Eucaloric diets enriched in palm olein, cocoa butter, and soybean oil did not differentially affect liver fat concentration in healthy participants: a 16-week randomized controlled trial

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ABSTRACT

Background: Effects of dietary fat quality on liver fat remain to be elucidated. Inconsistent evidence may be influenced by fatty acid saturation, chain-length, and regio-specificity within triacylglycerol (TAG) molecules.

Objectives: We aimed to compare eucaloric diets enriched in palm olein (POo), cocoa butter (COB), and soybean oil (SBO) on liver fat concentration in healthy participants. Secondary outcomes included visceral (VAT) and abdominal subcutaneous (aSCAT) adipose tissue, plus other obesity and cardiometabolic health outcomes.

Methods: Eighty-three healthy participants (20–45 y, BMI 18.5–27.5 kg/m^2) commenced and 64 completed a 16-wk randomized parallel intervention, preceded by a 2-wk run-in. Participants consumed identical eucaloric background diets differing in test fats [contributing 20% total energy intake (%E)], providing 33%E total fat with the following ratios for PUFAs/SFAs/MUFAs: POo, 4.2/13.5/15%E; SBO, 14.4/8.8/9.4%E; COB, 2.3/19.5/11%E. Liver fat and abdominal adiposity were measured at weeks 0 and 16 using ^{1}H-magnetic resonance spectroscopy/imaging; all other outcomes were measured at 0, 4, 8, 12, and 16 wk.

Results: Fat quality did not affect liver fat concentration, VAT, aSCAT, obesity indexes, blood pressure, liver enzymes, leptin, or fasting glucose. Body fat mass decreased with SBO and COB compared with POo. SBO decreased serum total cholesterol (TC), LDL cholesterol, and TC:HDL cholesterol relative to POo [estimated marginal mean (95% CI) differences: −0.57 (−0.94, −0.20) mmol/L; −0.37 (−0.68, −0.07) mmol/L; and −0.42 (−0.73, −0.11) mmol/L, respectively]. No diet differences were observed on HDL cholesterol, TAG, apoA1, apoB, apoB:apoA1, or fecal free fatty acids (FFAs), except for lower FFA pentadecanoic acid (15:0) with COB than with SBO and POo.

Conclusions: In healthy adults, when consumed as part of eucaloric typical Australian diets, 3 different dietary fat sources did not differentially affect liver fat concentration and amounts of adipose tissue. Effects on serum lipids were inconsistent across lipid profiles. The findings must be confirmed in metabolically impaired individuals before recommendations can be made. *Am J Clin Nutr* 2021;113:324–337.

Keywords: palm olein, cocoa butter, soybean oil, saturated fatty acids, polyunsaturated fatty acids, liver fat, visceral adipose tissue, subcutaneous adipose tissue, fecal fat, regio-specificity

Introduction

Excessive accumulation of fat in the liver and adipose tissue, especially visceral adipose tissue (VAT), represents a prominent risk factor for insulin resistance and type 2 diabetes (T2D) as well as cardiovascular disease (CVD) (1, 2). Regular aerobic exercise participation, body weight changes, caloric and dietary fat restrictions, or overfeeding have all been shown to modulate liver fat content (3–9). However, the effect of dietary fat quality on liver fat in the absence of overfeeding and metabolic

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Abbreviations used: ALT, alanine aminotransferase; aSCAT, abdominal subcutaneous adipose tissue; AST, aspartate aminotransferase; BAI, body adiposity index; BIA, bioelectrical impedance analysis; COB, cocoa butter; CSIRO, Commonwealth Scientific and Industrial Research Organisation; CVD, cardiovascular disease; FFA, free fatty acid; FM, fat mass; HC, hip circumference; HSI, hepatic saturation index; IHL, intrahepatic lipid; LP(a), lipoprotein (a); MPOB, Malaysian Palm Oil Board; NAFLD, nonalcoholic fatty liver disease; POo, palm olein; SBO, soybean oil; *sn*, stereological number; TAG, triacylglycerol; TC, total cholesterol; T2D, type 2 diabetes; VAI, visceral adiposity index; VAT, visceral adipose tissue; WC, waist circumference; WHR, waist-to-hip ratio; ^{1}H-MRS, proton magnetic resonance spectroscopy; %E, percentage of total energy intake.

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abnormalities remains to be further elucidated. The limited amount of evidence from human studies suggests that diets high in unsaturated fatty acids result in lower liver fat concentrations (10–12) as well as abdominal subcutaneous adipose tissue (aSCAT) (13) and VAT (12) than SFA-rich diets. This was particularly evident when the dietary fats were consumed in excess relative to daily energy requirements (11, 12) and/or when studies were performed in obese participants (10, 13). On the contrary, evidence from nutrition intervention studies in animal models has been inconsistent. Animals fed diets high in SFAs (beef tallow, lard) compared with MUFA (olive oil, canola oil) or PUFA sources [safflower oil, soybean oil (SBO), sunflower oil] showed greater body weight gain, body fat accumulation, aSCAT, VAT, and liver fat (14–17). In contrast, other studies reported greater body weight and fat gain in rodents fed diets high in n–6 PUFAs (SBO, sunflower oil) as opposed to high-SFA diets (palm oil or lard) (18, 19).

Differences in SFA source characteristics may explain these discrepancies; they differ in the proportion of SFAs relative to unsaturated fatty acids, chain length, and the positional distribution of fatty acids on the triacylglycerol (TAG) backbone, also referred to as regio-specificity. The latter may affect the rate of fatty acid absorption and their bioavailability for deposition in tissues (20). Accordingly, fatty acids at the stereological number (*sn*)-2 position are absorbed more efficiently than those at the *sn*-1, 3 positions (21). Furthermore, long-chain SFAs released from *sn*-1, 3 positions seem to be poorly absorbed compared with unsaturated fatty acids with the same regio-specificity. Indeed, long-chain saturated nonesterified fatty acids were more inclined to form insoluble calcium and magnesium soaps in the intestine of rats (22, 23), a process which inhibits their absorption and consequent metabolic effects. In line with this notion, mice consuming SFAs esterified at the *sn*-1, 3 positions [palm olein (POo)] displayed lower adiposity and aSCAT gain and greater excretion of SFAs than those consuming SFAs esterified at the *sn*-2 position (chemically interesterified POo) or PUFAs (SBO) (24). In a follow-up mouse study, Gouk et al. (25) reported that the dietary fat source with the highest proportions of oleic acid at *sn*-1, 3 positions (high-oleic sunflower oil) resulted in greater fat deposition than dietary lipid sources containing SFAs at the same regio-specific position. Furthermore, this effect appeared to be more marked for stearic acid (18:0) than for palmitic acid (16:0) (25). Indeed, fats with high proportions of stearic acid at *sn*-1, 3 positions [cocoa butter (COB)] exhibited the lowest VAT and total body fat deposition (25). Whether this postulation holds true and results in physiologically relevant outcomes in humans remains to be elucidated.

The present study aimed to compare eucaloric diets enriched in POo, COB, and SBO on liver fat concentration in healthy participants. SFA-enriched POo and COB were chosen because most of their SFAs are in the *sn*-1, 3 positions, with the *sn*-2 position mostly (exclusively for COB) occupied by unsaturated fatty acids. PUFA-enriched SBO was chosen as the comparison fat because it was previously reported to affect fat deposition compared with POo and COB in animal models (24, 25), a paradigm not yet confirmed in humans. We hypothesized that the 3 dietary fats would not differentially affect liver fat concentration. Secondary outcomes included VAT, aSCAT, as well as other obesity and cardiometabolic risk markers.

Methods

The clinical trial was approved by the Commonwealth Scientific and Industrial Research Organisation (CSIRO) Human Research Ethics Committee (Adelaide, South Australia) (reference number 11/17) and registered with the Australian Clinical Trials Registry (ACTRN12618000201279). The trial was conducted within the CSIRO Nutrition and Health Research Clinic, Adelaide, South Australia, Australia in accordance with International Conference on Harmonisation Good Clinical Practice guidelines. The dietary intervention phase of the study was executed from March 2018 and completed in December 2018.

Participants

Healthy men and women, aged 20–45 y, were recruited via social media and local advertisement. Oral and written information about the study objectives and protocol were provided to each individual and written informed consent was obtained before their participation in the trial. Prospective participants were screened according to the following criteria: BMI (in kg/m^2) 18.5–27.5; no self-reported history of chronic disease [T2D, hypertension, ischemic heart disease, hyperlipidemia, liver disease, cancer (excluding skin cancer), hemochromatosis]; no pancreatic insufficiency or other condition affecting fat malabsorption; not consuming medication or supplements/nutraceuticals that may affect liver function, blood lipids, blood pressure, or body weight; and nonsmoking for the 6 mo before study commencement. Further, women were excluded if pregnant or currently lactating, or receiving stable hormone replacement therapy or hormone-based contraceptives for less than 3 mo before study commencement. Individuals were assessed at screening for the following exclusion criteria: hyperlipidemia [fasting serum total cholesterol (TC) > 6.2 mmol/L or TAG > 2.0 mmol/L], abnormal serum liver enzymes [alanine aminotransferase (ALT), aspartate aminotransferase (AST)], and mean blood pressure > 140/90 mm Hg.

Study design

The 18-wk study was designed as a 16-wk single-blinded, randomized, 3-arm parallel feeding trial, preceded by a 2-wk run-in period. After the run-in period, participants were block-matched for gender and randomly assigned in a 1:1 ratio to 1 of 3 treatments (either POo or COB or SBO) using computer-generated random numbers (http://www.randomization.com/) generated by an individual independent to study allocations. Treatments were color coded by a staff member not directly involved in the study. Clinic staff involved in the study, as well as research staff involved in the data collection and statistical analyses, were blinded to treatment allocation. Research dietitians and participants were not fully blinded because the appearance and flavor of test fats differed despite efforts to mask this within meals and snacks.

Outcome measurements of liver fat, VAT, and aSCAT were collected at baseline (week 0) and the end of the intervention (week 16). After an overnight fast, blood and fecal samples, anthropometric measurements, body composition, and blood pressure were collected at weeks 0, 4, 8, 12, and 16.

TABLE 1 Fatty acid composition and regio-specific distribution of different fatty acids in the TAGs of dietary test fats[1]

% Fatty acids[2]	POo (IV72)[3]		POo (IV64)		SBO		COB	
14:0	0.45		0.54		—		—	
16:0	29.2		33.8		11.38		29.75	
18:0	1.54		1.97		2.74		34.5	
18:1*cis*	56.2		51.8		21.6		34.4	
18:1*trans*	2.08		1.97		—		—	
18:2*cis*	10.5		9.95		60.8		1.16	
18:3n–3	—		—		3.53		—	
20:0	—		—		—		0.25	
SFA	31.2		36.3		14.1		64.5	
MUFA	58.3		53.8		21.6		34.4	
PUFA	10.5		9.95		64.3		1.16	
TAG regio-specificity[4]	*sn*-2	*sn*-1, 3	*sn*-2	*sn*-1, 3	*sn*-2	*sn*-1, 3	*sn*-2	*sn*-1, 3
SFA	4.3	51.3	8.5	60.7	0	20.1	0	95.9
MUFA	67.1	41.9	63.9	34.5	21.4	26.6	87.4	4.1
PUFA	28.6	6.8	27.6	4.8	78.6	53.3	12.6	0

[1] Values are percentages of total fatty acids. COB, cocoa butter; POo, palm olein; SBO, soybean oil; *sn*, stereological number; TAG, triacylglycerol.
[2] Analyzed by the Commonwealth Scientific and Industrial Research Organisation laboratory.
[3] Run-in fat.
[4] Analyzed by Malaysian Palm Oil Board.

Intervention

For the duration of the 18-wk intervention, participants consumed the same background diet. Test diets differed in test fats only, containing either SBO [supplied by Malaysian Palm Oil Board (MPOB)], POo (IV64) (supplied by MPOB), or COB [sourced from a local importer (Maretai Organics)]. For the run-in period, all participants consumed a premium palm olein (IV72). This fat was selected because of its slightly different composition than the POo (IV64) test fat (slightly higher in unsaturated fatty acids and lower in SFAs) (**Table 1**). The purpose of the run-in fat was to bring study participants to an equilibrium by consuming the same type of fat. Participants were asked to maintain their usual physical activity patterns for the duration of the study, and where acceptable to not consume medications (e.g., not prescribed by their physician) during the study.

The experimental diets were planned to provide 30%–35% of total energy intake (%E) requirements as total fat with the test fats supplying 20%E [~44 g fat/d for an 8400-kJ (2000-kcal) diet], ~15%E as protein, and ~50%E as carbohydrate. These intakes were in line with the average macronutrient intakes of the Australian population based on the latest Australian Health Survey in 2011/2012 (26) and with the Australian National Health and Medical Research Council Nutrient Reference Values Acceptable Macronutrient Distribution Ranges of 20%–35%E as fat, 15%–25%E as protein, and 45%–65%E as carbohydrate (27).

The test fats were provided via 3 food items: 1 frozen meal consumed each day for either lunch or dinner and cookies and cake consumed daily at any time, with the amount of cookies varying according to the participant's energy requirements.

The nutrient composition of the diets was planned using Food Works Professional Edition version 9 (Xyris Software, 2017) and confirmed analytically before the commissioning and production of test meals and snacks. The final macronutrient composition of the manufactured products and whole diets was reconfirmed analytically using standard AOAC methods (**Supplemental Table 1**).

The test foods were prepared in commercial food manufacturing facilities (Community Chef and Emmaline's Country Kitchen) according to recipes developed by the research dietitian and all foods were certified fit for human consumption.

The prescribed diets were eucaloric in order to maintain body weight stability. Diets were designed at different levels of energy in increments of 1500 kJ for adjustment to individual energy requirements. Participants' individual energy requirements were determined using the Schofield equation (28) based on age, gender, and physical activity. Participants were then assigned the closest 1500-kJ bracket. Participants were requested to consume all the food supplied and not to consume any other high-fat foods. Breakfast was standardized, comprised of a choice of 3 cereals (Sanitarium Weetbix, Uncle Toby's Iron Plus, or Kellogg's Special K), and provided to be consumed with low-fat milk. The remainder of the diet was prescribed as units from the 5 food groups: fruit, low-fat dairy, lean meat or meat alternatives, vegetables, and low-fat breads and cereals, with the number of units from each food group calculated relative to the energy level. Although the diets contained cookies and cake, the remainder of the diet was made up of healthy foods in order to ensure overall nutritional adequacy (see **Supplemental Table 2** for selected daily nutrient intakes from an 8500-kJ diet). The 2-wk run-in period was included to minimize any potential impact that transitioning from habitual diets to intervention diets may have caused.

Before the run-in period, participants attended a diet consultation with a dietitian, who explained the procedures related to the dietary interventions and provided advice regarding how to manage "special occasions," for example when required to eat away from home. A sample meal plan was developed in consultation with the participant to demonstrate how to incorporate all study foods and other food units into their daily diet, with an emphasis on selecting low-fat options. Participants were also provided with a detailed and illustrated shopping list to assist with food selection and a set of measuring cups and spoons to assist with correct portioning of foods.

Participants were requested to record their weight measured at home (~3 times/wk) during the run-in phase and 1 time/wk for the 16-wk intervention phase. Participants were also asked to record their intake of study foods daily using a paper checklist and report this into an online platform at weekly intervals, with the results available to the dietitian in real time for monitoring. If a trend was observed in change of body weight (±~1 kg from baseline for the first 2 wk; ±~3 kg from baseline thereafter) or a deviation from the study dietary protocol was reported, participants were contacted by the dietitian to determine possible reasons for the change or deviation and adjustments made to the prescribed energy intake level accordingly.

Compliance to study treatments was assessed by self-reported intake checklists with data provided weekly to the dietitian via an online survey. In addition, compliance was cross-monitored by the dietitian at each visit. Percentage compliance scores were calculated for the run-in and intervention periods using data on daily consumption of test fat food items (frozen meals, cookies, and cake). The reported intake of test foods was summed and divided by the prescribed test food intake for each participant. For missing data, participants who had returned ≥80% of their compliance records (i.e., ≥14 of 18 wk of compliance records) had a mean value for each individual calculated and substituted for the missing values.

Study outcomes

All primary and secondary outcomes were investigated as predeclared at trial registration. The primary outcome was liver fat concentration, quantified as intrahepatic lipid (IHL) by proton magnetic resonance spectroscopy (^{1}H-MRS). Secondary outcomes were hepatic saturation index (HSI), VAT, aSCAT as measured by MRI, total body fat as inferred by bioelectrical impedance analysis (BIA), obesity indexes [BMI, waist circumference (WC), waist-to-hip ratio (WHR), visceral adiposity index (VAI), body adiposity index (BAI)], fasting serum lipid profiles {TC, LDL cholesterol, HDL cholesterol, TC:HDL cholesterol ratio, TAG, apoA1, apoB, apoB:apoA1 ratio, lipoprotein (a) [LP(a)]}, fasting leptin, liver enzymes (ALT, AST), fasting plasma glucose, fecal total fat and free fatty acids (FFAs), and blood pressure.

Body fat assessments

IHLs (liver fat concentration) were measured by ^{1}H-MRS using a 3.0 Tesla Achieva whole-body system (Philips Medical Systems). Liver fat concentration and HSI were measured as detailed previously (29) using a localized point resolved spectroscopy (PRESS) sequence in 2 voxel sites, with the mean of the 2 sites presented. For liver fat concentration and composition, a 5-resonance model was used including the "fat metabolite peaks" (allylic, methylene, and methyl), which allows for quantification of both liver fat percentage and liver fat composition (29). Hepatic water signal amplitudes were measured from the non-water-suppressed spectrum using Hankel Lanczos Squares Singular Values Decomposition, and liver fat concentration was determined as the ratio of the methylene resonance to water, expressed as a percentage (29). HSI was quantified as the complement of the signal amplitude from allylic hepatic lipid protons, divided by the signal amplitude of all hepatic lipid protons (i.e., methyl, methylene, allylic) as detailed elsewhere (29). We have previously shown that the CV for this technique is ~7% (29).

Abdominal subcutaneous and visceral fat volumes were quantified by MRI. Noninvasive, nonionizing MRI, along with computed tomography, are considered gold standard for VAT measurement, with a CV for repeated measures of ~2% (30). Cross-sectional areas of both VAT and aSCAT were determined using automated software (e.g., Hippo Fat^{TM}) (31) and summated for the abdomen to determine volumes.

Body composition, including total fat mass (FM) and fat-free mass, was assessed using a multifrequency BIA with 8 tactile electrodes (InBody 230, Biospace Co. Ltd.). Measurements were obtained after voiding of the bladder.

Anthropometric measurements, obesity indexes, and blood pressure

Height was measured using a stadiometer (Seca) and body weight measured using calibrated electronic digital scales (Mercury, AMZ 14). BMI was calculated from these measurements.

WHR was calculated after measurements with a plastic measuring tape of WC (top of the iliac crest, at minimal respiration for the mean of 3 consecutive readings) and hip circumference (HC) (at the largest circumference of the buttocks). VAI and BAI were calculated as follows (32, 33):

$$\text{VAI (men)} = [\text{WC}/39.68 + (1.88 \times \text{BMI})] \times (\text{TAG}/1.03) \times (1.31/\text{HDL cholesterol}) \quad (1)$$

$$\text{VAI (women)} = [\text{WC}/36.58 + (1.89 \times \text{BMI})] \times (\text{TAG}/0.81) \times (1.52/\text{HDL cholesterol}) \quad (2)$$

$$\text{BAI} = [\text{HC(cm)}/\text{height}(\text{m}^{1.5})] - 18 \quad (3)$$

Blood pressure was measured using an automated blood pressure monitor (Philips SureSigns VS3, Philips Medical Systems) with participants in a seated position, after a 5-min rest. The mean of 3 measurements (separated by 2 min) was recorded.

Biochemical analysis

Venous blood samples assessed at screening were sent directly to a commercial testing laboratory for analysis of serum TC, TAG, and liver enzymes (ALT, AST).

Venous blood samples collected during the study intervention (weeks 0, 4, 8, 12, and 16) were processed and stored at −70°C until study completion. Serum and plasma were prepared by centrifugation (GS-6R centrifuge; Beckman Coulter Inc.) at 2095 × *g* for 10 min at 4°C. Blood for serum was left at room temperature for 30 min to allow for clot formation and then centrifuged at 2850 × *g* for 15 min at 4°C. Vacutainers containing sodium fluoride were used for collection of blood samples for glucose analysis and centrifuged at 3000 × *g* for 10 min at 4°C. Samples from each subject were analyzed within the same analytic run to reduce variation.

A Beckman AU480 clinical analyzer (Beckman Coulter Inc.) and commercial enzymatic test kits were used for analysis of serum TC, TAG, HDL cholesterol, LDL cholesterol, ALT, AST, and plasma glucose; and immunoturbidimetric test kits for Lp(a),

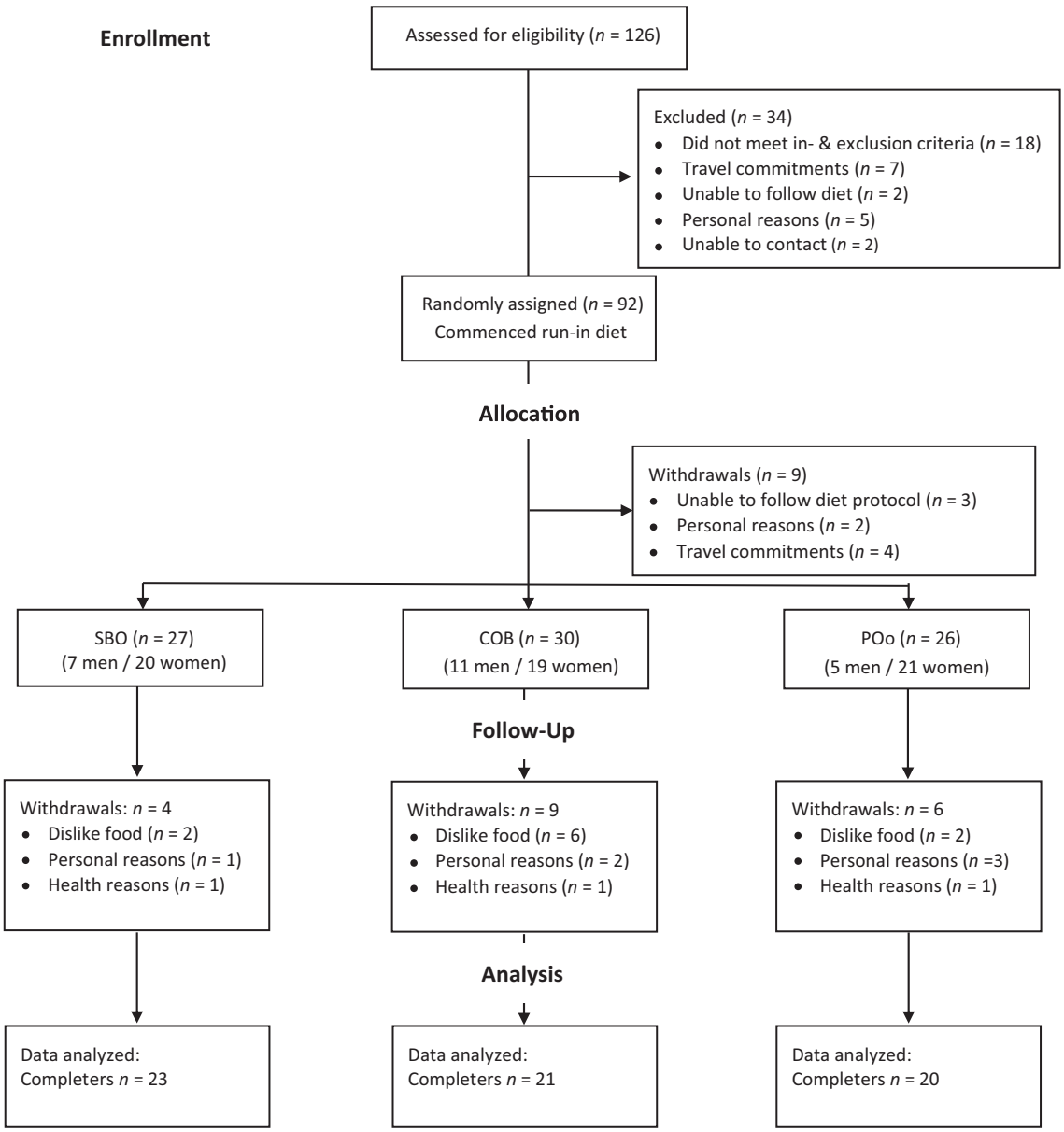

FIGURE 1 Flowchart of participants through the trial. COB, cocoa butter; POo, palm olein; SBO, soybean oil.

apoA1, and apoB. TC:HDL cholesterol and apoB:apoA1 ratios were calculated from these results. Serum leptin concentration was analyzed using AlphaLISA test kits on a microplate reader (PerkinElmer). Intra-assay CVs were TC: 0.53%; TAG: 0.86%; HDL cholesterol: 0.69%; LDL cholesterol: 1.79%; AST: 1.27%; ALT: 1.23%; glucose: 0.58%; Lp(a): 0.86%; apoA1: 0.73%; apoB: 0.98%; and leptin: 6.3%.

Fecal collection and fatty acid analysis

A one-off fecal sample was collected, stored in a cooler bag, and delivered to the Research Clinic within 24 h. Fecal samples were stored at −20°C until analyses at study completion. A 150-mg fecal sample was treated with 200 μL 0.2 M KOH/MeOH and 50 μL of a 1-mg/mL solution of margaric acid (17:0) (internal standard) followed by acidification with acetyl chloride (34). Methyl esters were extracted into 1 mL hexane and an aliquot (1.0 μL) was injected onto a gas chromatographic column (DB-FastFAME, 20 m × 0.18 mm; Agilent Technologies), using a PerkinElmer Clarus 690 gas chromatograph/Clarus SQ 8 T Mass Spectrometer with a split 50:1 injector. Fatty acids were identified by comparison with authentic Sepelco 37 component FAME mix (Sigma-Aldrich) and verified using the NIST MS Search database software. Peaks were measured using TurboMass software (PerkinElmer) and component peak area was expressed as a percentage of the total area of the known fatty acid peaks to give a fatty acid profile. Total fats were calculated against the internal standard. The mean intra-assay CV was 11.8%.

Sample study diets were also analyzed for fatty acid composition as described above.

TABLE 2 Baseline characteristics for completers[1]

	SBO ($n = 23$)	COB ($n = 21$)	POo ($n = 20$)
Gender, *n* (M/F)	6/17	9/12	5/15
Age, y	32.9 ± 7.79	33.7 ± 8.01	31.3 ± 8.13
Weight, kg	62.8 ± 9.41	68.9 ± 12.0	68.5 ± 11.5
BMI, kg/m^2	22.2 ± 2.44	22.6 ± 2.22	23.6 ± 2.54
Waist circumference, cm	73.4 ± 7.44	75.3 ± 8.29	77.4 ± 7.49
Hip circumference, cm	93.4 ± 5.51	94.0 ± 6.11	95.9 ± 7.06
Waist-to-hip ratio	0.78 ± 0.05	0.80 ± 0.07	0.81 ± 0.06
Systolic BP, mm Hg	108 ± 8.38	111 ± 8.05	106 ± 9.51
Diastolic BP, mm Hg	68.8 ± 7.49	71.4 ± 6.22	67.4 ± 8.02
Serum TC, mmol/L	4.56 ± 0.77	4.69 ± 0.72	4.52 ± 0.59
Serum HDL-C, mmol/L	1.44 ± 0.29	1.46 ± 0.35	1.41 ± 0.26
Serum TC:HDL-C ratio, mmol/L	3.12 ± 0.66	3.23 ± 0.67	3.11 ± 0.58
Serum TAG, mmol/L	0.90 ± 0.46	1.00 ± 0.46	0.76 ± 0.23
Serum LDL-C, mmol/L	2.70 ± 0.55	2.78 ± 0.65	2.75 ± 0.51
AST, U/L	21.5 ± 6.96	23.1 ± 9.16	22.2 ± 9.07
ALT, U/L	17.3 ± 10.7	19.3 ± 13.8	17.5 ± 11.1
IHL, %	0.86 ± 0.83	1.00 ± 0.78	1.46 ± 1.79
HSI, arbitrary units	0.93 ± 0.04	0.91 ± 0.04	0.93 ± 0.03
aSCAT, cm^3	3668 ± 1534	3521 ± 1686	4976 ± 2245
VAT, cm^3	796 ± 469	707 ± 425	969 ± 915
Body adiposity index	25.0 ± 2.27	23.1 ± 2.71	25.5 ± 3.61
Visceral adiposity index	1.03 ± 0.51	1.12 ± 0.65	0.91 ± 0.37
Body fat mass by BIA, kg	16.0 ± 4.73	14.5 ± 5.43	18.1 ± 6.76
Fat-free mass by BIA, kg	46.6 ± 7.77	54.2 ± 13.2	50.1 ± 11.3

[1] $n = 64$. Values are means ± SDs calculated from raw data unless indicated otherwise. ALT, alanine transaminase; aSCAT, abdominal subcutaneous adipose tissue; AST, aspartate transaminase; BIA, bioelectrical impedance analysis; BP, blood pressure; COB, cocoa butter; HDL-C, high-density lipoprotein cholesterol; HSI, hepatic saturation index; IHL, intrahepatic lipid; LDL-C, low-density lipoprotein cholesterol; POo, palm olein; SBO, soybean oil; TAG, triacylglycerol; TC, total cholesterol; VAT, visceral adipose tissue.

Statistical analysis

Statistical power for this study was based on the primary outcome: liver fat concentration. It was calculated that a sample size of 24 participants/group would provide 80% power at $\alpha = 0.05$ to detect a minimum difference of 1.5% in liver fat concentration between treatments. The calculation was based on a mean SD of 1.8% observed within healthy lean Caucasian populations (3, 35). Considering a dropout rate of ∼20% it was planned to recruit a total sample of 90 participants (30/group).

Statistical analyses were performed on changes from baseline calculated by subtracting 4-, 8- 12-, and 16-wk data from baseline data.

Completers' data (participants who completed the intervention) were analyzed. Differences between groups, expressed as changes from baseline to 16 wk, for IHL, HSI, aSCAT, VAT, and VAT:aSCAT (only measured at weeks 0 and 16), were performed using ANCOVA, controlling for baseline values of the respective outcome variable, baseline BMI, and gender. For all other variables (measured at 0, 4, 8, 12, and 16 wk), differences between groups in changes from baseline were assessed using mixed-effects longitudinal models, controlling for baseline values of the respective outcome variable, baseline BMI (except for anthropometric variables), and gender. An unstructured repeated covariance matrix structure was used. Treatment and time were included as fixed factors and analyzed for treatment × time interaction effects. Participant identifier was fitted as a random effect, i.e., 1 intercept per participant. Post hoc analysis was adjusted using Bonferroni adjustments. Mixed model assumptions were tested by checking residuals for normality by examining histograms and Q-Q plots. In cases where residuals were not normally distributed [including changes in serum TAG, Lp(a), leptin, ALT, AST, fecal FFA 18:1*cis*, 18:1*trans*, and 18:2*cis*] the specific dependent variable was logarithmically transformed, and analysis performed on the transformed variable.

Statistical analysis was performed using SPSS software version 26 (IBM Corporation). Results are presented as unadjusted means ± SDs or means (95% CIs). For all data analyses, statistical significance was determined at a P value < 0.05.

Results

Study population

Ninety-two participants were recruited of whom 9 withdrew during the run-in phase owing to inability to follow the diet protocol, personal reasons, and travel commitments. Eighty-three were randomly assigned of whom 64 completed the interventions (23% attrition rate) (**Figure 1**). Reasons for withdrawal included dislike of study foods, personal reasons, and health reasons. Withdrawal rates due to dislike of study foods seemed greater in the COB group ($n = 6$) than in the other groups ($n = 2$ for both SBO and POo groups). This was expected because COB has a distinct flavor and is not usually employed in the preparation of meals in Australia.

Baseline characteristics, summarized in **Table 2** ($n = 64$) and **Supplemental Table 3** ($n = 83$), reflect the inclusion criteria: namely, young healthy adults with anthropometrics, blood pressure, and serum lipid profiles within normal ranges. Baseline characteristics did not differ between completers and withdrawals ($P > 0.05$).

Three participants had liver fat concentration > 5% at baseline, consistent with the presence of nonalcoholic fatty liver disease (NAFLD) (36); all 3 were from the POo group. One of these participants had a liver fat concentration of 17.7%.

Baseline characteristics did not differ between the 3 groups for gender, age, blood pressure, lipid profiles, or liver enzymes, but several baseline anthropometric and body composition variables appeared higher and more variable in the POo group than in the SBO and COB groups, including BMI, WC, IHL, aSCAT, VAT, and body FM. Accordingly, statistical analyses were controlled for baseline BMI and baseline levels of the respective variables being analyzed.

Prescribed energy intake did not differ between groups.

Compliance

Mean compliance during the run-in period (POo IV72) was 93.3% ± 6.6% and during the intervention period was 98.4% ± 2.4% for SBO, 98.9% ± 2.9% for COB, and 98.9% ± 1.2% for POo for completers.

The total number of protocol deviations related to consuming other high-fat foods (defined as a food item containing >5 g fat) recorded over the 18-wk study period was 749. The mean ± SD number of deviations per participant was 11 ± 8.12 ($n = 24$) for SBO, 10 ± 9.13 ($n = 27$) for COB, and 9 ± 7.52 ($n = 24$) for POo. This equated to only 1 deviation every fortnight over a period of 18 wk, which is relatively small and unlikely to have affected the overall results of the trial.

Body fat assessments by ^{1}H-MRS/MRI

One participant in the POo group had a liver fat concentration of 17.7% and 26.3% at baseline and 16 wk, respectively. Because this was clearly an outlier in this sample, this participant's liver fat concentration values were omitted from the analyses.

No significant differences were seen between groups for IHL, HSI, aSCAT, VAT, or VAT:aSCAT ratio (**Table 3**). Individuals' responses (**Figure 2**) were inconsistent, displaying small increases, decreases, or no change in IHL from baseline. Results did not differ when the participants with liver fat concentration > 5% were omitted (data not shown).

Anthropometric measurements and obesity indexes

No significant differences were seen between diet groups for any of the anthropometric and obesity indexes (**Table 4**). However, body FM was significantly reduced with the SBO and COB diets compared with the POo diet. The estimated marginal mean (95% CI) differences in changes were as follows: at 4 wk between SBO and COB: 0.74 (0.02, 1.46) kg; between COB and POo: −0.80 (−1.56, −0.06) kg; at 12 wk between SOB and POo: −0.98 (−1.95, −0.003) kg; at 16 wk between SBO and POo: −1.35 (−2.39, −0.30) kg; and between COB and POo: −1.18 (−2.25, −0.11) kg. **Supplemental Table 4** summarizes descriptive statistics calculated from the raw data.

Biochemical markers and blood pressure

Serum TC, LDL cholesterol, and TC:HDL cholesterol were significantly lower at 16 wk in participants on the SBO diet than in those on the POo diet (**Table 5**). Estimated marginal mean

TABLE 3 Changes in body fat assessments by ^{1}H-MRS and MRI during the study and comparisons between diets[1]

	SBO ($n = 23$)			COB ($n = 20$)			POo ($n = 19$)			
Variables	Baseline	Week 16	Change	Baseline	Week 16	Change	Baseline	Week 16	Change	P value[2]
IHL, %	0.86 ± 0.83	0.97 ± 1.14	0.11 (−0.22, 0.43)	1.00 ± 0.78	0.80 ± 0.62	−0.18 (−0.36, 0.00)	1.46 ± 1.7[3]	1.45 ± 1.6[3]	0.08 (−0.45, 0.61)[3]	0.18
HSI, arbitrary units	0.93 ± 0.04	0.92 ± 0.05	−0.01 (−0.04, 0.01)	0.91 ± 0.04	0.90 ± 0.08	−0.01 (−0.04, 0.02)	0.93 ± 0.03	0.94 ± 0.04	0.01 (−0.01, 0.03)	0.56
aSCAT, cm^3	3668 ± 1534	3707 ± 1589	39.5 (−170, 249)	3521 ± 1686	3371 ± 1788	−80.9 (279, 117)	4976 ± 2245	5116 ± 2369	78.0 (−112, 268)	0.26
VAT, cm^3	796 ± 469	775 ± 483	−20.6 (−91.5, 50.3)	707 ± 425	738 ± 443	18.1 (−54.6, 90.8)	969 ± 915	1013 ± 789	62.7 (−68.6, 194)	0.16
VAT:aSCAT	0.22 ± 0.10	0.21 ± 0.09	−0.01 (−0.03, 0.01)	0.22 ± 0.14	0.24 ± 0.12	0.01 (−0.01, 0.03)	0.20 ± 0.21	0.21 ± 0.17	0.01 (−0.02, 0.04)	0.63

[1] Values are unadjusted means ± SDs unless indicated otherwise; change values are means (95% CIs). aSCAT, abdominal subcutaneous adipose tissue; COB, cocoa butter; HSI, hepatic saturation index; IHL, intrahepatic lipid; POo, palm olein; SBO, soybean oil; VAT, visceral adipose tissue; ^{1}H-MRS, proton magnetic resonance spectroscopy.

[2] Comparisons between treatment groups were performed using ANCOVA; changes from baseline to 16 wk were calculated by subtracting 16-wk data from baseline data and compared between groups while controlling for the baseline values of the respective outcome variable, baseline BMI, and gender. P value refers to the differences between groups in change from baseline to 16 wk.

[3] $n = 18$, excludes 1 participant with IHL > 17%.

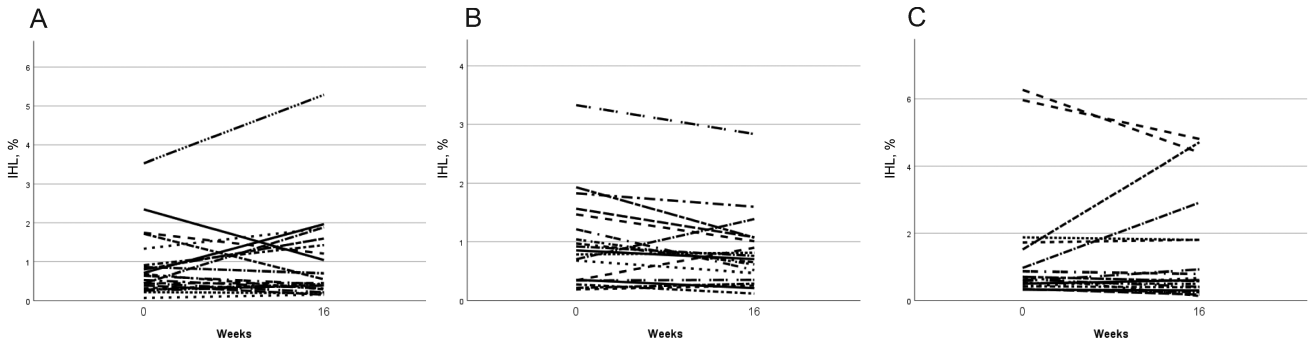

FIGURE 2 Individual responses in IHL percentages to soybean oil diet (A), cocoa butter diet (B), and palm olein diet (C). IHL, intrahepatic lipid.

(95% CI) differences in change from baseline to 16 wk were as follows: −0.57 (0.94, −0.20) mmol/L for TC, −0.37 (−0.68, −0.07) mmol/L for LDL cholesterol, and −0.42 (−0.73, −0.11) mmol/L for TC:HDL cholesterol (**Supplemental Table 5**). These differences were due to significant reductions in these variables over time on the SBO diet whereas changes over time on the POo diet were nonsignificant. No differences were seen between diets for HDL cholesterol, TAG, apoA1, apoB, and apoB:apoA1 ratio. Serum Lp(a), leptin, plasma glucose, and diastolic blood pressure also did not differ between diets. Although the difference in systolic blood pressure between groups was overall statistically significant, post hoc tests were not significant. **Supplemental Table 6** summarizes descriptive statistics calculated from the raw data.

Fecal total fat and FFAs

Fecal FFA pentadecanoic acid (15:0) decreased in the group consuming COB compared with SBO and POo. Estimated marginal mean (95% CI) differences in changes were as follows: at 4 wk between COB and POo: −1.43% (−2.32%, −0.55%); at 8 wk between COB and SBO: −0.90% (−1.61%, −0.18%); at 16 wk between COB and SBO: −0.89% (−1.69%, −0.08%). Total fecal fat and other fecal FFA concentrations did not differ significantly between groups (**Table 6**). Although not statistically significant, at face value, fecal FFA palmitic acid and FFA stearic acid appeared to increase with the POo and COB diets, respectively (**Supplemental Figure 1**). The estimated marginal mean (95% CI) difference in change at 16 wk for fecal FFA stearic acid between COB and SBO was 18.3% (7.4%, 29.2%) and between COB and POo was 22.7% (11.3%, 34.0%); and for palmitic acid, the difference in change between POo and SBO was 7.75% (−0.50%, 16%) and between POo and COB was 8.60% (−0.02%, 17.21%). **Supplemental Table 7** summarizes descriptive statistics calculated from the raw data.

Adverse events

Over the duration of the trial, 2 adverse events were reported that were possibly related to the study diets: 1 case of constipation, mild in severity (COB group); and 1 case of vomiting, moderate in severity (POo group).

Discussion

The present investigation provides, to our knowledge, the first comparison between diets enriched in SFA sources, with the majority of SFAs on the *sn*-1, 3 position, and PUFA-rich SBO in metabolically healthy participants in the absence of overfeeding or changes in body weight. Results showed that POo, COB, and SOB diets, over a period of 16 wk, did not differentially affect liver fat concentration, abdominal adipose tissue, or several other obesity outcomes, although total body FM was reduced with SBO and COB relative to POo (−3.6% and −2.83% with SBO and COB, respectively, compared with 3.6% with POo). SBO decreased serum TC, LDL cholesterol, and TC:HDL cholesterol relative to POo (−7.46% compared with 6.0% for TC; −9.63% compared with 5.45% for LDL cholesterol; −3.85% compared with 6.75% for TC:HDL cholesterol). No differences were observed between diets on serum HDL cholesterol, TAG, apoA1, apoB, apoB:apoA1, Lp(a), leptin, and plasma glucose. Fecal FFA pentadecanoic acid was significantly less excreted with COB than with SBO and POo, whereas COB and POo appeared to increase fecal excretion of FFA stearic acid and palmitic acid, respectively, but this did not reach statistical significance.

In contrast to the current study, results from previous clinical trials showed lower liver fat concentrations in response to high–unsaturated fatty acid than in response to high-SFA diets (10–12). Several study design differences may explain the discrepancies. Previous findings were observed in the presence of hypercaloric diets and weight gain (11, 12) or were conducted in obese participants at greater risk of developing metabolic abnormalities (10, 11, 13). Under hypercaloric conditions SFAs may promote the build-up of fat in the liver through increased circulating ceramides, insulin resistance, increased adipose tissue lipolysis, and circulating FFA availability (11) and lower β-oxidation rates compared with unsaturated fatty acids (37). Furthermore, the total dietary fat content of the current study (33%E) was modest and aligned with typical Australian diets and recommendations. Other studies, however, used much higher total fat proportions ranging from ∼40%E to ∼60%E (7, 10, 11, 13), often with disproportionate contents between diets with the SFA diets often higher in fat than the PUFA diets (7, 10, 13). It is known that high-fat diets increase liver fat (9). Utzschneider et al. (7) showed no differences in liver fat between isocaloric low-fat/low-SFA/low-glycemic-index and high-fat/high-SFA/high-glycemic-index diets in older adults, albeit it is impossible to disentangle the SFA effects from the other dietary changes. Participants in the present study were metabolically healthy; they had BMI, lipid profiles, plasma glucose concentrations, and blood pressure levels within normal reference ranges, only 3 participants met NAFLD criteria, and none had a history of chronic

TABLE 4 Changes in anthropometric measurements and obesity indexes from baseline and comparisons between diets[1]

Variable	Wk	SBO ($n = 22$)	COB ($n = 21$)	POo ($n = 20$)	P value[2]
BMI, kg/m^2	0	22.2 (21.7, 22.8)	22.6 (22.2, 23.1)	23.6 (23.1, 24.2)	—
	Δ4	0.07 (−0.07, 0.20)	− 0.08 (−0.22, 0.05)	0.01 (−0.14, 0.16)	0.35
	Δ8	− 0.01 (−0.18, 0.16)	− 0.09 (−0.32, 0.13)	0.07 (−0.09, 0.23)	
	Δ12	0.13 (−0.05, 0.31)	− 0.14 (−0.38, 0.11)	0.21 (0.00, 0.42)	
	Δ16	0.05 (−0.20, 0.30)	− 0.14 (−0.37, 0.09)	0.20 (−0.04, 0.45)	
WC, cm	0	73.4 (71.8, 74.9)	75.3 (73.6, 77.1)	77.4 (75.8, 79.0)	—
	Δ4	− 0.63 (−1.47, 0.21)	− 0.74 (−1.66, 0.18)	− 0.36 (−1.47, 0.75)	0.53
	Δ8	− 0.85 (−2.20, 0.50)	− 0.63 (−2.00, 0.74)	− 0.70 (−1.74, 0.33)	
	Δ12	− 1.28 (−2.59, 0.04)	− 1.28 (−2.73, 0.17)	− 0.16 (−1.11, 0.78)	
	Δ16	− 1.31 (−2.53, −0.08)	− 1.23 (−2.26, −0.20)	− 0.60 (−1.48, 0.28)	
WHR	0	0.78 (0.77, 0.80)	0.80 (0.79, 0.82)	0.81 (0.80, 0.82)	—
	Δ4	0.00 (−0.02, 0.02)	− 0.01 (−0.02, 0.00)	0.00 (−0.02, 0.01)	0.66
	Δ8	0.01 (−0.01, 0.03)	0.00 (−0.01, 0.01)	− 0.01 (−0.02, 0.01)	
	Δ12	0.00 (−0.02, 0.02)	− 0.01 (−0.02, 0.01)	− 0.01 (−0.02, 0.01)	
	Δ16	0.00 (−0.02, 0.02)	0.00 (−0.02, 0.01)	− 0.01 (−0.03, 0.01)	
BAI	0	25.0 (24.5, 25.5)	23.1 (22.5, 23.7)	25.5 (24.7, 26.2)	—
	Δ4	− 0.56 (−1.39, 0.27)	0.18 (−0.39, 0.74)	− 0.12 (−0.68, 0.43)	0.31
	Δ8	− 0.91 (−1.76, −0.06)	− 0.30 (−0.81, 0.21)	− 0.15 (−0.94, 0.64)	
	Δ12	− 0.82 (−1.65, 0.02)	− 0.20 (−0.67, 0.26)	0.04 (−0.75, 0.83)	
	Δ16	− 0.55 (−1.48, 0.37)	− 0.40 (−0.92, 0.13)	0.07 (−0.77, 0.91)	
VAI	0	1.03 (0.92, 1.13)	1.12 (0.98, 1.26)	0.91 (0.83, 0.99)	—
	Δ4	− 0.12 (−0.25, 0.01)	− 0.16 (−0.32, 0.01)	0.04 (−0.09, 0.17)	0.29
	Δ8	0.01 (−0.11, 0.13)	− 0.03 (−0.16, 0.11)	0.14 (−0.03, 0.32)	
	Δ12	− 0.13 (−0.27, 0.02)	0.10 (−0.02, 0.22)	0.28 (−0.05, 0.61)	
	Δ16	0.01 (−0.18, 0.21)	− 0.05 (−0.22, 0.12)	0.12 (−0.02, 0.26)	
FM, kg	0	16.0 (15.1, 17.0)	14.5 (13.4, 15.7)	18.1 (16.7, 19.6)	—
	Δ4	− 0.08 (−0.49, 0.34)[a]	− 0.62 (−1.08, −0.17)[b]	− 0.07 (−0.49, 0.35)[a]	0.01
	Δ8	− 0.36 (−0.83, 0.11)	− 0.40 (−1.01, 0.21)	− 0.14 (−0.70, 0.42)	
	Δ12	− 0.37 (−0.83, 0.10)[a]	− 0.54 (−1.01, −0.06)	0.43 (−0.20, 1.07)[b]	
	Δ16	− 0.57 (−1.13, 0.00)[a]	− 0.41 (−0.90, 0.08)[a]	0.65 (−0.10, 1.39)[b]	
FFM, kg	0	46.6 (45.0, 48.2)	54.2 (51.3, 57.0)	50.1 (47.7, 52.5)	—
	Δ4	0.15 (−0.22, 0.52)	0.45 (0.07, 0.83)	0.12 (−0.32, 0.56)	0.05
	Δ8	0.31 (−0.13, 0.75)	0.13 (−0.38, 0.64)	0.48 (0.09, 0.86)	
	Δ12	0.75 (0.38, 1.12)	0.13 (−0.49, 0.76)	0.42 (0.01, 0.83)	
	Δ16	0.80 (0.38, 1.21)	0.06 (−0.49, 0.61)	0.19 (−0.22, 0.60)	

[1] Values are unadjusted means (95% CIs) unless indicated otherwise. BAI, body adiposity index; COB, cocoa butter; FFM, fat-free mass; FM, fat mass; POo, palm olein; SBO, soybean oil; VAI, visceral adiposity index; WC, waist circumference; WHR, waist-to-hip ratio; Δ, change from baseline.

[2] Comparisons between treatment groups were performed using mixed-effects longitudinal models; changes from baseline were calculated by subtracting 4-, 8-, 12-, and 16-wk data from baseline data and compared while controlling for the baseline values of the respective outcome variable and gender. P value refers to treatment × time interaction.

[a,b] Different superscripts indicate significant differences between groups (Bonferroni adjusted). No superscript means no difference compared with any other group.

metabolic disease. Hence, it may be argued that they have been able to adapt to the dietary challenge, precluding unfavorable changes in liver fat concentration. However, previous trials, albeit employing different dietary interventions compared with this study, demonstrated changes in liver fat concentration in healthy, non-NAFLD populations within timeframes of ≤12 wk (6, 38).

The dietary sources from which SFAs were derived may provide further insight. POo contains a high proportion of unsaturated fatty acids (54% MUFAs, 10% PUFAs) (Table 1), which have been reported to increase β-oxidation rates (37, 39) and reduce liver fat (40). Other studies investigating the effects of SFAs on liver fat used mostly butter to increase the diet's SFA content (10, 11, 13). Palmitic acid in butterfat occurs mostly in the *sn*-2 position, unlike POo where most palmitic acid is in the *sn*-1, 3 positions which may in turn reduce its bioavailability (20).

Fecal excretion of total fat and individual FFAs was assessed in the current study to provide support to the regio-specificity hypothesis, according to which increased excretion may be an indication of reduced bioavailability arising from the specific positional location of fatty acids. However, except for differences in fecal FFA pentadecanoic acid, an odd-chain SFA present in dairy fat and not synthesized in vivo (41), no other fecal FFA or total fecal fat concentrations were statistically significantly different between diets. Albeit, at face value fecal FFA stearic acid and palmitic acid concentrations appeared to increase with the COB and POo diets, respectively. Owing to large interindividual variability (based on wide 95% CIs) and a modest increase in the case of FFA palmitic acid, the trial may have had insufficient statistical power to detect significant effects (type 2 error). These trends are consistent with Gouk et al. who showed greater excretion of FFA palmitic acid

TABLE 5 Changes in biochemical markers and blood pressure from baseline and comparisons between diets[1]

Variable	Week	SBO ($n = 22$)	COB ($n = 21$)	POo ($n = 20$)	P value[2]
Serum TC, mmol/L	0	4.56 (4.40, 4.72)	4.69 (4.54, 4.84)	4.52 (4.39, 4.64)	—
	Δ4	− 0.20 (−0.37, −0.02)	− 0.26 (−0.42, −0.09)	0.09 (−0.13, 0.32)	0.04
	Δ8	− 0.03 (−0.23, 0.18)	0.03 (−0.17, 0.24)	0.26 (0.01, 0.50)	
	Δ12	− 0.18 (−0.39, 0.03)	0.11 (−0.13, 0.35)	0.13 (−0.08, 0.34)	
	Δ16	− 0.34 (−0.58, −0.11)[a]	0.02 (−0.17, 0.22)	0.27 (−0.01, 0.55)[b]	
Serum LDL-C, mmol/L	0	2.70 (2.59, 2.81)	2.78 (2.64, 2.92)	2.75 (2.64, 2.86)	—
	Δ4	− 0.10 (−0.24, 0.05)	− 0.13 (−0.28, 0.02)	0.09 (−0.11, 0.29)	0.01
	Δ8	0.00 (−0.15, 0.14)	0.09 (−0.10, 0.28)	0.22 (0.02, 0.41)	
	Δ12	− 0.07 (−0.22, 0.08)	0.14 (−0.05, 0.33)	0.00 (−0.21, 0.22)	
	Δ16	− 0.26 (−0.43, −0.08)[a]	0.07 (−0.09, 0.22)	0.15 (−0.10, 0.40)[b]	
Serum HDL-C, mmol/L	0	1.44 (1.38, 1.50)	1.46 (1.38, 1.53)	1.41 (1.36, 1.47)	—
	Δ4	− 0.04 (−0.08, 0.01)	− 0.07 (−0.12, −0.02)	0.02 (−0.04, 0.08)	0.45
	Δ8	− 0.02 (−0.07, 0.04)	− 0.04 (−0.09, 0.00)	0.02 (−0.04, 0.07)	
	Δ12	− 0.05 (−0.13, 0.02)	− 0.04 (−0.11, 0.03)	0.02 (−0.04, 0.09)	
	Δ16	− 0.08 (−0.17, 0.01)	− 0.02 (−0.10, 0.05)	0.06 (0.00, 0.12)	
Serum TC:HDL-C	0	3.12 (2.98, 3.26)	3.23 (3.09, 3.38)	3.11 (2.98, 3.23)	—
	Δ4	− 0.16 (−0.31, −0.01)	− 0.19 (−0.33, −0.04)	0.07 (−0.12, 0.26)	0.05
	Δ8	− 0.01 (−0.16, 0.15)	0.08 (−0.11, 0.26)	0.24 (0.03, 0.45)	
	Δ12	− 0.12 (−0.27, 0.03)	0.15 (−0.06, 0.35)	0.10 (−0.09, 0.29)	
	Δ16	− 0.26 (−0.43, −0.09)[a]	0.05 (−0.11, 0.20)	0.21 (−0.05, 0.47)[b]	
Serum TAG,[3] mmol/L	0	0.90 (0.81, 1.00)	1.00 (0.90, 1.10)	0.76 (0.71, 0.81)	—
	Δ4	− 0.12 (−0.23, −0.01)	− 0.14 (−0.25, −0.03)	0.02 (−0.08, 0.11)	0.08
	Δ8	0.00 (−0.10, 0.10)	− 0.04 (−0.15, 0.07)	0.09 (−0.02, 0.21)	
	Δ12	− 0.10 (−0.20, 0.00)	0.03 (−0.12, 0.17)	0.24 (0.00, 0.47)	
	Δ16	− 0.02 (−0.14, 0.10)	− 0.08 (−0.24, 0.09)	0.14 (0.01, 0.28)	
Serum apoA1, g/L	0	1.48 (1.44, 1.53)	1.53 (1.47, 1.60)	1.45 (1.41, 1.48)	—
	Δ4	− 0.04 (−0.08, 0.00)	− 0.06 (−0.11, −0.02)	0.02 (−0.02, 0.07)	0.26
	Δ8	− 0.02 (−0.08, 0.04)	− 0.05 (−0.10, 0.00)	0.03 (−0.02, 0.08)	
	Δ12	− 0.06 (−0.12, 0.01)	− 0.03 (−0.10, 0.03)	0.07 (0.03, 0.11)	
	Δ16	− 0.07 (−0.15, 0.00)	− 0.04 (−0.09, 0.02)	0.08 (0.04, 0.12)	
Serum apoB, g/L	0	0.77 (0.74, 0.80)	0.77 (0.74, 0.80)	0.76 (0.73, 0.78)	—
	Δ4	− 0.04 (−0.07, 0.00)	− 0.02 (−0.05, 0.01)	0.03 (−0.01, 0.06)	0.12
	Δ8	− 0.02 (−0.05, 0.01)	0.01 (−0.02, 0.05)	0.04 (0.00, 0.08)	
	Δ12	− 0.03 (−0.06, −0.01)	0.01 (−0.03, 0.05)	0.01 (−0.03, 0.04)	
	Δ16	− 0.06 (−0.10, −0.03)	− 0.01 (−0.03, 0.02)	0.03 (−0.02, 0.08)	
Serum apoB:apoA1	0	0.53 (0.51, 0.55)	0.53 (0.49, 0.57)	0.53 (0.51, 0.56)	—
	Δ4	− 0.01 (−0.04, 0.01)	0.01 (−0.01, 0.04)	0.01 (−0.01, 0.04)	0.27
	Δ8	− 0.01 (−0.03, 0.02)	0.03 (0.00, 0.05)	0.02 (−0.01, 0.05)	
	Δ12	− 0.01 (−0.04, 0.02)	0.02 (−0.01, 0.05)	− 0.02 (−0.05, 0.01)	
	Δ16	− 0.02 (−0.04, 0.01)	0.01 (−0.02, 0.04)	0.00 (−0.04, 0.03)	
Serum Lp(a),[3] nmol/L	0	65.0 (50.4, 79.5)	58.3 (44.0, 72.6)	55.8 (41.9, 69.7)	—
	Δ4	− 0.10 (−3.23, 3.04)	− 3.62 (−11.1, 3.88)	0.97 (−1.74, 3.68)	0.62
	Δ8	− 3.66 (−7.24, −0.07)	1.47 (−6.37, 9.32)	0.60 (−2.53, 3.74)	
	Δ12	− 2.96 (−8.57, 2.66)	− 9.81 (−30.7, 11.11)	− 2.58 (−5.69, 0.52)	
	Δ16	− 4.49 (−11.1, 2.11)	− 8.70 (−28.1, 10.71)	− 1.78 (−5.82, 2.27)	
Serum leptin,[3] pg/mL	0	4020 (3377, 4664)	3593 (2928, 4258)	4442 (3734, 5151)	—
	Δ4	267 (−616, 1151)	345 (−108, 798)	− 175 (−705, 356)	0.31
	Δ8	43.7 (−643, 731)	188 (−238, 614)	250 (−505, 1006)	
	Δ12	231 (−657, 1120)	212 (−274, 698)	699 (−26, 1425)	
	Δ16	60.7 (−672, 794)	− 35.7 (−508, 437)	557 (−402, 1516)	
Plasma glucose, mmol/L	0	5.22 (5.15, 5.28)	5.11 (5.02, 5.19)	5.06 (5.00, 5.12)	—
	Δ4	− 0.02 (−0.14, 0.09)	0.16 (0.04, 0.27)	0.04 (−0.09, 0.16)	0.20
	Δ8	0.11 (0.02, 0.20)	0.21 (0.07, 0.35)	0.06 (−0.07, 0.18)	
	Δ12	0.08 (−0.05, 0.21)	0.16 (0.02, 0.31)	0.06 (−0.07, 0.19)	
	Δ16	0.01 (−0.10, 0.12)	0.25 (0.11, 0.38)	0.10 (−0.02, 0.22)	
Systolic BP, mm Hg	0	108 (106, 109)	111 (109, 113)	106 (104, 108)	—
	Δ4	− 0.32 (−2.80, 2.17)	− 0.81 (−3.89, 2.27)	4.46 (2.80, 6.11)	0.04
	Δ8	0.67 (−2.02, 3.35)	1.38 (−0.98, 3.73)	1.00 (−0.80, 2.80)	
	Δ12	2.09 (−0.78, 4.96)	0.87 (−2.04, 3.78)	1.90 (−0.35, 4.16)	
	Δ16	2.03 (−0.83, 4.89)	1.14 (−1.55, 3.84)	2.80 (−0.13, 5.73)	
Diastolic BP, mm Hg	0	68.8 (67.2, 70.3)	71.4 (70.1, 72.7)	67.4 (65.7, 69.1)	—
	Δ4	− 0.97 (−3.77, 1.82)	− 1.23 (−4.24, 1.78)	4.18 (2.27, 6.09)	0.06
	Δ8	1.15 (−1.83, 4.14)	0.68 (−1.36, 2.73)	1.26 (−0.55, 3.07)	

(*Continued*)

TABLE 5 (*Continued*)

Variable	Week	SBO ($n = 22$)	COB ($n = 21$)	POo ($n = 20$)	P value[2]
	Δ12	2.30 (−0.13, 4.73)	1.35 (−0.14, 2.84)	1.90 (0.31, 3.50)	
	Δ16	1.54 (−1.03, 4.11)	0.94 (−1.46, 3.34)	2.67 (0.73, 4.60)	
Serum ALT,[3] U/L	0	17.3 (15.1, 19.5)	19.3 (16.3, 22.2)	17.5 (15.1, 19.9)	—
	Δ4	− 1.23 (−4.33, 1.87)	− 1.68 (−5.78, 2.43)	3.05 (−4.75, 10.85)	0.35
	Δ8	2.54 (−5.15, 10.2)	− 2.11 (−6.90, 2.68)	5.54 (−1.58, 12.7)	
	Δ12	0.22 (−5.85, 6.28)	3.09 (−6.64, 12.8)	5.99 (−8.80, 20.8)	
	Δ16	0.52 (−6.48, 7.52)	0.97 (−7.35, 9.29)	3.30 (−7.11, 13.7)	
Serum AST,[3] U/L	0	21.5 (20.2, 22.9)	23.1 (21.1, 25.0)	22.2 (20.3, 24.2)	—
	Δ4	− 0.80 (−2.75, 1.15)	− 0.53 (−2.46, 1.41)	0.96 (−3.38, 5.31)	0.42
	Δ8	1.91 (−2.60, 6.42)	− 1.97 (−4.51, 0.58)	1.31 (−2.10, 4.73)	
	Δ12	− 1.42 (−4.22, 1.38)	− 0.10 (−4.06, 3.86)	1.00 (−4.85, 6.84)	
	Δ16	− 1.01 (−4.55, 2.53)	− 0.71 (−4.54, 3.13)	1.25 (−4.27, 6.78)	

[1]All values are unadjusted means (95% CIs) unless indicated otherwise. ALT, alanine aminotransferase; AST, aspartate aminotransferase; BP, blood pressure; COB, cocoa butter; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; Lp(a), lipoprotein (a); POo, palm olein; SBO, soybean oil; TAG, triacylglycerol; TC, total cholesterol; Δ, change from baseline.

[2]Comparisons between treatment groups were performed using mixed-effects longitudinal models; changes from baseline were calculated by subtracting 4-, 8-, 12-, and 16-wk data from baseline data and compared while controlling for baseline values of the respective outcome variable, baseline BMI, and gender. P value refers to treatment × time interaction.

[3]Analyses were performed on log-transformed data.

[a,b]Different superscripts indicate significant differences between groups (Bonferroni adjusted). No superscript means no difference compared with any other group.

with POo than with SBO (24) and FFA stearic acid with COB than with palm-mid fraction and high-oleic sunflower oil (25). In these mouse models increased FFA excretion was accompanied by lower adiposity and aSCAT (24) and lower VAT and total body fat deposition (25). Although the current results suggest that fatty acid regio-specificity may have played a role in mitigating potential unfavorable effects of SFA on liver fat concentration, its biological or clinical relevance needs to be determined in larger study populations over the longer term.

In the current study most adiposity/anthropometry outcomes were not differentially affected by diets. However, body FM was significantly reduced with SBO and COB compared with POo. Other studies showed diets high in n–6 PUFAs lowered aSCAT (13), VAT (12), and the VAT:aSCAT ratio (10) compared with SFA-rich diets, potentially due to increased β-oxidation (37), but these effects were not consistently seen across trials. Considering no changes were observed in other adiposity outcomes, including serum leptin concentrations, these effects on body FM may be chance effects.

Serum lipid profiles were significantly and differentially modulated by the test fats. Whereas SBO decreased serum TC, LDL cholesterol, and TC:HDL cholesterol relative to POo, these effects were not accompanied by significant differences in other lipid counterparts, apoB, and the apoB:apoA1 ratio (42). Serum apoB, an emerging CVD risk biomarker, has been shown to be a stronger predictor of CVD risk than LDL cholesterol (43–46). Emerging evidence suggested the apoB:apoA1 ratio to be the best overall predictor of CVD risk compared with other lipid markers (44, 47, 48). The fact that the results were not consistently shown across lipid profiles reduces the confidence in the overall results. A meta-analysis reported that when POo was compared with PUFA-rich fats, TC, LDL cholesterol, apoB, HDL cholesterol, and apoA1 were increased without affecting TC:HDL cholesterol (49). COB is well known for its cholesterol-neutral effect (50) which may be explained by rapid conversion of stearic acid to 18:1 (51) and enhanced fecal excretion of stearic acid (52), likely due to its longer chain length (53) and regio-specificity (54).

A strength of the current study is the highly controlled feeding protocol which allowed us to assess the effect of the test fats as isolated variables, independent from overall energy availability. Furthermore, the test fats provided 20% of the daily energy requirement, thereby ensuring that if a clinically significant effect was present it would have been detected. Importantly, POo and COB are not fat sources typically consumed in the Australian diet and therefore the amount of these fats provided during the present study was higher than the amount generally consumed. Using a healthy population may be perceived as both a strength and a limitation. It allowed us to assess whether dietary fats with different fatty acid profiles affect liver and body fat concentrations independently of other confounding factors such as metabolic abnormalities and mitochondrial dysfunction, which are key metabolic defects that impair fatty acid catabolism (55, 56). Other limitations included restricted generalizability to healthy adults aged 18–45 y; bias due to completers rather than intention-to-treat analysis; the ^{1}H-MRS technique used for assessing liver fat concentrations cannot differentiate contributions from different types of fatty acids; and because P values were not adjusted for multiple secondary outcomes, type 1 errors may have occurred.

In conclusion, the present study suggests that in healthy adults, when consumed as part of eucaloric, typical Australian diets, different dietary fat sources did not differentially affect liver fat concentration. Although no differences were seen between diets, it should be noted that SFA-rich POo and COB diets did not induce unfavorable effects on any outcomes. SBO and COB favorably affected body FM relative to POo, but no other

TABLE 6 Changes in fecal total fat and FFAs (as percentage of total FFA) from baseline and comparisons between diets[1]

Variable	Week	SBO ($n = 22$)	COB ($n = 21$)	POo ($n = 20$)	P value[2]
FFA 15:0	0	2.0 (1.6, 2.3)	1.9 (1.7, 2.2)	1.9 (1.6, 2.3)	—
	Δ4	−0.23 (−0.89, 0.44)	−0.97 (−1.43, −0.51)[a]	0.50 (−0.40, 1.40)[b]	0.003
	Δ8	0.10 (−0.52, 0.72)[a]	−0.84 (−1.41, −0.27)[b]	−0.52 (−1.29, 0.26)	
	Δ12	−0.52 (−1.18, 0.15)	−1.05 (−1.66, −0.45)	−0.42 (−1.21, 0.36)	
	Δ16	−0.09 (−0.90, 0.72)[a]	−1.12 (−1.71, −0.54)[b]	−0.53 (−1.21, 0.16)	
FFA 15:1	0	1.6 (1.4, 1.9)	1.2 (1.0, 1.4)	1.5 (1.3, 1.7)	—
	Δ4	−0.06 (−0.53, 0.41)	−0.60 (−0.94, −0.25)	0.33 (−0.34, 0.99)	0.12
	Δ8	0.31 (−0.22, 0.84)	−0.41 (−0.97, 0.14)	−0.40 (−0.92, 0.13)	
	Δ12	−0.26 (−0.88, 0.35)	−0.60 (−1.02, −0.18)	0.04 (−0.60, 0.68)	
	Δ16	−0.15 (−0.79, 0.48)	−0.64 (−1.07, −0.22)	−0.32 (−0.87, 0.23)	
FFA 16:0	0	35.5 (33.2, 37.9)	34.8 (32.4, 37.1)	32.4 (30.3, 34.4)	—
	Δ4	−3.01 (−9.60, 3.58)	−3.97 (−7.72, −0.23)	6.42 (−0.51, 13.35)	0.92
	Δ8	−0.31 (−4.75, 4.13)	−2.68 (−7.19, 1.83)	5.47 (−2.02, 12.95)	
	Δ12	−3.66 (−8.63, 1.30)	−2.50 (−6.52, 1.52)	5.95 (−1.08, 12.98)	
	Δ16	−3.19 (−8.03, 1.65)	−2.56 (−7.65, 2.54)	6.53 (−1.02, 14.08)	
FFA 18:0	0	30.7 (26.8, 34.5)	36.6 (33.1, 40.0)	32.3 (28.5, 36.2)	—
	Δ4	−0.38 (−7.02, 6.26)	17.7 (11.0, 24.4)	−3.66 (−9.35, 2.03)	0.79
	Δ8	0.08 (−6.25, 6.41)	15.2 (6.78, 23.6)	−0.50 (−9.60, 8.61)	
	Δ12	−2.17 (−8.92, 4.58)	14.0 (5.44, 22.6)	−2.74 (−11.5, 6.05)	
	Δ16	1.43 (−6.42, 9.27)	15.9 (7.30, 24.5)	−4.04 (−14.8, 6.73)	
FFA 18:1*cis*[3]	0	5.4 (4.7, 6.1)	5.5 (4.5, 6.5)	5.9 (4.8, 6.9)	—
	Δ4	1.69 (−0.40, 3.79)	−0.52 (−2.18, 1.13)	−0.93 (−3.57, 1.71)	0.82
	Δ8	0.67 (−1.36, 2.71)	−2.02 (−3.55, −0.49)	−2.13 (−4.57, 0.32)	
	Δ12	1.27 (−2.46, 4.99)	−2.25 (−3.78, −0.73)	−2.16 (−4.53, 0.20)	
	Δ16	2.40 (−0.65, 5.44)	−2.68 (−4.47, −0.88)	−0.33 (−4.68, 4.01)	
FFA 18:1*trans*[3]	0	16.3 (14.1, 18.4)	14.9 (12.1, 17.7)	18.0 (14.7, 21.2)	—
	Δ4	0.89 (−4.55, 6.33)	−8.74 (−15.2, −2.25)	−3.31 (−10.4, 3.76)	0.72
	Δ8	−0.08 (−5.37, 5.20)	−7.80 (−14.8, −0.81)	−2.50 (−10.6, 5.56)	
	Δ12	5.50 (−1.89, 12.90)	−5.46 (−12.6, 1.65)	−1.72 (−8.65, 5.21)	
	Δ16	−0.05 (−5.74, 5.65)	−6.43 (−13.8, 0.96)	−0.85 (−10.3, 8.63)	
FFA 18:2*cis*[3]	0	8.1 (6.7, 9.4)	4.7 (4.1, 5.4)	7.5 (6.1, 8.9)	—
	Δ4	1.03 (−3.96, 6.01)	−3.06 (−4.59, −1.52)	0.74 (−3.27, 4.76)	0.73
	Δ8	−0.76 (−3.64, 2.11)	−1.65 (−3.47, 0.17)	0.11 (−4.94, 5.15)	
	Δ12	−0.18 (−2.85, 2.50)	−2.35 (−4.58, −0.12)	0.98 (−4.56, 6.53)	
	Δ16	−0.70 (−4.11, 2.72)	−2.62 (−4.62, −0.62)	−0.48 (−3.39, 2.44)	
Total fat, g/100 g	0	2.5 (2.1, 2.9)	2.5 (2.1, 2.8)	1.7 (1.5, 1.9)	—
	Δ4	0.04 (−0.67, 0.74)	1.45 (0.96, 1.94)	0.38 (−0.53, 1.29)	0.21
	Δ8	−0.70 (−1.39, −0.01)	1.32 (0.50, 2.14)	1.10 (0.03, 2.17)	
	Δ12	−0.20 (−1.10, 0.70)	1.23 (0.44, 2.02)	0.96 (−0.23, 2.15)	
	Δ16	−0.47 (−1.19, 0.26)	1.71 (0.75, 2.66)	0.72 (−0.20, 1.63)	

[1] All values are unadjusted means (95% CIs) unless indicated otherwise. COB, cocoa butter; FFA, free fatty acid; POo, palm olein; SBO, soybean oil; Δ, change from baseline.

[2] Comparisons between treatment groups were performed using mixed-effects longitudinal models; changes from baseline were calculated by subtracting 4-, 8-, 12-, and 16-wk data from baseline data and compared while controlling for baseline values of the respective outcome variable, baseline BMI, and gender. P value refers to treatment × time interaction.

[3] Analyses were performed on log-transformed data.

[a,b] Different superscripts indicate significant differences between groups (Bonferroni adjusted). No superscript means no difference compared with any other group.

anthropometric or obesity markers were differentially affected by diets. Although SBO reduced TC, LDL cholesterol, and TC:HDL cholesterol relative to POo, these effects were not consistently shown for other CVD lipid markers, apoB, and the apoB:apoA1 ratio. The findings must be confirmed in cardiometabolically at-risk study populations.

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The authors' responsibilities were as follows—WS, DS, BB-E, GJ-M, and MA: designed the research (developed the protocol and overall research plan); BB-E and GJ-M: delivered the dietary intervention; CHT: conducted medical oversight of the research; NJ: acquired the liver fat and adiposity data by ^{1}H-MRS/MRI and performed blinded analysis of the ^{1}H-MRS data; WS: performed the statistical analysis and had primary responsibility for the

final content; WS and DS: drafted the first version of the manuscript; and all authors: read and approved the final manuscript. The authors report no conflicts of interest.

Data Availability

Data described in the manuscript, code book, and analytic code will be made available upon request pending application and approval.

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