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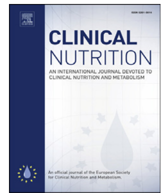

Randomized Control Trials

Postprandial lipaemia following consumption of a meal enriched with medium chain saturated and/or long chain omega-3 polyunsaturated fatty acids. A randomised cross-over study☆

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SUMMARY

Background & aims: Postprandial lipaemic response has emerged as a risk factor for cardiovascular disease. Dietary fats such as medium-chain saturated fatty acids (MCSFA) and long-chain omega-3 polyunsaturated fatty acids (LCn-3PUFA) are known to reduce postprandial lipaemic responses. The combination of the two could potentially have complementary and/or synergistic effects for optimising cardiovascular health. This study aims to investigate the effects of MCSFA (coconut oil) with or without LCn-3PUFA (fish oil) inclusion in the test meal on postprandial blood lipids in healthy adults.

Methods: In a randomised, double-blinded, placebo-controlled, 2 × 2 factorial cross-over study, participants (n = 15) were randomised to receive four standardised isocaloric test meals. Test meals include: placebo [PL, containing no fish oil (0 g EPA & DHA) or coconut oil (0 g MCSFA)], fish oil [FO, 6 g fish oil (3.85 g EPA & DHA), containing no coconut oil (0 g MCSFA)], coconut oil [CO, 18.65 g coconut oil (15 g MCSFA), containing no fish oil (0 g EPA & DHA)] and coconut oil + fish oil [COFO, 18.65 g coconut oil (15 g MCSFA) + 6 g fish oil (3.85 g EPA & DHA)]; all providing a total fat content of 33.5 g. Participants received all four treatments on four separate test days with at least 3 days washout in between. Blood parameters were measured by finger pricks at 7 timepoints between 0 and 300min. The primary outcome of this study was the change in postprandial TG concentrations with secondary outcomes as total cholesterol, high-density lipoprotein cholesterol and blood glucose concentrations.

Results: TG area under the curve (AUC) (mmol/L/min) was significantly lower for FO (383.67, $p = 0.0125$) and COFO (299.12, $p = 0.0186$) in comparison to PL (409.17) only. TG incremental area under the curve (iAUC) (mmol/L/min) was significantly lower with COFO (59.67) in comparison to CO (99.86), ($p = 0.0480$). Compared to PL, the change in absolute TG concentrations (mmol/L) from baseline to post TG peak time (180min) after FO were significantly less at 240min (0.39 vs 0.15), 270min (0.2 vs 0.1), and 300min (0.28 vs 0.06), and after COFO was significantly less at 300min (0.28 vs 0.16) ($p < 0.05$). No significant differences in postprandial AUC and iAUC for any other blood parameters were reported.

Conclusions: Our study demonstrated that MCSFA with or without LCn-3PUFA but not MCSFA alone are effective in reducing postprandial TG in healthy individuals.

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☆ This trial has been approved by the Human Research Ethics Committee [H-2018-0477] and is registered with the Australian New Zealand Clinical Trials Registry [ACTRN12619000921189] at http://www.anzctr.org.au/.

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Abbreviations

AUC	area under the curve
BG	blood glucose
CHO	carbohydrates
CO	coconut oil
CVD	cardiovascular disease
DHA	docosahexaenoic acid
COFO	coconut oil + fish oil
EPA	eicosapentaenoic acid
FO	fish oil
iAUC	incremental area under the curve
LCn-3PUFA	long-chain omega-3 polyunsaturated fatty acids
LCSFA	long-chain saturated fatty acids
MCSFA	medium-chain saturated fatty acids
MUFA	monounsaturated fatty acids
PL	placebo
SFA	saturated fatty acids
TC	total cholesterol
TG	triglycerides
VLDL	very low-density lipoprotein

1. Introduction

Elevated levels of circulating triglycerides (TG) have been recognised as a risk factor for cardiovascular disease (CVD) independent of low-density lipoprotein cholesterol (LDL-cholesterol) concentrations [1]. The fasting state, reflecting endogenous metabolism, has traditionally been used for clinical CVD risk assessment [2] with 19% of Australian adults having high TG concentrations ≥ 2.0 mmol/L [3]. The postprandial state (non-fasting) has recently emerged as an independent risk factor for CVD [4]. TG concentration increases significantly from fasting to 2 h of meal consumption and reaches a peak at 3–4 h and returns to baseline values within 6–8 h [5,6]. This response is normal; however, impaired postprandial clearance and prolonged elevated TG concentrations are associated with increased risk of CVD [4,7,8].

Elevated fasting and postprandial lipaemia are closely correlated [9] and are common amongst cardio-metabolic disease states including obesity, insulin resistance and type 2 diabetes [6,10]. Current guidelines to reduce CVD mainly focus on lowering LDL-cholesterol in the fasting state through pharmacological agents and changes to diet and lifestyle [11]. Safe and effective management strategies are required to lower postprandial lipaemia and CVD risk.

Fish oils are a rich source of long-chain omega-3 polyunsaturated fatty acids (LCn-3PUFA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) [12]. Systematic reviews have demonstrated that LCn-3PUFA supplementation improves cardiovascular health by reducing inflammation and platelet aggregation, and favourably modulating circulating blood lipids, including fasting TG [13–15]. Recent RCTs in healthy individuals have reported suppressed elevations of postprandial TG concentrations after 4 g/day supplementation of fish oils for 4 weeks [16,17]. A large body of evidence suggests that the key biochemical mechanism underpinning LCn-3PUFA reduction of postprandial lipaemia is decreased production of endogenous very low-density lipoprotein (VLDL) particles as well as TG content in chylomicrons, leading to significantly suppressed postprandial peaking of TG and faster clearance time [6,10,13].

It has been demonstrated that chain length of saturated fatty acids (SFA) determines lipid responses [18]. MCSFA (6–12 carbons) have been shown to lower TG concentrations and raise HDL-cholesterol concentrations compared to long-chain saturated fatty acids (LCSFA, 16–18 carbons) in pre-clinical studies [19,20]. LCSFAs, namely palmitic (16:0) and stearic acid (18:0) found in dairy and animal fats (e.g. tallow and lard) follow a complex pathway of incorporation into chylomicrons to reach the bloodstream via the lymphatic circulation [18,19]. In contrast, MCSFA, prominently found in coconut and palm kernel oil are directly transported to the liver via portal circulation [21], thus achieving higher rates of mitochondrial oxidation as they surpass the carnitine shuttle [18]. Limited number of studies in healthy adults report that consumption of MCSFA from coconut oil reduces peaking and clearance of postprandial TG concentrations up to 60% in comparison to LCSFA from butter [22,23]. In addition, a meta-analysis on healthy individuals showed that diets rich in MCSFA significantly increased fasting concentrations of HDL-cholesterol and apolipoprotein-A1 [18].

Since MCSFA and LCn-3PUFA possess different biochemical mechanisms for reducing postprandial lipaemia, we hypothesise that in combination they could potentially produce complementary and/or synergistic effects. Therefore, the primary aim of this study was to investigate in healthy adults the effects of MCSFA with or without LCn-3PUFA supplementation on postprandial lipaemia. Findings from this trial could provide a relatively safe and effective management strategy for targeting postprandial lipaemia in not only healthy individuals, but individuals at high risk of developing CVD. Furthermore, findings could support evidence of the potential differential effects of MCSFA and therefore influence dietary guidelines surrounding saturated fats.

2. Materials and methods

2.1. Recruitment

Participants were recruited from Newcastle (NSW, Australia) and surrounding areas via recruitment flyers and contacting participants (who meet the eligibility criteria) from the database held by the Nutraceuticals Research Program at the University of Newcastle. Participants were screened via telephone interviews using an eligibility checklist containing the inclusion and exclusion criteria. Participants were eligible if they were: aged between 18 and 70 years and otherwise healthy. Individuals were excluded if they were: morbidly obese (body mass index, BMI ≥40 kg/m^2); currently taking lipid lowering and/or anti-inflammatory medications or supplements known to influence blood lipids; taking fish oil supplements or consume oily fish > 2 times per week; consuming ≥ 15 g coconut oil ≥ 4 times per week; diagnosis of any form of cardiovascular, gastrointestinal or neurological disorder; history of liver/renal disease; diabetes mellitus; pregnant or planning to become pregnant; breast feeding; smoking; participating in moderate-vigorous intensity exercise ≥ 4 times per week and have any allergies/intolerances to trial ingredients e.g. seafood, dairy, lactose, wheat, gluten. Upon enrolment participants were de-identified and assigned a numeric identification code for data handling. Participants provided written informed consent prior to commencing the trial which was mandatory for participation. Procedures were performed in accordance with Good Clinical Practice (GCP) conditions. The study protocol was approved by the University of Newcastle Human Research Ethics Committee (H-2018-0477) and the trial was registered with the Australian New Zealand Clinical Trials Registry (ACTRN12619000921189) at http://www.anzctr.org.au/.

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2.2. Study design

This study was a randomised, placebo-controlled, double-blinded, crossover study. The trial consisted of 4 test days, with each participant receiving all four treatment groups and a washout period of at least 3 days between the test days. Following screening, eligible participants were asked not to change their habitual diet, lifestyle and exercise routines for the duration of the study period. Prior to each visit, participants received necessary instructions for each test day: 10-h overnight fast, avoiding a high fat meal the night before (meal ideas provided in participant information form) and abstaining from alcohol consumption and vigorous physical activity for 24 h prior to test days. A 24-h food recall was collected at every test day by the lead investigator (GA) to assess habitual food and beverage intake on the previous day. Dietary information was analysed using FoodWorks Version 8.0.3551 (Xyris Software, Australia, Pty Ltd) to evaluate consistency of intake prior to test days. FoodWorks was also used to calculate nutrient composition of the test meals and the accompanied bioactive powders that were added to the meals (Table 1). The test meals provided approximately 2800 kJ and a total fat content of 33.5 g. Dietary components analysed included: energy, protein, carbohydrate (CHO), sugars, starch, total fat, saturated fats, trans fats, monounsaturated fatty acids (MUFAs), polyunsaturated fatty acids (PUFAs), dietary fibre and sodium. Physical activity was assessed using the validated International Physical Activity Questionnaire (IPAQ, short form) which captures intensity, duration and frequency of physical activity undertaken in the past 7 days. Physical activity data was interpreted as metabolic equivalent of task minutes per week (MET/week) to measure the energy cost of physical activities. At each study visit, participants were provided with a standard breakfast which had to be consumed within 15 min: one slice of wholemeal bread (30 g) (Coles Bakery Wholemeal Toast Bread Loaf), raspberry jam (14 g) (Coles raspberry jam, made in Poland) one cavendish banana (100 g) (commercially available at Coles) and vanilla greek yoghurt (160 g) (Farmers Union Vanilla Greek Yoghurt, made in Morwell, VIC, Australia). On each test day one of four bioactive powders (50 g) were mixed with yoghurt. All standard breakfast meals were prepared by lead investigator (GA). The four bioactive powders were de-identified through relabelling by the senior investigator (MG) using alphabetical codes (A, B. C, D) to ensure treatment allocation was concealed to both the lead investigator (GA) and participants. According to a computer-generated sequence (block size of four), participants received test meals in a random order: Placebo [PL, containing no fish oil (0 g EPA & DHA) or coconut oil (0 g MCSFA)], Fish Oil [FO, 6 g fish oil (3.85 g EPA & DHA), containing no coconut oil (0 g MCSFA)], Coconut Oil [CO, 18.65 g coconut oil (15 g MCSFA), containing no fish oil (0 g EPA & DHA)] and Coconut Oil + Fish Oil [COFO, 18.65 g coconut oil (15 g MCSFA) + 6 g fish oil (3.85 g EPA & DHA)]. Extra Virgin Coconut Oil (Olivado Ltd, New Zealand) was purchased from a local supermarket and fish oil (EPAX 1500 TG/N) was from EPAX Norway. The bioactive powders were isocaloric and prepared using micro-emulsification technology using milk derived sodium caseinate. The dispersible powder containing coconut oil and/or fish oil were packaged under good manufacturing practice conditions by Riddet Institute, Massey University, New Zealand who have extensive expertise in developing functional foods containing bioactive compounds. Tallow was used as the placebo for coconut oil and olive oil was used as the placebo for fish oil. Participants were allocated 200 mL of tap water during their test meal at 0min, 120min and 240 min h totalling 600 mL at each visit. Time taken to finish the test meal was recorded and made consistent for subsequent visits.

Table 1
Nutrient composition of the test meals.

Dietary component	Placebo	Coconut oil	Fish oil	Coconut + fish oil
Energy (kJ)	2793	2793	2793	2793
Protein (g)	16.7	16.7	16.7	16.7
CHO (g)	71.3	71.3	71.3	71.3
Sugars (g)	37.5	37.5	37.5	37.5
Starch (g)	33.8	33.8	33.8	33.8
Total fat (g)	33.5	33.5	33.5	33.5
Saturated (g)	15.3	23.2	14.5	22.4
Trans fats (g)	0.3	0.2	0.3	0.2
MUFAs (g)	14.6	7.0	10.9	3.3
PUFAs (g)	1.8	1.1	6.3	5.6
DHA (mg)	0	0	3108	3108
EPA (mg)	0	0	738	738
Cholesterol (mg)	51.2	30.9	51.2	30.9
Dietary fibre (g)	5.8	5.8	5.8	5.8
Sodium (mg)	224.4	224.4	224.4	224.4

Test meals for each visit include: one slice (30 g) of commercial wholemeal bread, 14 g raspberry jam, 100 g Cavendish banana, 160 g vanilla Greek yoghurt fortified with one of four 50 g bioactive powders. Extra Virgin Coconut Oil (Olivado Ltd, New Zealand) was purchased from a local supermarket and fish oil (EPAX 1500 TG/N) was from EPAX, Norway.

2.3. Medical history, dietary intake, physical activity and anthropometric measures

Participants attended each clinic visit at the Nutraceuticals Research Program clinical trial facility in a fasting state (>10 h) and medical history/demographics, physical activity levels, 24-h food recall and consent were collected from self-administered paper questionnaires. Blood pressure including systolic blood pressure (SBP) and diastolic blood pressure (DBP) was collected three times, 3–5 min apart in the seated position at every visit. An automated blood pressure monitor (Microlife BP 3AD1-A, Heerbrugg Switzerland) was used and an average of the second and third measures were used for analysis. Height (cm), waist (cm) were measured to the nearest 0.5 and weight (kg) was measured to the nearest 0.1 units. Height was measured once at each visit using a using wall-mounted stadiometer with a movable head piece (Seca 206 Bodymeter Wall Height Measure Ruler). Waist circumference was measured twice at each visit using a tensible tape measure positioned at midway between the lower rib margin and the iliac crest (approximately in line with the belly-button) horizontally. An average of both measurements was used for data analysis. At each visit, body weight and other parameters of body composition (skeletal muscle mass, fat mass, total body water etc) were determined using direct segmental multi-frequency bioelectrical impedance (InBody 230, Biospace Co.Ltd. Seoul, Korea) in the standing position, lightly clothed with any metal, electronic devices, shoes and socks removed. BMI was calculated as weight/height (kg/m^2).

2.4. Blood sampling

Blood samples were collected by finger prick to measure postprandial lipid levels of the primary outcome (TG) and secondary outcomes such as total cholesterol (TC), HDL-cholesterol, LDL-cholesterol, TC:HDL ratio and blood glucose (BG). A total of 7 finger pricks at 0 min (fasting, before ingestion of test meal), 2, 3, 3.5, 4, 4.5 and 5 h were collected at every test visit. BG was measured at 0 min and 2 h using Freestyle glucometer (Optimum Neo, Abbotts Diabetes Care, Doncaster VIC). Collection of blood using a 40 μl capillary tube and CardioChek PA Analyser (Polymer Technology Sydney Inc) machine was used to measure blood lipids. Minimum and maximum ranges were TC: 2.59–10.36 mmol/L, HDL-

cholesterol: 0.65–2.20 mmol/L, TG: 0.56–5.65 mmol/L, BG 1.11–33.33 mmol/L. Prior to use in the study, the Freestyle Optimum Neo and CardioChek PA Analyser were calibrated and standardised according to product guidelines to ensure they fulfil adequate requirements for calibration. The CardioChek PA is a reliable measure of plasma lipids, with no significant differences in results compared to venous collection [24].

2.5. Statistical methods

The primary outcome of this study is change in blood TG concentration and the secondary outcomes are changes in total, HDL-cholesterol, LDL-cholesterol levels and BG concentrations. Based on the anticipated 30% difference in primary outcome, incremental area under the curve (iAUC) for postprandial TG levels, at 0.05 level of significance, 80% power, standard deviation (SD) of 1.14 in iAUC [25] and 10% dropout rate, $n = 15$ were required for the crossover design. The data of all variables included in the analysis was tested for normality by Shapiro-Wilk's test and visual plots such as histograms. Data are presented as mean ± SEM (Standard error or measure) or median (interquartile range (IQR)) where appropriate. Change in area under the curve (AUC's) and iAUC for postprandial TG, TC, LDL-cholesterol, HDL-cholesterol, TC:HDL ratio and BG were calculated by the trapezoid rule. Outliers were removed based on 1.5x interquartile range below 25th quartile and above the 75th quartile for TG variation values at each timepoint. Statistically significant differences between treatment groups were tested using ANOVA with one-repeated measure for parametric data or Friedman's Test for non-parametric data. When analysis was significant a paired t-test or Wilcoxon signed-rank test was preformed to compare treatment groups pairwise. Analysis of confounders on AUC and iAUC for primary outcome (TG) including differences between fasting concentrations of biochemical variables, diet, PA and macronutrient intake were identified firstly via Spearman's correlation to assess the relationship between explanatory variables. Variables with correlation coefficients greater than 0.8 were assessed more closely for multicollinearity and the number of potential predictors to include in the analyses was reduced accordingly. Analysis of covariance (ANCOVA) was performed on each outcome with the inclusion of treatment group as a factor and the corresponding potential predictors of the outcome variable as covariates. Significance was set at $p < 0.05$ and adjusted for Gessier-Greenhouse (g–g) to indicate statistical significance for all tests. This adjustment tends to be slightly conservative in small samples and is corrected for degrees of freedom for sphericity of the repeated measures. All statistical analyses and trapezoid calculations for AUC's were conducted using STATA version 14.1 (STATACorp, Texas, USA).

3. Results

3.1. Baseline characteristics

Sixteen healthy participants (5 male, 11 female) were recruited during the period April–July 2019 (Fig. 1). Recruitment ceased after 16 participants were recruited. One participant was lost to follow up after receiving one treatment (COFO) due to family circumstances. 20 data points were removed as outliers based on 1.5x interquartile range below 25th quartile or above the 75th quartile for TG variation values. Median (IQR) age of the participants was 54 [35] years, BMI 25.51 ± 3.99 kg/m^2, waist circumference 91.07 ± 14.50 cm, waist-hip ratio 0.92 ± 0.08, skeletal muscle mass 26.91 ± 5.04 kg, body fat percentage 31.34 ± 9.79% and fasting TG concentrations 1.12 ± 0.14 mmol/L (Table 2). Anthropometric measurements and blood parameters were similar across treatment groups and across visits, except fasting HDL-cholesterol concentrations which was significantly different across visits ($p = 0.0086$).

3.2. Dietary intake and physical activity

Overall participants METs were 2603 ± 501 (Table 2). Physical activity analysis indicated no significant differences between visits and treatments. There were no significant differences in nutrient intakes prior to the test days across visits and treatments (Table 3).

3.3. Change in postprandial triglyceride AUC and iAUC

Changes in the primary outcome postprandial TG (AUC/iAUC (mmol/L/min)) concentrations are shown in Table 4. Postprandial AUC (0–300min) and iAUC (0–300min) for TG were significantly different between treatments ($P < 0.0001$ and $P = 0.0014$, respectively). A significantly lower AUC was demonstrated with FO (383.67, $p = 0.0125$) and COFO (299.12, $p = 0.0186$) in comparison to PL (409.17) only. iAUC was significantly higher with CO (99.86) in comparison to COFO (59.67, $p = 0.0480$). No other statistically significant changes in iAUC were reported between the other treatments.

3.4. Postprandial triglyceride concentrations at each timepoint

Compared to PL, the change in absolute TG concentrations (mmol/L) from baseline post TG peak time (180min) after FO were significantly less at 240min (0.39 vs 0.15), 270min (0.2 vs 0.1), and 300min (0.28 vs 0.06), and after COFO was significantly less at 300min (0.28 vs 0.16) ($p < 0.05$) (Fig. 2).

3.5. Postprandial cholesterol and blood glucose

No significant differences in postprandial AUC and iAUC for TC, HDL-cholesterol, LDL-cholesterol and TC:HDL ratio were observed between treatments (Table 4). Absolute change (120-0min) in BG significantly differed across treatments ($P = 0.0006$), however, when post hoc comparisons were performed, significance was lost yielding no significant difference between treatments.

3.6. Effects of baseline data on AUC and iAUC for postprandial TG

Baseline data including fasting blood lipids, fasting BG, physical activity level, age, BMI, SBP, DBP and dietary components: saturated fats, trans fats, cholesterol, CHO, sugars, starch, alcohol, fibre, PUFAs, MUFAs were analysed as confounding factors. Baseline characteristics of participants on their first visit did not significantly influence the net AUC for TG. After adjusting for confounders across all treatments only age and fasting TG (0min) were significant predictors of AUC ($p < 0.05$).

4. Discussion

Postprandial lipaemia is an independent risk factor for CVD [4], and perhaps an even stronger predictor for CVD than fasting TG concentrations [26]. Results presented in this paper suggest that combined MCSFA + LCn-3PUFA treatment resulted in the most significant reduction in postprandial TG, followed by LCn-3PUFA alone. MCSFA treatment alone did not lower postprandial lipaemia and the synergy between the two fats was not apparent in this study.

LCn-3PUFAs have been recently explored in clinical trials to determine its effectiveness in lowering postprandial lipaemia [8]. An RCT in healthy individuals reported 4 g/day of fish oil

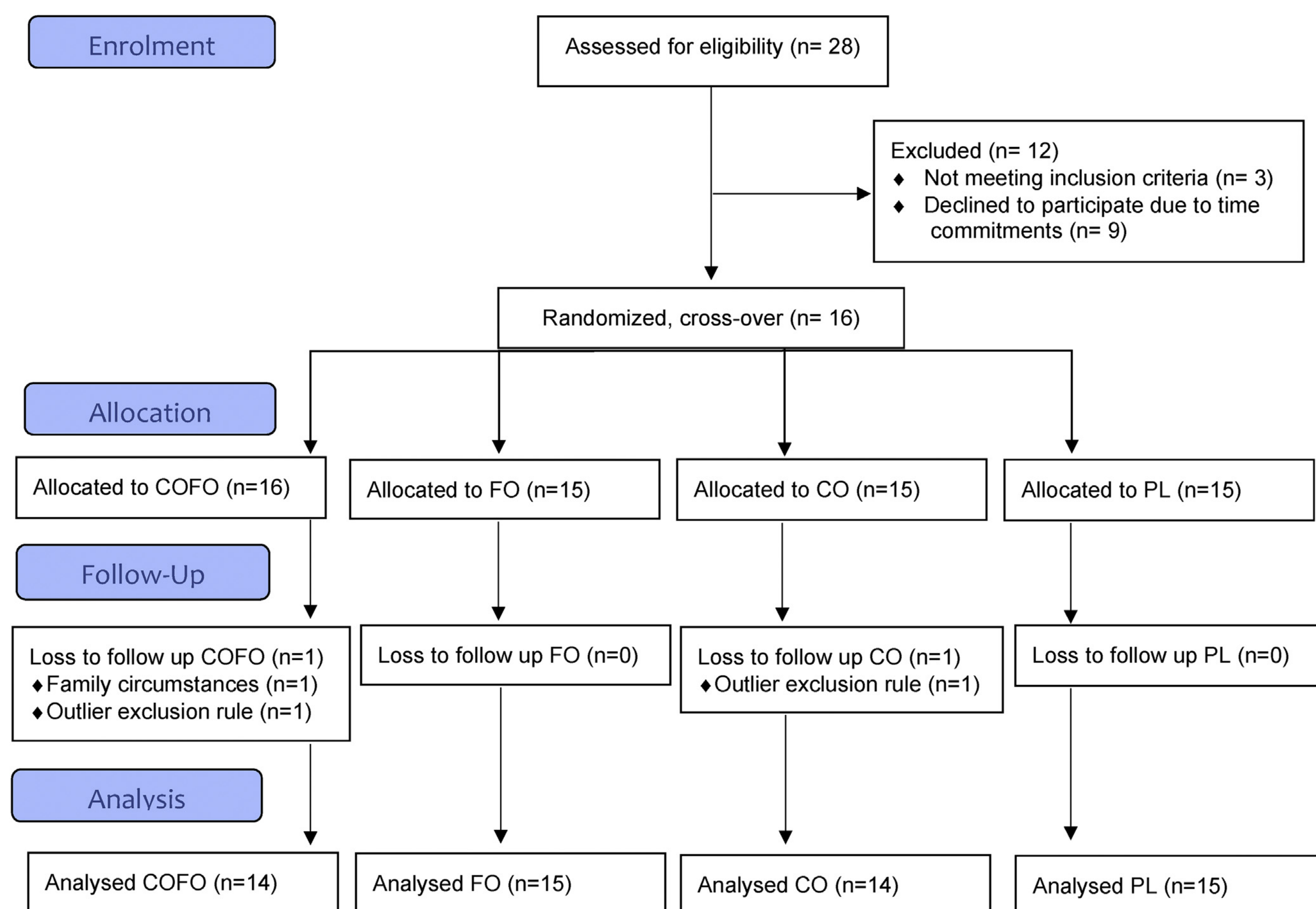

Fig. 1. CONSORT schematic of participant recruitment, screening and assessment.

Table 2
Characteristics of participants at baseline.

Variables	All participants (n = 15)
Males	5 (33.3%)
Age (years)	54 (30, 65)
Height (cm)	167.26 ± 2.10
Weight (kg)	71.54 ± 3.37
BMI (kg/m^2)	25.51 ± 1.03
Waist circumference (cm)	91.07 ± 3.74
Waist-to-hip ratio	0.92 ± 0.02
Skeletal muscle mass (kg)	26.91 ± 1.30
Body fat mass (kg)	23.02 ± 2.50
Percentage body fat (%)	31.34 ± 2.53
Fasting TG (mmol/L)	1.12 ± 0.14
Fasting TC (mmol/L)	5.96 ± 0.30
Fasting HDL (mmol/L)	1.55 ± 0.13
Fasting LDL (mmol/L)	3.78 ± 0.42
Fasting TC/HDL ratio (mmol/L)	4.16 ± 0.32
Fasting BG (mmol/L)	5.47 ± 0.32
SBP (mmHg)	118 ± 13.10
DBP (mmHg)	75.2 ± 2.03
Physical activity (MET)	2603 ± 501

Data reported as means ± SEM or median (25th and 75th percentile) as appropriate. Physical activity levels of participants were measured using standard international physical activity questionnaire - short form. CO; coconut oil, COFO; coconut and fish, FO; fish oil, MET: Metabolic equivalent of task minutes, PL; placebo. BG; blood glucose, BMI; body max index, DBP; diastolic blood pressure, HDL; High-density lipoprotein, LDL; low-density lipoprotein, TC; total cholesterol, TC/HDL; total cholesterol high-density lipoprotein ratio, TG; triglycerides, SBP; systolic blood pressure.

supplementation via capsules for 4 weeks, significantly suppressed postprandial TG elevation (AUC 220 ± 209 vs 374 ± 216 mg/h/dL) [16]. Another RCT in 33 in healthy males showed that dietary supplementation with fish oil capsules significantly reduced postprandial TG concentrations by 16% in 4 weeks [27]. Our results, where LCn-3PUFA are delivered in a meal are comparable to the aforementioned findings since LCn-3PUFA produced significantly lower AUC and lower change in TG concentrations at 240, 270 and 300 min compared with placebo. In addition, we reported the lowest TG peak value at 210 min, almost returning to baseline values in comparison to all other treatments. These findings are likely indicative of LCn-3PUFAs ability to decrease TG content in chylomicrons as well as reduce endogenous production of VLDL [16,27,28]. Moreover, LCn-3PUFA have been shown to increase extracellular lipolysis by lipoprotein lipase which enhances the hepatic and skeletal muscle β-oxidation, leading to reduced FA delivery to the liver [29]. In light of previous findings and results of our study, the use of LCn-3PUFA supplementation proves to be an effective postprandial lipaemic-lowering agent in healthy individuals. Future studies could investigate the potential beneficial effects acute supplementation of LCn-3PUFA may have on postprandial lipaemia in high risk individuals such as those with hyperlipidaemia, pancreatitis and type 2 diabetes.

The novel combination of MCSFA (coconut oil) with LCn-3PUFA (fish oil) in the current study lowered some markers of postprandial lipaemia. We demonstrated that this treatment led to the most significant reduction in AUC, and the absolute change in TG concentrations at 300 min compared to placebo. Since the single

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Table 3
Reported dietary intake from 24 recall records of participants prior to each treatment.

Dietary Component	Placebo	Coconut oil	Fish oil	Coconut + fish oil	P-value (treatments)	P-value (visits)
Energy (kJ)	8637 ± 331	8648 ± 278	8281 (2105)	8372 ± 227	0.414	0.053
Protein (g)	90 (15.60)	87.80 ± 7.18	97.87 ± 6.40	91.28 ± 6.53	0.694	0.900
Carbohydrate (g)	234.67 ± 17.79	241.51 ± 15.99	213.83 ± 11.85	221.17 ± 12.56	0.480	0.353
Sugars (g)	94.93 ± 15.90	97.61 ± 9.52	93.24 ± 9.94	92.49 ± 8.98	0.982	0.117
Starch (g)	138.94 ± 13.78	143.15 ± 14.23	119.07 ± 8.70	127.22 ± 11.31	0.475	0.093
Total fat (g)	78.6 ± 3.88	75.20 (20.80)	77.23 ± 6.46	77.71 ± 4.39	0.992	0.066
Saturated (g)	27.79 ± 2.89	27.20 (8.20)	22.50 (9)	26.86 ± 2.14	0.089	0.145
Trans fats (g)	1.36 ± 0.22	1.10 (0.60)	1.27 ± 0.21	1.20 ± 0.12	0.884	0.374
MUFAs (g)	29.74 ± 1.83	31.47 ± 2.49	29.22 ± 2.60	31.90 ± 2.68	0.820	0.190
PUFAs (g)	14.21 ± 1.43	11.09 ± 1.21	13.37 ± 71.89	11.65 ± 0.80	0.222	0.173
Cholesterol (mg)	205.40 (120.10)	180.80 ± 25.0	197.30 (229.50)	201 (244)	0.347	0.204
Dietary fibre (g)	28.37 ± 1.38	29.25 ± 2.54	24.21 ± 1.98	26.57 ± 2.13	0.165	0.504
Alcohol (mL)	0.00	0.00	0.00	0.00	–	0.8091

Data reported as mean ± SEM or median (IQR) as appropriate. Significance changes in dietary intake across treatment groups and visits were assessed using ANOVA or Friedman Test where appropriate. MUFAs; Monounsaturated fatty acids, PUFAs; Polyunsaturated fatty acids.

Table 4
Change in AUC (mmol/L/min) and iAUC (mmol/L/min) for blood lipid parameters and glucose.

Parameters	Placebo (PL)	Coconut oil (CO)	Fish oil (FO)	Coconut + fish oil (COFO)	P-value (ANOVA)	PL vs CO	PL vs FO	PL vs COFO	CO vs COFO
TG AUC	409.17 (520.24)	336.9 (322.84)	383.66 (276.05)	299.12 (289.14)	P = 0.0014	0.1094	0.0125	0.0186	–
TG iAUC	101.40 (96.36)	99.86 (58.18)	66.47 (79.10)	59.67 (43.51)	P = 0.0025	–	–	–	0.0480
TC AUC	1731.12 ± 77.38	1731.18 ± 84.14	1709.52 ± 90.91	1705.14 ± 84.10	P = 0.585	–	–	–	–
TC iAUC	8.12 ± 19.84	−27.46 ± 25.90	6.72 ± 16.25	−43.22 ± 22.84	P = 0.2843	–	–	–	–
HDL AUC	465.19 ± 42.93	450.09 ± 35.28	431.51 ± 37.02	460.13 ± 36	P = 0.6109	–	–	–	–
HDL iAUC	1.35 (33.3)	−14.32 (23.1)	−11.4 (17.4)	−14.18 (37.95)	P = 0.7148	–	–	–	–
LDL AUC	1033.40 ± 72.78	1028.50 ± 83.89	1056.01 ± 88.46	929.87 ± 93.41	P = 0.1473	–	–	–	–
LDL iAUC	−34.05 (83.4)	−56.5 (233.4)	−22.2 (88.8)	−34.12 (586.2)	P = 0.3869	–	–	–	–
TC/HDL AUC	1258 ± 88.56	1274 ± 102.70	1285.1 ± 101.30	226.5 ± 97.78	P = 0.4185	–	–	–	–
TC/HDL iAUC	22 ± 24.15	32 ± 16.39	29.1 ± 14.12	18.5 ± 20.67	P = 0.8758	–	–	–	–
BG (change 120-0min)	0.5 (1.3)	0.8 (1.4)	1.1 (1.3)	0.8 (1.2)	P = 0.0006	0.4776	0.2558	0.9320	–

Data are presented as mean ± SEM or median (IQR) where appropriate. One-way ANOVA and Friedman's test were used to compare change in AUC and iAUC across different treatment groups. P < 0.05 (adjusted for Geisser-Greenhouse) indicated statistically significant differences between groups. Paired t-test and Wilcoxon's signed-rank test were used for pairwise comparisons between groups where appropriate. BG; blood glucose, CO; coconut oil, COFO; coconut and fish oil, FO; fish oil, HDL; high-density lipoprotein, LDL; low-density lipoprotein, PL; placebo, TC; total cholesterol, TC/HDL; total cholesterol high-density lipoprotein ratio, TG; triglycerides.

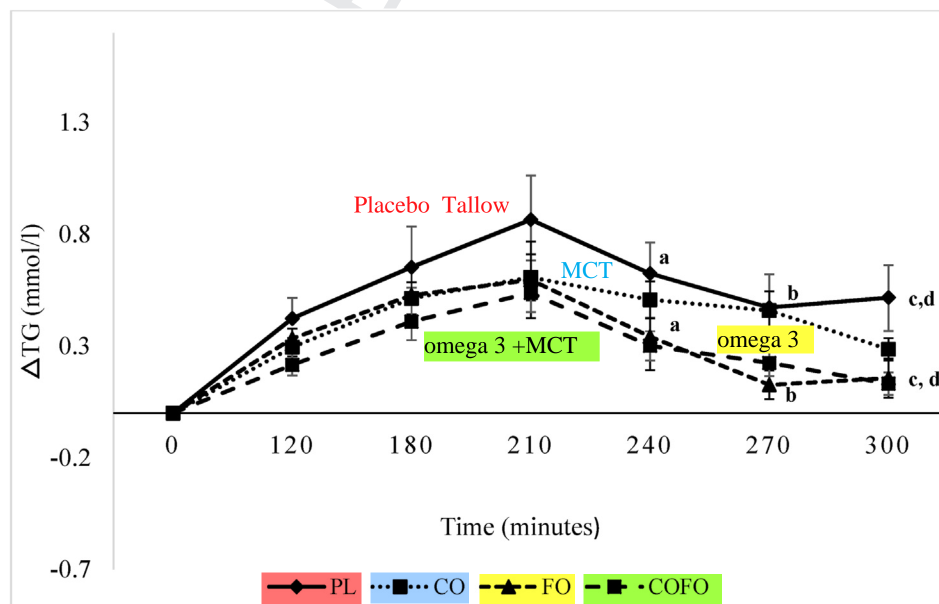

Fig. 2. Change in absolute TG concentrations (△TG) at each time point across 300 min in response to PL; placebo, CO; coconut oil, FO; fish oil, COFO; coconut and fish oil. Data reported as mean and SEM as displayed as error bars. Lower case letters represent the difference between treatment groups: [a] PL vs FO at 240 min ($p = 0.00264$); [b] PL vs FO at 270 min ($p = 0.0227$); [c] PL vs FO at 300 min ($p = 0.0082$); [d] PL vs COFO at 300min ($p = 0.0480$).

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bioactive treatment groups had opposing effects i.e. MCSFA did not influence postprandial lipaemia and LCn-3PUFA favourably lowered postprandial lipaemia; it is likely that the combined treatment effects are driven primarily by LCn-3PUFA.

Our research group recently reported that change in postprandial TG after consumption of 40 g coconut oil was 59.8% lower compared to 40 g of butter and 58.8% lower compared to 40 g of lard [23]. In the current study, we were unable to replicate our previous findings for the postprandial TG lowering effects of MCSFA. Another RCT in healthy individuals reported that dietary saturated fats of varying chain length had differential effects on lowering postprandial TG with results showing MCSFA to be significantly more effective than LCSFA [22]. It is well-established that the key mechanism by which MCSFA lowers postprandial lipaemia is that they are directly transported via the portal circulation with little incorporation into chylomicrons [30,31]. Preclinical studies have suggested that MCSFA decrease the expression of apolipoprotein B-48 which is used to transport dietary lipids from the intestine to the circulatory system [32]. In addition, it has been shown MCSFA undergo rapid β-oxidation in the liver resulting in decreased TG synthesis, consequently increasing clearance of VLDL and remnant lipoproteins [8]. In the present study, delivery of MCSFA was in the form of a dispersible powder which is different compared to other studies that used enriched biscuits [23]. Isocaloric food matrix studies have shown that solid foods lead to a slower release in serum postprandial TG concentrations compared to liquid and semi-solid foods [33]. In the current study, MCSFA was delivered as a dispersible powder via a semi-solid food matrix (i.e. yoghurt), discrepancies between the studies could be a result of different delivery mediums (semi-solid vs solid). Since food structure is a key determinant of the rate of fat digestion and absorption [33], it is possible that the semi-solid food matrix used in the current study influenced the lipaemic effects of MCSFA. Moreover, the dose of MCSFA used in this study (18.65 g) was much lower in comparison to the previous study (40 g) and thus may account for some inconsistencies in findings. Age and fasting TG were reported to be significant predictors of the change in TG AUC in this study. This agrees with previous literature as it is well established that age and fasting TG level influence postprandial lipaemic response [34,35]. It is noteworthy that the spread of age (22–69 years) was quite broad in this study, and future studies may need to focus on a narrow age range. Fasting TG levels were in the healthy range (<2 mmol/L), perhaps individuals with higher baseline fasting TG concentrations would have had a greater postprandial TG lowering after treatments. Further research in human clinical trials are warranted to investigate these discrepancies and establish more conclusive evidence for the effects of MCSFA from coconut oil on postprandial lipaemia.

There were no significant differences in other postprandial lipid parameters including HDL-cholesterol, LDL-cholesterol, TC, TC/HDL ratio and BG. Previous studies exploring the effects of MCSFA or LCn-3PUFA on other postprandial lipid markers in humans are limited, however, the fasting state has been investigated. A recent systematic review and meta-analysis conducted by our group reported that diets rich in MCSFA from coconut oil led to significantly higher HDL-cholesterol and apolipoprotein A-1 concentrations, with no significant increase in TC when compared to diets rich in LCSFA from lard and butter [18,36,37]. Intervention studies and systematic reviews have reported LCn-3PUFA supplementation to have mild effects on TC levels due to raised LDL-cholesterol concentrations, however, this is attributed to an increase in LDL particle size (less atherogenic) and mild increases in HDL-cholesterol concentrations [13,38]. As this study did not show any significant effects of MCSFA or LCn-3PUFA on postprandial lipid parameters other than TG this suggests that a single dose of bioactive lipids is insufficient to induce changes in LDL-cholesterol particle size and/or lipid parameters other than TG.

Preclinical studies have reported MCSFA from coconut oil dampens the rise in BG levels in high-fructose fed rats and diabetic rat models in comparison to placebo (17% vs 47% increase) [39,40]. MCSFA has been proven to be an effective strategy in preventing the development of diet-induced insulin resistance as the fatty acids are not stored in skeletal muscle and regenerate oxidative damage of islet cells [41]. However, the effects of LCn-3PUFA via fish oil supplementation on BG were ambiguous and should be used with caution in subjects with non-insulin-dependent diabetes mellitus [42]. Further investigations are warranted to understand the potential effects of MCSFA on glycaemic parameters both postprandially and fasting in humans.

This study was adequately powered, and the number of required participants were obtained for all treatments. In addition, the novelty of using a dispersible powder as a functional food allows it to be translational to diet. However, this study is a preliminary investigation exploring the use of MCSFA + LCn-3PUFA on postprandial lipaemia, and some limitations need to be addressed. The small sample size and acute length may affect validity and long-term effects remain unestablished. Only healthy individuals were included in the study, therefore translation of results to other disease populations is unknown. The significant confounding variables (age and fasting TG) may also affect validity of results. In light of these limitations, future studies may lengthen the duration of the trial to determine long-term effects and potential additional benefits e.g. increase HDL-cholesterol, increase LDL-cholesterol particle size and anti-inflammatory effects. Specific population groups such as those with metabolic syndrome, hyperlipedaemia and pancreatitis where fasting TG are hugely elevated may benefit from the combined treatment. The postprandial lipaemic-lowering achieved in our study in healthy individuals consuming LCn-3PUFA (with or without MCSFA) provides preliminary findings for an efficacious strategy for modulating the post-meal rise in blood lipids, which is a conundrum in several non-communicable diseases such as overweight/obesity, metabollic syndrome, type 2 diabetes, cardiovascular disease, non-alcoholic fatty liver disease and pancreatic diseases. We would suggest that follow-on studies investigate 3 g EPA + DHA per meal as an effective therapy for reducing fasting TG le It is possible that the combination of MCSFA and LCn-3PUFA may provide more pronounced lipaemic-lowering in these patient populations, given their elevated basal lipaemic levels. Human trials in these population groups are warranted to examine the effects of this dietary combination on postprandial lipaemia at main mealtimes, the feasibility of implementing this intervention at more than one meal time as well as investigate other potential cardiometabolic benefits attributed to the addition of MCSFA that were unable to demonstrated in the current study in healthy individuals.

In conclusion our study demonstrated that MCSFA with or without LCn-3PUFA but not MCSFA alone are effective in reducing postprandial TG in healthy individuals. There were no complementary and/or synergistic lipid lowering effects of LCn-3PUFA and MCSFA at the doses tested in this trial, however, results are still reliable as they hold proof of concept. Our previous studies have shown that MCSFA lower postprandial TG in healthy individuals, therefore, the controversy noted in this study needs to be investigated further both postprandially and in the fasting state [18]. Findings from this trial could provide a safe and effective treatment and/or management strategy for targeting postprandial lipaemia in not only healthy individuals, but individuals at high risk of developing CVD.

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Statement of authorship

MG and JF conceptualised and designed the research. GA conducted the research and analysed the data with statistical support from JF and RT. HS provided the bio-active powders required for the study. GA wrote the manuscript. MG, JF, RT, HS, TB revised the manuscript. GA had primary responsibility for the finial content. All authors read and approved the finial manuscript.

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Conflicts of interest

The authors have no conflicts of interest to declare.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.clnu.2020.06.027.

References

[1] Peng J, Luo F, Ruan G, Peng R, Li X. Hypertriglyceridemia and atherosclerosis. Lipids Health Dis 2017;16(1):233.
[2] Lopez-Miranda J, Williams C, Lairon D. Dietary, physiological, genetic and pathological influences on postprandial lipid metabolism. Br J Nutr 2007;98(3):458–73.
[3] [internet] 4364.0.55.005 - Australian health survey: biomedical results for chronic diseases, 2011-12: triglycerides. Australian Beureau of Statistics; 2013 [updated 21st Dec 2015][cited 2019 Jun 5]. Available from: https://www.abs.gov.au/ausstats/abs@.nsf/Lookup/4364.0.55.005main+features12011-12.
[4] Hyson D, Rutledge JC, Berglund L. Postprandial lipemia and cardiovascular disease. Curr Atherosclerosis Rep 2003;5(6):437–44.
[5] Tiihonen K, Rautonen N, Alhoniemi E, Ahotupa M, Stowell J, Vasankari T. Postprandial triglyceride response in normolipidemic, hyperlipidemic and obese subjects - the influence of polydextrose, a non-digestible carbohydrate. Nutr J 2015;14:23.
[6] Weintraub MS, Eisenberg S, Breslow JL. Different patterns of postprandial lipoprotein metabolism in normal, type IIa, type III, and type IV hyperlipoproteinemic individuals. Effects of treatment with cholestyramine and gemfibrozil. J Clin Invest 1987;79(4):1110–9.
[7] Jackson KG, Poppitt SD, Minihane AM. Postprandial lipemia and cardiovascular disease risk: interrelationships between dietary, physiological and genetic determinants. Atherosclerosis 2012;220(1):22–33.
[8] Dias CB, Moughan PJ, Wood LG, Singh H, Garg ML. Postprandial lipemia: factoring in lipemic response for ranking foods for their healthiness. Lipids Health Dis 2017;16(1):178.
[9] Seo Hee Lee B-WL, Won Hee Kwan, Moon Jae Hoon, Kim Kwang Joon, Kang Eun Seok, Cha Bong Soo, et al. Postprandial triglyceride is associated with fasting triglyceride and HOMA-IR in Korean subjects with type 2 diabetes. Diabetes Obes Metabol 2011;35(4).
[10] Chan DC, Pang J, Romic G, Watts GF. Postprandial hypertriglyceridemia and cardiovascular disease: current and future therapies. Curr Atherosclerosis Rep 2013;15(3):309.
[11] Diabetes Australia KHA, Heart foundation, Stroke Foundation. Absolute cardiovascular disease risk: Australian government. National health and Medical Research Council; 2012 [updated 2012; cited 2019 09.08]. Available from: https://www.heartfoundation.org.au/images/uploads/publications/Absolute-CVD-Risk-Full-Guidelines.pdf.
[12] Roche HM, Gibney MJ. Long-chain n-3 polyunsaturated fatty acids and triacylglycerol metabolism in the postprandial state. Lipids 1999;34(1):S259–65.
[13] Thota RN, Ferguson JJA, Abbott KA, Dias CB, Garg ML. Science behind the cardio-metabolic benefits of omega-3 polyunsaturated fatty acids: biochemical effects vs. clinical outcomes. Food Funct 2018;9(7):3576–96.
[14] Wang C, Harris WS, Chung M, Lichtenstein AH, Balk EM, Kupelnick B, et al. n-3 Fatty acids from fish or fish-oil supplements, but not alpha-linolenic acid, benefit cardiovascular disease outcomes in primary- and secondary-prevention studies: a systematic review. Am J Clin Nutr 2006;84(1):5–17.
[15] Marik PE, Varon J. Omega-3 dietary supplements and the risk of cardiovascular events: a systematic review. Clin Cardiol 2009;32(7):365–72.
[16] Miyoshi T, Noda Y, Ohno Y, Sugiyama H, Oe H, Nakamura K, et al. Omega-3 fatty acids improve postprandial lipemia and associated endothelial dysfunction in healthy individuals - a randomized cross-over trial. Biomed Pharmacother 2014;68(8):1071–7.
[17] Schirmer SH, Werner CM, Binder SB, Faas ME, Custodis F, Bohm M, et al. Effects of omega-3 fatty acids on postprandial triglycerides and monocyte activation. Atherosclerosis 2012;225(1):166–72.
[18] Panth N, Abbott KA, Dias CB, Wynne K, Garg ML. Differential effects of medium- and long-chain saturated fatty acids on blood lipid profile: a systematic review and meta-analysis. Am J Clin Nutr 2018;108(4):675–87.
[19] Arunima S, Rajamohan T. Virgin coconut oil improves hepatic lipid metabolism in rats-compared with copra oil, olive oil and sunflower oil. Indian J Exp Biol 2012;50(11):802–9.
[20] Lidiani Figueiredo Santana KWC, Soares Fabíola Lacerda Pires, Cássia Freitas Karine de. Coconut oil increases HDL-c and decreases triglycerides in wistar rats. Acta Sci 2016;38(2):185.
[21] Bach AC, Babayan VK. Medium-chain triglycerides: an update. Am J Clin Nutr 1982;36(5).
[22] Karupaiah T, Tan CH, Chinna K, Sundram K. The chain length of dietary saturated fatty acids affects human postprandial lipemia. J Am Coll Nutr 2011;30(6):511–21.
[23] Panth N, Dias CB, Wynne K, Singh H, Garg ML. Medium-chain fatty acids lower postprandial lipemia: a randomized crossover trial. Clin Nutr 2019. Q3
[24] Gao Y, Zhu CG, Wu NQ, Guo YL, Liu G, Dong Q, et al. Study on the reliability of CardioChek PA for measuring lipid profile. J Peking Univ (Heal Sci) 2016;48(3):523–8.
[25] Berry SE, Tydeman EA, Lewis HB, Phalora R, Rosborough J, Picout DR, et al. Manipulation of lipid bioaccessibility of almond seeds influences postprandial lipemia in healthy human subjects. Am J Clin Nutr 2008;88(4):922–9.
[26] Boudewijn Klop SP, Mamo John, Botham Kathleen M, Castro Cabezas Manuel. Understanding postprandial inflammation and its relationship to lifestyle behaviour and metabolic diseases. Int J Vasc Med 2012;947417(9):11–2.
[27] Park Y, Harris WS. Omega-3 fatty acid supplementation accelerates chylomicron triglyceride clearance. J Lipid Res 2003;44(3):455–63.
[28] Brown AJ, Roberts DC. Moderate fish oil intake improves lipemic response to a standard fat meal. A study in 25 healthy men. Arterioscler Thromb 1991;11(3):457–66.
[29] Shearer GC, Savinova OV, Harris WS. Fish oil - how does it reduce plasma triglycerides? Biochim Biophys Acta 2012;1821(5):843–51.
[30] Swift LL, Hill JO, Peters JC, Greene HL. Medium-chain fatty acids: evidence for incorporation into chylomicron triglycerides in humans. Am J Clin Nutr 1990;52(5):834–6.
[31] Aoyama T, Nosaka N, Kasai M. Research on the nutritional characteristics of medium-chain fatty acids. J Med Invest 2007;54(3–4):385–8.
[32] Nicolosi RJ, Stucchi AF, Kowala MC, Hennessy LK, Hegsted DM, Schaefer EJ. Effect of dietary fat saturation and cholesterol on LDL composition and metabolism. In vivo studies of receptor and nonreceptor-mediated catabolism of LDL in cebus monkeys. Arteriosclerosis 1990;10(1):119–28.
[33] Dias CB, Zhu X, Thompson AK, Singh H, Garg ML. Effect of the food form and structure on lipid digestion and postprandial lipaemic response. Food Funct 2019;10(1):112–24.
[34] Issa JS, Diament J, Forti N. Postprandial lipemia: influence of aging. Arq Bras Cardiol 2005;85(1):15–9.
[35] Lee SH, Lee BW, Won HK, Moon JH, Kim KJ, Kang ES, et al. Postprandial triglyceride is associated with fasting triglyceride and HOMA-IR in Korean subjects with type 2 diabetes. Diabetes Metab J 2011;35(4):404–10.
[36] Chinwong S, Chinwong D, Mangklabruks A. Daily consumption of virgin coconut oil increases high-density lipoprotein cholesterol levels in healthy volunteers: a randomized crossover trial. Evid base Compl Alternative 2017;2017:7251562.
[37] Khaw K-T, Sharp SJ, Finikarides L, Afzal I, Lentjes M, Luben R, et al. Randomised trial of coconut oil, olive oil or butter on blood lipids and other cardiovascular risk factors in healthy men and women. BMJ Open 2018;8(3):e020167.
[38] Yanai H, Masui Y, Katsuyama H, Adachi H, Kawaguchi A, Hakoshima M, et al. An improvement of cardiovascular risk factors by omega-3 polyunsaturated fatty acids. J Clin Med Res 2018;10(4):281–9.
[39] Mohammed A, Luka C, Gyang S, Ngwen. A evaluation of the effect of coconut oil (Cocos nucifera) on some biochemical parameters in alloxan-induced diabetic rats. Saudi J Med Pharm Sci 2017;3(4):218–319.
[40] Narayanankutty A, Mukesh RK, Ayoob SK, Ramavarma SK, Suseela IM, Manalil JJ, et al. Virgin coconut oil maintains redox status and improves glycemic conditions in high fructose fed rats. J Food Sci Technol 2016;53(1):895–901.
[41] Maidin NQHA N. Protective and antidiabetic effects of virgin coconut oil (VCO) on blood glucose concentrations in alloxan induced diabetic rats. Int J Pharm Pharmaceut Sci 2015;7:57–60.
[42] Borkman M, Chisholm DJ, Furler SM, Storlien LH, Kraegen EW, Simons LA, et al. Effects of fish oil supplementation on glucose and lipid metabolism in NIDDM. Diabetes 1989;38(10):1314–9. Q4