



# Palmolein and olive oil consumed within a high protein test meal have similar effects on postprandial endothelial function in overweight and obese men: A randomized controlled trial



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## ABSTRACT

**Objective:** This study assessed the postprandial effects of high fat, high protein meals containing either palmolein or olive oil on endothelial function in overweight/obese men.

**Design:** 28 men (32–65 yr; 25–35 kg/m<sup>2</sup>) consumed, in random order 1 wk apart, isocaloric high protein, high fat meals (2791 kJ, 40 g protein (~3 g L-arginine), 44 g fat, 21 g carbohydrate) prepared with either 40 g palmolein or 40 g olive oil after an overnight fast. The SFA:MUFA:PUFA ratio of the oils were: palmolein, 42:47:12; olive oil, 17:76:7. Brachial artery flow-mediated dilatation (FMD), circulating endothelial function markers, nitrotyrosine (oxidative stress marker), triglycerides, glucose and insulin were assessed pre-meal and hourly for 5 h. Mixed model procedures were used to analyze the data.

**Results:** Meal consumption increased serum triglycerides (time effect,  $P < 0.001$ ); with no meal differences (meal  $\times$  time interaction,  $P = 0.93$ ). Serum insulin peaked 1 h post-consumption and returned to pre-meal concentrations by 5 h with both meals (time effect,  $P < 0.001$ ; meal  $\times$  time effect,  $P = 0.68$ ). FMD, serum intercellular adhesion molecule-1 (ICAM-1) and E-selectin did not change (meal  $\times$  time effect,  $P > 0.4$ ). Olive oil transiently increased plasma nitrotyrosine after 1 h compared to palmolein (meal  $\times$  time interaction,  $P = 0.002$ ) whereas both meals increased serum vascular cell adhesion molecule-1 (VCAM-1) after 1 h (time effect,  $P < 0.001$ ; meal  $\times$  time interaction,  $P = 0.98$ ). Both nitrotyrosine and VCAM-1 returned to pre-meal concentrations after 2 h.

**Conclusion:** In the context of a high protein meal, palmolein similarly to olive oil did not affect postprandial endothelial function in overweight/obese men.

**Trial registration:** Australian New Zealand Clinical Trials Registry (ANZCTR) (<http://www.anzctr.org.au/default.aspx>). Trial ID: ACTRN12613000136707.

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## 1. Introduction

Reducing saturated fatty acid (SFA) intake has been a key dietary strategy for lowering coronary heart disease (CHD) risk based primarily on its cholesterol raising effects [1,2]. However, recent evidence suggests this relationship may not be as straightforward [1,3]

**Abbreviations:** %E, percentage of total energy; CHD, coronary heart disease; CVD, cardiovascular disease; FMD, flow-mediated dilation; ICAM-1, intercellular adhesion molecule-1; MUFA, monounsaturated fatty acids; PAI-1, plasminogen activator inhibitor 1; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids; TG, triglycerides; tPA, tissue plasminogen activator; VCAM-1, vascular cell adhesion molecule-1.

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and may be affected by co-consumption of other dietary components. Meals or foods high in SFA may contain other constituents that counteract its CHD potential [1]. Furthermore, dietary effects on CHD risk are mediated through multiple pathways; it is therefore insufficient to base dietary recommendations solely on its lipid modulating effects and evaluating the effects of SFA on multiple biomarkers will assist to characterize its CHD potential [1].

Endothelial dysfunction, involving increased endothelial permeability to lipoproteins and other plasma constituents, reduced vasodilatation and activation of thrombotic and inflammatory pathways has been proposed as the earliest identifiable event in the atherosclerosis process and therefore represents an important clinical target for cardiovascular disease (CVD) prevention through dietary interventions [4,5]. To date, the effects of SFA on vascular function have not been well-researched [2,6].

Considering most humans spend the majority of their day in the postprandial state, it is important to investigate the acute effects of fat-containing meals on endothelial function. High-fat meals have previously been shown to impair postprandial vascular function as indicated by reduced brachial artery flow-mediated dilatation (FMD) [6], a recognized marker of endothelial dysfunction [7], and increased markers of vascular function such as cell adhesion molecules including vascular cell adhesion molecule-1 (VCAM-1), intercellular adhesion molecule-1 (ICAM-1) and E-selectin [8]. This effect may be ascribed to increased inflammation and oxidative stress mediated by elevated non-esterified fatty acids and postprandial triglyceride rich lipoproteins [6]. However, evidence for the differential postprandial effects of SFA vs. unsaturated fatty acids on vascular function is inconclusive [6] and may be dependent on other concurrent meal components such as protein. Westphal et al. [9] showed that consumption of SFA from dairy (whipped cream) had a neutral effect on FMD postprandially when consumed with a 50 g protein load (soy protein, caseinate). This response may be due to the presence of the amino acid L-arginine in the meals [10]. This suggests consumption of SFA in combination with a high protein and/or L-arginine load provided by dietary sources such as fish, meat (beef, poultry, pork), seeds, nuts and soy [11] may not impair FMD and endothelial function.

Palmolein, derived from dry fractionation of palm oil is a rich source of both SFA (42%) and unsaturated fat (47% monounsaturated fatty acids (MUFA), 12% polyunsaturated fatty acids (PUFA)) (Table 1). Its inherent stability and resistance to oxidation makes it a popular choice for food manufacturers and as replacement for *trans* fats [12,13]. Hence, assessing its health effects is important.

The aim of this study was to assess postprandial effects of typical meals high in protein containing either palmolein or olive oil on endothelial function in overweight or obese men, a target group with high prevalence globally [14] with increased risk of endothelial dysfunction [15]. We hypothesized that palmolein relative to olive oil, in the context of a high protein meal, will have similar effects on endothelial function as assessed by FMD as primary outcome. Secondary outcomes were circulating markers of endothelial function including serum adhesion molecules (VCAM-1, ICAM-1 and E-Selectin), endothelium derived fibrinolytic factors (plasminogen activator inhibitor-1 [PAI-1], tissue plasminogen activator [tPA]), a marker of oxidative stress (nitrotyrosine formation), insulin, glucose and triglycerides.

## 2. Methods

The dietary intervention (<http://www.anzctr.org.au>; ACTRN12613000136707) was conducted at CSIRO's Nutrition and

Health Research Clinic, Adelaide, South Australia between September and December 2013 according to the guidelines of the National Health and Medical Research Council National Statement on Ethical Conduct in Human Research. Ethical approval for the trial was obtained from the CSIRO Human Ethics Committee reference 12/13 and written informed consent was obtained from all participants.

### 2.1. Participants

Twenty eight [28] overweight and obese men (aged 18–65 yr, BMI between 25 and 35 kg/m<sup>2</sup>) who were weight stable over the previous 3 months were recruited by local advertisement and a pre-existing participant database. Overweight and obesity is associated with endothelial dysfunction [15] and it was therefore considered important to establish the effects of the interventions being tested in this population. Women were excluded to remove the potential confounding effects of the menstrual cycle on FMD [7]. Other exclusion criteria were known medical conditions or diseases including type 1 or 2 diabetes, kidney, respiratory, gastrointestinal, cardiovascular or peripheral vascular disease, smoking, history of heavy alcohol consumption (>5 standard drinks/d), use of nitrate medication, non-steroidal anti-inflammatory medication or medication and supplements that may affect the study outcomes and gastrointestinal function (such as antibiotics, laxatives, fish oil, etc) during the 3 months prior to the study.

### 2.2. Study design

This acute (5 h) nutrition intervention was conducted using a randomized double-blind cross-over study design. Participants attended the CSIRO clinic on two occasions separated by 1 wk during which they consumed two high protein, high fat test meals prepared with either palmolein or olive oil. Participants were randomly assigned by computer generation (<http://www.randomisation.com>) to treatment orders matched on age and BMI. Medication usage remained constant throughout the study and during test days. Prior to each test day participants were requested to avoid alcohol and strenuous exercise for 24 h and 48 h respectively, and to consume the same evening meal. Participants recorded the content of their evening meal and research staff verified whether the same meal was consumed. Two participants deviated from this protocol, but their outcome responses did not differ from other participants. Participants arrived at the clinic between 8.00 and 8.30 am after an overnight fast; height (at first visit), weight and blood pressure were recorded before an intra-venous cannula was inserted in the left arm for venous blood collection. A baseline blood sample and FMD measurement (of the right arm) were then obtained after which participants consumed the test meals within 15 min. After 1 h from commencing consumption of the test meal and hourly thereafter for 5 h, blood samples and FMD measurements were obtained.

### 2.3. Test meals

The test meals were prepared in the CSIRO clinic kitchen using safe food handling practices. The test meals were identical in appearance and energy and macronutrient content and only differed in the oil type used to prepare the meals. No obvious difference in flavor between the meals was apparent. The meals consisted of 200 g (raw weight) of lean chicken strips fried in 40 g test oil, either palmolein (RBD Palmolein) or olive oil (Moro Pure Olive Oil 100% pure, produced and packed in Spain for Conga Foods Pty. Ltd). The cooked chicken was served with lightly fried white bread (fried in the remaining oil after cooking the chicken to absorb

**Table 1**  
Fatty acid composition of the test oils (% of total fatty acids).

Fatty acids	Palmolein	Olive oil
Lauric acid (C12:0)	0.20	0.00
Myristic acid (C14:0)	0.98	0.00
Palmitic acid (C16:0)	36.2	11.7
Palmitoleic acid (C16:1n-9)	0.22	0.69
Stearic acid (C18:0)	4.14	4.20
Elaidic acid (C18:1n-9t)	0.25	1.04
Oleic acid (C18:1n-9)	46.1	74.1
Linoleic acid (C18:2n-6)	11.3	6.80
Alpha-linolenic acid (C18:3n-3)	0.19	0.45
Arachidic acid (C20:0)	0.37	0.51
Gondoic acid (C20:1n-9)	0.21	0.33
Behenic acid (C22:0)	0.00	0.16
Total SFA	41.9	16.6
Total MUFA	46.8	76.2
Total PUFA	11.5	7.25

all fat) and a small salad (20 g lettuce, 10 g tomato and 10 g cucumber). The nutrient composition of the meals as estimated using the computer program FoodWorks Professional Edition version 4 (Xyris Software, 2009) were 2791 kJ, 40 g (30% or total energy [%E]) protein (~3 g from L-arginine [11]), 44 g (58%E) fat and 21 g (11%E) carbohydrate. Fatty acid compositions of the test oils are summarized in Table 1. Research staff preparing the meals was not involved in the data collection or analysis processes. Treatment allocation was fully concealed from all other researchers and participants. Unblinding occurred after statistical analysis was completed.

#### 2.4. Anthropometric and physiological measurements

Height was measured using a stadiometer (SECA, Hamburg, Germany); body weight using calibrated electronic digital scales (A & D Mercury HW-PW200 Platform Scales, A & D Australasia) and BMI ( $\text{kg}/\text{m}^2$ ) calculated. Blood pressure was measured using an automated blood pressure monitor (Philips SureSigns VS3, Philips Medical Systems, Andover, MA) in a seated position after a 5 min rest. The average of three measurements (separated by 2 min) was recorded.

Endothelial-dependent brachial artery FMD was measured as previously described [16] using 2 dimensional B-mode ultrasound with a 5–13 MHz linear array transducer (Logiq e, GE Healthcare, Wauwatosa, WI, USA). To induce ischemia a sphygmomanometer cuff was placed around the right forearm and inflated to 200 mmHg for 5 min. Snapshots (2 cardiac cycle length videos) of the brachial artery were recorded 30 s prior to cuff inflation (baseline) and every 15 s for 3 min after deflation. End-diastole brachial artery diameter for each snapshot was measured manually using the measurement function of the ultrasound instrument. Peak diameter was defined as the highest value obtained following cuff deflation with FMD expressed as the percentage change from baseline in brachial artery diameter prior to cuff deflation. Manual image assessments were conducted by the same operator to reduce variation. The intra-observer coefficient of variation for FMD in this operator's hands was 15% based on repeated scans performed on 10 healthy individuals on two separate occasions under similar circumstances prior to study commencement. This CV is similar to that reported in other laboratories in similar populations [17].

#### 2.5. Blood sample collection and assays

Venous blood samples were collected into vacutainers with no additive for the analysis of adhesion molecules, triglycerides and insulin; sodium fluoride for glucose; EDTA for nitrotyrosine; and into CTAB tubes containing sodium citrate, theophylline, adenosine and dipyridamole to maximize platelet stabilization for the analysis of PAI-1 and tPA. Blood for serum samples (tubes without additive) were allowed to clot at room temperature for 30 min before processing. Other tubes were kept on ice; CTAB tubes were processed within 20–30 min; all other tubes were processed within 60 min by centrifugation at 4 °C on a Beckman Coulter Allerga X-12R (Beckman Coulter, Inc., CA, USA). Sodium fluoride tubes were centrifuged for 10 min at 2500 rpm; EDTA tubes for 10 min at 3000 rpm; tubes without additive for 15 min at 3500 rpm and CTAD tubes for 20 min at 3300 rpm. Aliquots of serum and plasma samples were stored at –80 °C until the end of the study for analysis in one batch.

Biochemical assays were performed within the CSIRO laboratory. Serum ICAM-1 and VCAM-1 were measured using the Luminex 100/200 system with xPONENT software (Luminex, Texas, USA) and the human cardiovascular disease panel 2 magnetic bead kit (Cat. no. HCVD2MAG-67K; Milliplex, EMD Millipore Corporation, Missouri, USA) (intra-assay CVs were for ICAM-1: low 1.9% and high 5.1 and for VCAM: low 2.0% and high 2.7%). Serum E-selectin,

plasma PAI-1, tPA, nitrotyrosine and insulin were measured using a multilabel plate reader (PerkinElmer, Massachusetts USA) and the following test kits: serum E-selectin, quantikine human sE-Selectin/CD62E immunoassay (Cat. no. DSLE00; R&D Systems Inc, Minneapolis, USA) (intra-assay CV 4.9%); plasma PAI-1, quantikine human serpin E1/PAI-1 immunoassay (Cat. no. DSE100; R&D Systems Inc, Minneapolis, USA) (intra-assay CV, low 3%, mid 3.6%, high 1.8%); plasma tPA, soluble tPA ELISA Kit (Cat. no. QIA99; Calbiochem, Millipore, San Diego, CA, USA) (intra-assay CV 4.4%); plasma nitrotyrosine, oxiSelect nitrotyrosine ELISA kit (Cat. no. STA-305; Cell Biolabs Inc, San Diego, CA, USA) (intra-assay CV, 20.9%); serum insulin, Mercodia insulin ELISA and diabetes antigen control (Cat. no's. 10-1113-01 and 10-1164-01, respectively; Mercodia AB Uppsala, Sweden) (intra-assay CV, low 2.2%, high 1.1%). Serum TG and plasma glucose were measured using a Beckman Coulter AU480 Chemistry Analyzer and test kits (TRIGLYCERIDES OSR60118, GLUCOSE OSR6121, Beckman Coulter, Inc., CA, USA) (intra-assay CV for TG, low 2.9%, high 2.2%; for glucose, low 2.8%, high 1.2%).

#### 2.6. Analysis of test oils

Test oils were analyzed within the CSIRO laboratory for fatty acid content by mixing 20 mg oil with 8 mL chloroform. A 250  $\mu\text{l}$  sample of the oil/chloroform mix was then extracted using the standard fatty acid method by Folch et al. [18] with the exceptions that no internal standard was used and the methyl esters were reconstituted with 150  $\mu\text{l}$  iso-octane. An aliquot (2.5  $\mu\text{l}$ ) was injected onto a gas chromatographic column of vitreous silica (BPX-70, 30 m  $\times$  0.53 mm from SGE, Australia) using an Agilent 6890 gas chromatograph equipped with split 10:1 injector. Fatty acids were identified by comparison with authentic Supelco 37 component FAME mix (Sigma–Aldrich, Australia). Peak areas were measured using ChemStation software and component peak area are expressed as a percentage of the total area of the known fatty acid peaks to give a fatty acid profile.

#### 2.7. Statistical analysis

Statistical power was based on detecting differences for the *a priori* hypothesis based on pairwise comparison between the palmolein vs. olive oil on the primary outcome measure of FMD; 27 participants were required to provide sufficient statistical power (80%,  $\alpha$  0.05) to detect a minimum absolute difference of 2% between the pairwise group comparisons for FMD. In the absence of information indicating the clinical relevance specific to post-prandial and transient changes in FMD the difference in FMD of 2% (absolute) was based on previous research showing this difference to be associated with a clinical relevant change in CVD risk [19].

Statistical analysis was performed using SPSS software version 20 (IBM Corporation, New York, USA). The variables were tested for normality using the Kolmogorov–Smirnov, Shapiro–Wilk tests and normality plots. Non-normally distributed data were transformed into approximate normal distributions by logarithmic transformations. Data are reported as mean (95% CI) and transformed variables were back transformed into mean (95% CI) from summary statistics.

Statistical comparisons were performed using a generalized linear model for repeated measures mixed models procedure with first-order autoregressive as the repeated covariance matrix structure. Meal type and time were included as fixed effects and the interactions between meal and time were tested. The data was examined for interaction effects due to the order in which the meals were consumed by including order of meal as fixed effect in the model and investigating its interaction effects with treatment, time and meal  $\times$  time. Since no meal order interactions were

evident, this variable was removed from the models. When significant effects of time were observed, post-hoc analysis with Bonferroni adjustments were performed to determine which time points differed significantly. AUC was calculated for variables that showed significant differences between meals using the trapezoid method. Pairwise differences between treatments in AUC were tested using the mixed models procedure and choosing the compound symmetry option as repeated covariance matrix structure. A *P*-value of <0.05 was considered statistically significant.

### 3. Results

The participant flow through the study is summarized in Fig. 1. A total of 28 participants completed both treatments and were included in the statistical analysis. Participant baseline characteristics are summarized in Table 2. FMD levels were similar to those previously reported in overweight/obese populations [20,21].

The postprandial effects of the test meals on FMD are illustrated in Fig. 2. The postprandial FMD response did not change, and there were no differences in FMD responses between the test oils.

After meal consumption, serum triglyceride concentrations increased significantly with both meals reaching peak concentrations at 4 h (time effect,  $P < 0.001$ ), with no difference in response between meals (meal  $\times$  time interaction,  $P = 0.93$ ) (Table 3). Serum insulin concentrations increased 1 h after meal consumption that returned to pre-meal concentrations by 5 h (time effect,  $P < 0.001$ ) with no significant differences between meals (Table 3). After an initial rise 1 h post-meal consumption, plasma glucose reduced after 2 h and was maintained at the lower concentration over the duration of the experiment (time effect,  $P < 0.001$ ) with no differences between meals (Table 3).

Circulating markers of vascular function and oxidative stress are summarized in Table 4. Plasma PAI-1 and tPA concentrations decreased after meal consumption (time effect,  $P < 0.001$ ), and VCAM-1 transiently increased after 1 h with concentrations returning to pre-meal concentrations thereafter; with no differential responses between meals. Serum ICAM-1 remained unchanged throughout the experimental period. Serum E-selectin remained unchanged until 4 h after which concentrations

**Table 2**  
Baseline characteristics ( $n = 28$ ).

Variable	Mean (95% CI)
Age (years)	56.8 (53.7, 59.8)
Height (m)	1.75 (1.72, 1.78)
Weight (kg)	92.7 (87.8, 97.6)
BMI ( $\text{kg}/\text{m}^2$ )	30.0 (28.7, 31.3)
Systolic blood pressure (mmHg)	126 (121, 132)
Diastolic blood pressure (mmHg)	84.4 (80.4, 88.4)
Flow-mediated dilatation (%)	5.99 (4.95, 7.03)
Serum insulin (mU/L)	10.7 (8.57, 13.4) <sup>a</sup>
Plasma glucose (mmol/L)	5.87 (5.68, 6.06)
Serum TG (mmol/L)	1.91 (1.55, 2.28)
Plasma PAI-1 (ng/mL)	4.13 (3.07, 5.46) <sup>a</sup>
Plasma tPA (ng/mL)	1.79 (1.53, 2.10) <sup>a</sup>
Serum ICAM-1 (ng/mL)	61.5 (54.1, 68.8)
Serum VCAM-1 (ng/mL)	500 (455, 545)
Serum E-selectin (ng/mL)	46.7 (41.7, 51.8)
Plasma nitrotyrosine (nM)	4.47 (3.71, 5.24)
Taking lipid lowering medication	$n = 1$
Taking anti-hypertensive medication	$n = 1$

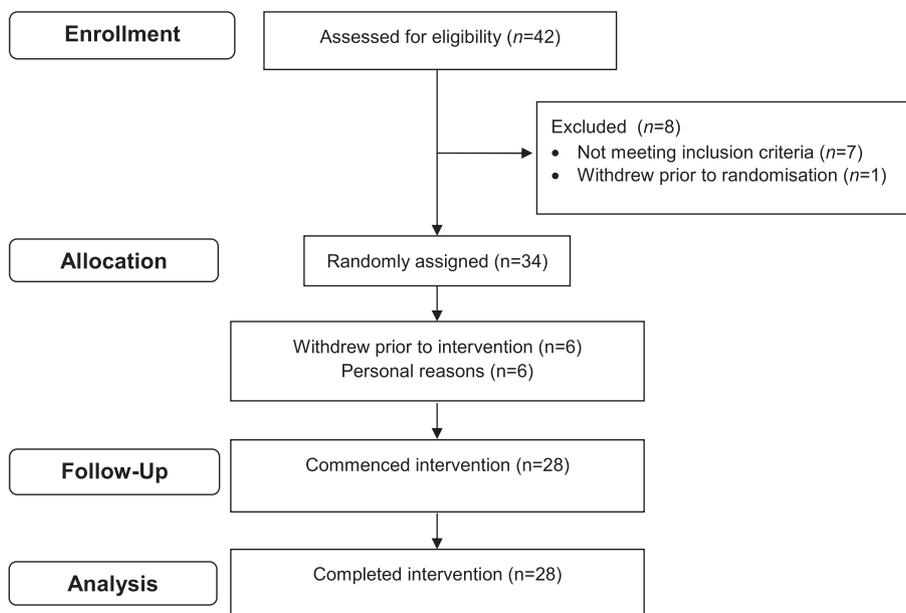
ICAM-1, intercellular adhesion molecule-1; PAI-1, plasminogen activator inhibitor 1; TG, triglycerides; tPA, tissue plasminogen activator; VCAM-1, vascular cell adhesion molecule-1.

<sup>a</sup> Variables that were transformed into approximately normal distributions by logarithmic transformations were back transformed into mean (95% CI) from summary statistics.

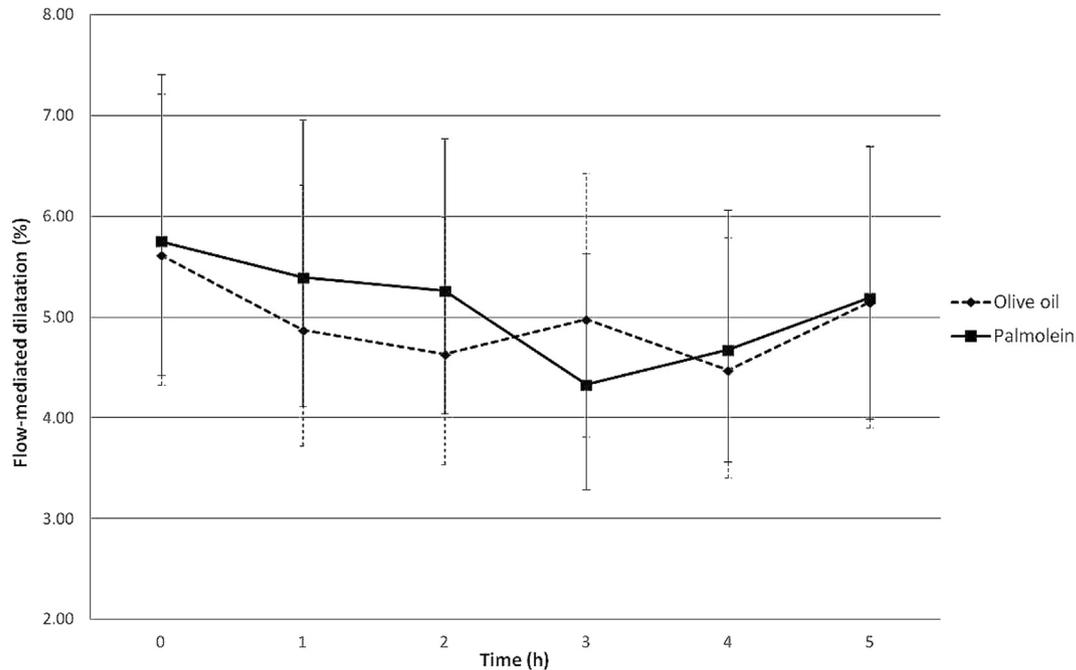
decreased slightly, with no differential response between meals. Plasma nitrotyrosine concentrations significantly increased 1 h after consuming the olive oil meal compared to the palmolein meal, but concentrations subsequently returned to pre-meal concentrations at 2 h with no further differences between meals. AUC for nitrotyrosine did not differ between meals (mean [95%CI] Palmolein, 19.8 [15.4, 24.2] vs. Olive oil, 22.3 [17.8, 26.7],  $P = 0.10$ ).

### 4. Discussion

This study showed consumption of a high protein meal containing fats differing in saturated fat, palmolein or olive oil, had no effect on postprandial FMD and markers of vascular function (serum ICAM-1, E-selectin) and similar increases in triglycerides.



**Fig. 1.** Flow chart of participants through the trial.



**Fig. 2.** Flow-mediated dilatation (FMD) following a high protein meal containing either olive oil or palmolein ( $n = 28$ ). Data were log transformed and are presented as mean (95% CI) calculated by back transforming summary statistics. Comparisons within and between treatments were made using mixed effects models. Meal effect,  $P = 0.8$ ; time effect,  $P = 0.48$ ; meal  $\times$  time interaction,  $P = 0.69$ .

Both meals decreased plasma tPA and PAI-1 which may have been related to a diurnal response [22], unrelated to meal consumption. Plasma nitrotyrosin, a marker of oxidative stress, transiently increased 1 h after consumption of the olive oil meal compared to the palmolein meal which coincided with increases in serum VCAM-1 for both meals. Both variables returned to pre-meal concentrations after 2 h. AUC for plasma nitrotyrosine did not differ between meals suggesting these transient increases are unlikely to be of clinical significance. The data support our hypothesis that palmolein and olive oil consumed in combination with a high protein meal does not impair or alter vascular function.

High fat meals ranging from  $\sim 26$  g ( $\sim 34\%$ E) to  $>50$  g ( $>50\%$ E) fat have been shown to impair endothelium-dependent vasodilatation or vasoactivity [9,23–31]. The effect seems to be independent of fat type, although inconsistencies have been observed. Rueda-Clausen et al. [28] showed similar impairments in FMD with olive, soybean and palm oil consumption, either in fresh or deep-fried forms. In contrast, some studies have shown impairment in FMD with high

MUFA meals [24–26,32] while others have shown no effect [30,32–34]. High SFA meals mainly from cream [9,10], butter [30], palm oil [28], palmstearin [35] or McDonalds take-away [27,31] have been shown to impair postprandial FMD while others have failed to show any effect with SFA from tallow fat [33] or palm kernel fat [36]. The postprandial effects of palmolein on vascular function have not yet been investigated. The inconsistent findings previously reported could be in part explained by disparities in other meal components. Addition of anti-oxidant vitamins or food sources rich in polyphenols [23,25,30,37], protein (particularly those rich in L-arginine) [9,10,25] or omega-3 fatty acids [35] have been shown to counteract postprandial FMD suppression associated with ingestion of high-fat meals. The addition of 2.5 g L-arginine to a cream-based fat load prevented any deterioration in FMD that was shown with the high fat load alone [10]. Cortes et al. [25] showed that the addition of 40 g walnuts (providing  $\sim 1$  g L-arginine and polyphenols) to a high SFA meal improved FMD compared to the same meal with the addition of olive oil.

**Table 3**

Mean (95% CI) changes in triglycerides, insulin and glucose following test meals containing olive oil or palmolein ( $n = 28$ ).

Variable	Meal	Pre-meal	1 h	2 h	3 h	4 h	5 h	P-value <sup>b</sup>		
								M	T	M $\times$ T
Serum TG (mmol/L)	Olive oil	1.84 <sup>a</sup> (1.39, 2.29)	1.87 <sup>a</sup> (1.42, 2.32)	2.28 <sup>b</sup> (1.83, 2.73)	2.97 <sup>c</sup> (2.52, 3.42)	3.10 <sup>c</sup> (2.65, 3.55)	2.82 <sup>d</sup> (2.37, 3.27)	0.22	<0.001	0.93
	Palmolein	1.94 <sup>a</sup> (1.49, 2.39)	2.00 <sup>a</sup> (1.55, 2.45)	2.53 <sup>b</sup> (2.08, 2.98)	3.24 <sup>c</sup> (2.79, 3.69)	3.34 <sup>c</sup> (2.89, 3.79)	2.96 <sup>d</sup> (2.51, 3.41)			
Serum insulin (mU/L) <sup>a</sup>	Olive oil	9.43 <sup>a</sup> (7.51, 11.8)	37.8 <sup>b</sup> (30.1, 47.4)	23.8 <sup>c</sup> (18.9, 29.8)	17.2 <sup>d</sup> (13.7, 21.5)	12.4 <sup>a,e</sup> (9.89, 15.6)	9.07 <sup>a,f</sup> (7.22, 11.4)	0.49	<0.001	0.68
	Palmolein	12.1 <sup>a</sup> (9.67, 15.3)	41.1 <sup>b</sup> (32.8, 51.7)	23.9 <sup>c</sup> (19.1, 30.0)	18.5 <sup>d</sup> (14.7, 23.2)	11.9 <sup>a,e</sup> (9.49, 15.0)	9.43 <sup>a,f</sup> (7.26, 11.8)			
Plasma glucose (mmol/L)	Olive oil	5.75 <sup>a</sup> (5.52, 5.99)	6.02 <sup>b</sup> (5.79, 6.25)	5.09 <sup>c</sup> (4.86, 5.32)	5.19 <sup>c</sup> (4.96, 5.43)	5.18 <sup>c</sup> (4.94, 5.41)	5.11 <sup>c</sup> (4.88, 5.34)	0.93	<0.001	0.99
	Palmolein	5.81 <sup>a</sup> (5.58, 6.05)	6.01 <sup>b</sup> (5.78, 6.24)	5.03 <sup>c</sup> (4.79, 5.26)	5.19 <sup>c</sup> (4.96, 5.42)	5.14 <sup>c</sup> (4.91, 5.37)	5.12 <sup>c</sup> (4.88, 5.35)			

TG, triglycerides; M, meal; T, treatment; M  $\times$  T, meal treatment interaction.

<sup>a,b,c,d,e,f</sup> Values with different superscript letters indicate significant differences over time (Post-hoc analysis with Bonferroni adjustments).

<sup>a</sup> Data were log transformed and are presented as mean (95% CI) calculated by back transforming summary statistics.

<sup>b</sup> Comparisons within and between treatments were made using mixed effects models.

**Table 4**Mean (95% CI)<sup>a</sup> changes in biochemical markers of endothelial function and oxidative stress following test meals containing olive oil or palmolein (n = 28).

Variable	Meal	Pre-meal	1 h	2 h	3 h	4 h	5 h	P-value <sup>b</sup>		
								M	T	M × T
Plasma PAI-1 (ng/mL)	Olive oil	3.49 <sup>a</sup> (2.80, 4.29)	2.49 <sup>b</sup> (1.96, 3.12)	2.09 <sup>c</sup> (1.61, 2.64)	1.75 <sup>d</sup> (1.33, 2.24)	1.25 <sup>e</sup> (0.91, 1.66)	1.02 <sup>f</sup> (0.71, 1.38)	0.73	<0.001	0.89
	Palmolein	3.82 <sup>a</sup> (3.09, 4.69)	2.62 <sup>b</sup> (2.06, 3.27)	2.22 <sup>c</sup> (1.72, 2.79)	1.75 <sup>d</sup> (1.33, 2.24)	1.26 <sup>e</sup> (0.92, 1.67)	0.97 <sup>f</sup> (0.67, 1.33)	0.703	<0.001	0.75
Plasma tPA (ng/mL)	Olive oil	1.63 <sup>a</sup> (1.40, 1.89)	1.58 <sup>b</sup> (1.36, 1.83)	1.47 <sup>c</sup> (1.27, 1.71)	1.42 <sup>c,d</sup> (1.22, 1.64)	1.38 <sup>d</sup> (1.19, 1.60)	1.29 <sup>e</sup> (1.11, 1.49)	0.73	<0.001	0.75
	Palmolein	1.71 <sup>a</sup> (1.47, 1.98)	1.55 <sup>b</sup> (1.33, 1.80)	1.51 <sup>c</sup> (1.30, 1.75)	1.47 <sup>c,d</sup> (1.26, 1.70)	1.41 <sup>d</sup> (1.21, 1.64)	1.31 <sup>e</sup> (1.13, 1.52)	0.47	0.20	0.83
Serum ICAM-1 (ng/mL)	Olive oil	56.7 (50.5, 63.5)	58.8 (52.4, 66.0)	57.4 (51.2, 64.4)	56.8 (50.6, 63.6)	55.8 (49.8, 62.6)	59.4 (53.0, 66.7)	0.47	0.20	0.83
	Palmolein	60.8 (54.2, 68.2)	60.3 (53.8, 67.6)	59.5 (53.0, 66.7)	58.0 (51.7, 65.0)	57.9 (51.7, 65.0)	59.7 (53.3, 67.0)	0.78	<0.001	0.98
Serum VCAM-1 (ng/mL)	Olive oil	472 <sup>a</sup> (433, 514)	488 <sup>b</sup> (448, 532)	481 <sup>a,b</sup> (441, 524)	473 <sup>a</sup> (434, 516)	468 <sup>a,c</sup> (430, 510)	475 <sup>a,b</sup> (436, 517)	0.78	<0.001	0.98
	Palmolein	474 <sup>a</sup> (435, 516)	488 <sup>b</sup> (448, 532)	483 <sup>a,b</sup> (443, 526)	477 <sup>a</sup> (437, 520)	475 <sup>a,c</sup> (436, 517)	478 <sup>a,b</sup> (438, 521)	0.38	0.01	0.39
Serum E-selectin (ng/mL)	Olive oil	41.8 (37.3, 46.7)	43.0 <sup>a</sup> (38.4, 48.1)	43.0 <sup>a,c</sup> (38.4, 48.1)	42.9 <sup>a,c</sup> (38.3, 48.0)	40.2 <sup>b</sup> (35.9, 44.9)	41.0 <sup>b,c</sup> (36.7, 45.9)	0.38	0.01	0.39
	Palmolein	44.7 (39.9, 50.0)	45.5 <sup>a</sup> (40.7, 50.9)	43.0 <sup>a,c</sup> (38.5, 48.1)	43.2 <sup>a,c</sup> (38.6, 48.3)	42.0 <sup>b</sup> (37.6, 47.0)	42.0 <sup>b,c</sup> (37.6, 47.0)	0.27	0.50	0.002
Plasma Nitrotyrosine (nM)	Olive oil	4.45 (3.64, 5.41)	5.59 <sup>a</sup> (4.60, 6.76)	4.17 (3.40, 5.07)	4.39 (3.59, 5.33)	3.83 (3.11, 4.67)	4.51 (3.70, 5.47)	0.27	0.50	0.002
	Palmolein	4.02 (3.28, 4.89)	3.73 <sup>b</sup> (3.01, 4.58)	4.26 (3.48, 5.18)	4.29 (3.49, 5.22)	4.25 (3.45, 5.18)	3.80 (3.09, 4.62)			

ICAM-1, intercellular adhesion molecule-1; PAI-1, plasminogen activator inhibitor-1; tPA, tissue plasminogen activator; VCAM-1, vascular cell adhesion molecule-1; M, meal; T, treatment; M × T, meal treatment interaction.

<sup>a,b,c,d,e,f</sup> Values with different superscript letters indicate significant differences over time or between meals (Post-hoc analysis with Bonferroni adjustments).

<sup>a</sup> Data were log transformed and are presented as mean (95% CI) calculated by back transforming summary statistics.

<sup>b</sup> Comparisons within and between treatments were made using mixed effects models.

Based on prior evidence (as discussed above), meals used in the current study contained sufficiently high amounts of fat to promote an impaired FMD response. This is supported by the observed elevated postprandial triglyceride concentrations. However, FMD was not affected by either meal. It is hypothesized, that the lack of effect may be ascribed to the high protein content of the meals which provided 30% of total energy from protein and ~3 g L-arginine. L-arginine is a potent vasodilator, required for the production of nitric oxide by nitric oxide synthase, a critical substance produced by the vascular endothelium for regulation of vasodilatation [38]. However, to confirm this hypothesis a low protein/L-arginine meal comparison would be required. Furthermore, protein has insulinotropic properties [39] which may contribute to vasodilatation through stimulation of nitric oxide release [40]. In the current study serum insulin concentrations increased in response to the meals which may have contributed to counteracting any FMD reduction associated with ingestion of high-fat meals.

A major cause of endothelial dysfunction is oxidative stress, inflammation, increased expression of cell adhesion molecules, ICAM-1, VCAM-1 and E-selectin, that promotes adhesion of leucocytes to endothelial cells, an important initiation step in atherosclerosis [5,41]. Increased concentrations of circulatory cell adhesion molecules, hence, serve as markers of endothelial cell damage and/or activation, atherosclerosis and the development of CHD [42]. Postprandial lipemia results in the generation of reactive oxygen species including superoxide anion which reacts with nitric oxide to produce peroxynitrite consequently resulting in a reduction in nitric oxide bioavailability and impaired FMD [24]. Nitrotyrosine is formed during nitration of tyrosine residues with peroxynitrite. The presence of nitrotyrosine in plasma is therefore an indication of peroxynitrite production and considered an appropriate marker of oxidative stress at the vascular level [8]. Postprandial hypertriglyceridaemia in response to high fat meals have been shown to increase plasma ICAM-1, VCAM [8,43] and E-selectin and the common mediator of this phenomenon was oxidative stress as evaluated by nitrotyrosine formation [8]. In the current study serum ICAM-1 and E-selectin were unaffected, but the meal containing olive oil stimulated a transient increase in plasma nitrotyrosine after 1 h compared to the palmolein meal.

This increase coincided with increased serum VCAM-1 but the increase was seen for both meals. Both variables returned to baseline after 2 h and the AUC between meals for nitrotyrosine did not differ. These short and transient increases are therefore unlikely to be of any clinical significance.

Plasma tPA and PAI-1 are endothelium derived fibrinolytic factors and increased concentrations may reflect increased endothelial cell activation or damage [44]. tPA is primarily responsible for the degradation of fibrin clots while PAI-1 is the most important inhibitor of tPA [45]. However, higher plasma tPA antigen concentrations, as measured in the current study, represents largely inactive circulating tPA-PAI-1 complexes, and is therefore more reflective of PAI-1 concentrations [45]. Both tPA antigen and PAI-1 have been associated with increased risk of CHD [46,47]. In the current study both plasma tPA and PAI-1 decreased after consumption of the meals. The observed reductions was most likely due to normal circadian rhythm that characterize peak PAI-1 concentrations occurring in the morning that subsequently decline over the course of the day independent of behavioral and environmental factors [22].

A strength of the current study was the sample size which is larger than most other postprandial studies investigating the effects of high fat meals on FMD using sample sizes of  $n = \sim 8-20$  [9,10,23–25,27,28,31–34]. A post hoc power calculation using the SD of our sample confirmed that we had 85% power at  $\alpha = 0.05$  to detect an absolute difference in FMD of 2% which has been shown to represent a clinically relevant change in CVD [19]. Thereby, since the mean absolute difference in FMD between groups at any time point was smaller than 2% it confers a non-significant effect. However, a limitation was that the clinical significant difference of 2% is not based on post-prandial changes in FMD because the clinical relevance of postprandial and transient changes in FMD has not been established to date. Thus, it remains unclear whether this FMD difference of 2% is relevant under postprandial conditions. Further strengths were that overweight or obese men were studied, representing an important and relevant target group at increased risk of endothelial dysfunction [15]; and the effects were investigated in the context of a typically consumed meal (containing whole foods, namely chicken, bread and salad) that

achieve greater relevance to typical postprandial conditions. However, the effects of these oil sources on vascular function may be different in the context of a low protein meal. Further research is also required to determine the impact of postprandial FMD responses on chronic changes in endothelial function and cardiovascular disease risk. A limitation of the study was the use of manual measurement methods for FMD analysis. The use of edge detection software has the potential to facilitate more accurate and reproducible measurements [7]. It should be noted that the results of the study apply to palmolein and cannot be generalized to palm oil. In addition, applicability of the results is limited to overweight/obese men and cannot be generalized to normal weight individuals or to women.

In conclusion, in the context of a high protein meal, consumption of palmolein similarly to olive oil did not alter postprandial endothelial function in overweight or obese men assessed by FMD, cellular adhesion molecules, and oxidative stress, despite triglyceride elevating responses and a higher SFA content in the palmolein meal.

### Contribution of authors

WS: Main author of the manuscript; managed the conduct of the research; performed statistical analysis.

GB: Designed research (project conception, development of overall research plan, study oversight); oversight of statistical analysis; critical revision of the manuscript and approval of the final version.

MN: Designed research (project conception and development of overall research plan, and study oversight); critical revision of the manuscript and approval of the final version.

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### Disclosures

None of the authors had any conflict of interest in relation to this manuscript.

### Conflicts of interest

WS, GB, MN do not have any conflict of interest.

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