAG (Marinangeli et al., 2006). In this study, 10 days of daily walking reduced the 5-hr incremental TAG area by ~17% in Chinese men with elevated BMI, confirming previous observations (Maraki & Sidossis, 2013). However, when PS was combined with walking, the reduction increased to ~32%. Other summary measures support this and demonstrate that, as hypothesized, TAG was reduced in the postprandial period when at its peak.

Two studies have investigated the effects of PS on postprandial TAG (Baumgartner et al., 2016; Demonty et al., 2006). Combining PS (1.7 g/day for 4 weeks) with fish oils in hyperlipidemic individuals reduced postprandial TAG more than fish oils alone, although the independent effect of the PS was not elucidated and only an isolated 4-hr postprandial measure taken (Demonty et al., 2006). The second study found no reduction in postprandial TAG after 4 weeks of PS supplementation (3 g/day) in individuals with normal fasting TAG (Baumgartner et al., 2016). The conflicting findings may relate to the background TAG/lipid levels of participants. However, like the current investigation, the second study included an independent PS trial and with this study’s data suggest that PS do not reduce postprandial TAG in isolation.

Circulating TAG is transported in intestinal-derived chylomicrons and hepatic-derived VLDL and reflects a balance in the rate of appearance and clearance of these particles (Malkova & Gill, 2006). Exercise can improve lipoprotein lipase affinity for TAG.
hydrolysis, particularly in VLDL (Ghafouri et al., 2015), while PS can reduce plasma and hepatic TAG by interfering in intestinal fatty acid absorption (Rideout et al., 2010). While walking alone reduced postprandial TAG in our investigation, no reduction was seen with PS. However, some studies show reduced VLDL with PS or stanols without changes in overall circulating TAG (Plat & Mensink, 2009; Schonewille et al., 2014). This may explain why TAG was unaffected by PS alone, but for the combined intervention, lower VLDL with PS improved TAG clearance to a greater extent than walking alone.

Pooled analysis from 12 investigations in 935 hypercholesterolemic individuals provided 1.6–2.5 g/day of PS found a mean lowering of serum fasting TAG of ∼6%, with the largest decreases in individuals with the highest TAG (Demonty et al., 2013). In comparison with C-S, we did not observe any change in fasting TAG, or other blood lipids, with PS in isolation or when combined with walking. The normal lipid profiles of participants may explain why no change occurred along with the shorter period of supplementation (Demonty et al., 2006; Varady et al., 2004). Alternatively, the impact of PS on fasting lipids in Chinese may not be as great as in Western populations (Li et al., 2007). However, we also note that the intervention was not designed/powered to observe fasting lipid changes.

The within-subject randomized design is a study strength. Evaluation of PS on postprandial TAG in Chinese participants with elevated BMI is novel given that the efficacy of PS on blood lipids in Chinese is not as strong as for Western populations (Li et al., 2007) but that some public health recommendations advocate their use even in individuals at low or moderate cardiovascular risk. Several limitations exist including the previously mentioned short supplementation period. Nevertheless, supplementation with a comparable dose and type of PS as used here substantially elevated serum concentrations within a similar time scale (Clifton et al., 2008). However, we did not measure serum PS and cannot confirm the changes over the trials or associate them with changes in postprandial TAG. Similarly, serum PS concentrations after each washout period are uncertain, although washout time should have been sufficient (Clifton et al., 2008) and no trial order effects were noted. We did also not objectively measure physical activity outside of laboratory testing, control the time of exercise training, or conduct postprandial testing on Day 1 of each intervention. Potentially, physical activity or lifestyle habits could change over time affecting TAG concentrations, although no trial order effects were noted.

In conclusion, 10 days of daily walking reduces postprandial TAG, but further reductions are achieved with consumption of 2 g/day of PS in healthy Chinese men with elevated BMI.
Novelty Statement

This study demonstrates a novel strategy to lower postprandial TAG by combining daily PS with exercise.

Practical Applications

Individuals spend much of their day with elevated TAG. Consuming PS with moderate-intensity exercise represents a simple, achievable strategy to help control TAG levels.

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