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Ingestion of native and thermally oxidized polyunsaturated fats acutely increases circulating numbers of endothelial microparticles

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Abstract

Circulating numbers of endothelial microparticles (EMP) are an index of endothelial injury and dysfunction; and microparticles positive to CD31 antibody increase acutely after cooked, fatty fast-food meals that are rich in saturated fatty acids (SAFA) and lipid oxidation products. The aim of this study was to determine the acute effect of meals rich in SAFA and native and thermally oxidized polyunsaturated vegetable oil on circulating numbers of EMP positive to CD144 antibody, a more specific marker of EMP. Twenty-two apparently healthy subjects received isocaloric meals rich in cream (CR), unheated sunflower oil, or heated sunflower oil in a randomized crossover study design. Circulating numbers of CD144-EMP and plasma lipids and Svedberg unit of flotation (S_f) greater than 400 triglyceride content were measured before and 1 and 3 hours after the meals. Triglycerides in the plasma S_f greater than 400 fraction increased significantly ($P < .001$) after the meals, with a significantly ($P < .05$) larger increase after the CR meal. Plasma CD144-EMP increased significantly (20%, $P < .05$) after the unheated sunflower oil and heated sunflower oil meals and did not increase significantly ($P = .55$) after the CR meal. This response was significantly different among the meals ($P = .002$) when first-visit fasting plasma glucose was a covariate. In conclusion, these data suggest that ingestion of meals rich in n-6 polyunsaturated vegetable oil irrespective of whether it has been mildly thermally oxidized may acutely alter the state of the vascular endothelium, resulting in increased shedding of CD144-EMP. The physiologic implications of these findings remain to be determined.

1. Introduction

Endothelial dysfunction is widely regarded as an early stage in the development of atherosclerotic disease [1]. Vascular responsiveness to endothelium-dependent stimuli such as increased shear stress (reactive hyperemia) and pharmacologic agents has become a standard test of endothelial function. However, other markers of endothelial function have been developed to reflect the multiple roles of the endothelium in maintaining vascular homeostasis. Recently, numbers of circulating endothelial microparticles (EMP) have been used to detect endothelial activation, dysfunction, and/or injury in vivo [2]. Endothelial microparticles are small vesicles containing a portion of cell membrane and contents and are formed during activation, injury, and apoptosis of endothelial cells [3]. The formation of cell microparticles is thought to start with an increase in intracellular calcium followed by loss of plasma membrane phospholipid asymmetry and cytoskeleton rearrangement, leading to the development of membrane budding and bleb formation and finally to release of microparticles [2]. Circulating microparticles also appear to be capable of mediating changes in various biological functions including endothelial function [2]. For example, there is evidence that circulating EMP may impair endothelial function by attenuating endothelial release of nitric oxide [2,4,5]. High numbers of EMP have been detected in the blood of patients with diseases that are associated with endothelial dysfunction including acute coronary syndrome [6], malignant hypertension [7], and type 2 diabetes mellitus [8].

Ingestion of a fatty fast-food meal acutely impairs endothelial function as indicated by a decrease in endothelium-dependent vasodilation [9,10] and activates and/or

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injures the endothelium as indicated by an increase in numbers of circulating EMP [11]. Fast-food meals are often rich in saturated fat (SAFA) and lipid oxidation products derived from heat damage to fats during deep-frying. Both of these components of meals have been linked with postprandial endothelial dysfunction [12,13] and atherosclerotic disease. Furthermore, diets rich in SAFA [14] or oxidized fatty acids [15] accelerate the formation of atherosclerotic lesions in animals. Many fast-food restaurants have switched to polyunsaturated fats (PUFA) such as sunflower oil for deep-frying to avoid the atherogenic impact of dietary SAFA. However, PUFA-rich fats are readily oxidized at high temperature in air, leading to a marked increase in their content of lipid oxidation products. Few studies have tested the effect of the type and oxidative state of fat in fatty meals on postprandial numbers of circulating EMP. The aim of the present study was to determine the acute effect of meals rich in SAFA, thermally oxidized PUFA, and unheated n-6 PUFA on numbers of circulating EMP in healthy subjects.

2. Subjects and methods

2.1. Subjects

We recruited 25 apparently healthy subjects aged 20 to 70 years, predominantly from the staff of Dunedin Hospital and the University of Otago. Subjects were excluded if they smoked cigarettes, had a serious illness, or were taking medications or antioxidant supplements. Participants gave informed consent in writing. The project was approved by the Lower South Regional Ethics Committee.

2.2. Study design

The study had a randomized crossover design. Participants were randomized to 1 of 6 sequences of the 3 test meals. Randomization was performed by mutually orthogonal Latin squares procedure and was blinded to all investigators except SdJ who prepared the meals but did not measure EMP numbers or analyze the data. Participants fasted overnight then reported to the study center in the early morning. A blood sample was taken from participants who then immediately consumed a test meal within 10 to 15 minutes. Blood samples were then taken 1 and 3 hours later. Subjects were instructed not to consume food or beverages other than water during 3 hours after the meals. They were also instructed to maintain their usual lifestyle during the study.

Age, ethnicity, body weight, and height were measured at baseline (first visit). Plasma EMP numbers and plasma concentrations of peroxides, glucose, blood lipids, high-density lipoprotein cholesterol (HDL-C), conjugated dienes in the Svedberg unit of flotation (S_f) greater than 400 (S_f >400) fraction, and nonesterified fatty acids (NEFA) were measured at each time point.

2.3. Test meals

The n-6 PUFA–rich meals contained heated sunflower oil (HSO) or unheated sunflower oil (USO) (0.6 g/kg body weight) and 40 g dried instant mash potato reconstituted with 160 mL hot water. Sunflower oil (Sunfield; Tasti Products, Auckland, New Zealand) was heated at 180°C for 4 hours, and potato chips from 2 potatoes were cooked every 30 minutes. The SAFA meal contained cream (CR) (1.6 g/kg body weight) and 40 g instant mash potato reconstituted with 100 mL hot water. The composition of the sunflower oil meals for a 70-kg individual was as follows: energy, 2134 kJ; fat, 41 g; SAFA, 5 g; PUFA, 26 g; monounsaturated fat, 9 g; carbohydrate, 33 g; and protein, 3.3 g. The corresponding composition of the CR meal was as follows: energy, 2208 kJ; fat, 41 g; SAFA, 26 g; PUFA, 2 g; monounsaturated fat, 12 g; carbohydrate, 36 g; and protein, 5.7 g. The content of conjugated dienes, carbonyls, and vitamin E in HSO, USO, and CR are shown in Table 1. Heated sunflower oil contained 2- to 3-fold higher levels of lipid oxidation products and 18% lower vitamin E content compared with USO.

2.4. Endothelial microparticles

Numbers of vascular endothelial cadherin (CD144)–positive EMP (CD144-EMP) in platelet-poor plasma were determined by flow cytometric analysis. Platelet-rich plasma was prepared by centrifuging citrate whole blood for 6 minutes at 160*g*. The platelet-rich plasma was cooled on ice and then centrifuged for 1 minute at 6000*g* to give platelet-poor plasma for the determination of EMP numbers. Platelet-poor plasma was prepared immediately after venipuncture and was kept at 4°C until numbers of EMP were determined on the same day. Platelet-poor plasma (50 μL) was added to 12 × 75-mm polystyrene tubes; 8 μL of anti-human CD144-PE (VE-cadherin) (R&D Systems, Minneapolis, MN) and 4 μL of anti-human CD42b-FITC, platelet membrane glycoprotein (GPIb) (BD Biosciences, Auckland, New Zealand) fluorescent antibodies were added; and the tubes were gently mixed by hand and then were incubated for 45 minutes in an ice-water bath. Tubes containing platelet-poor plasma alone and with CD144-PE or CD42b-FITC were also incubated. Next, phosphate-buffered saline (1 mL) was added to all tubes; and the resulting solutions were subjected to flow cytometry on a FACSCalibur Flow Cytometer (Franklin Lakes, NJ). *CD144-EMP* were defined as particles less than 1.5 μm in size relative to standard beads (1 and 2 μm from Molecular Probes, Eugene, OR), and CD144 positive and

Table 1
Lipid oxidation products and vitamin E in HSO, USO, and CR

	HSO mean ± SD, n = 3	USO	CR
Conjugated dienes (mmol/kg)	33.4 ± 1.4	17.7	13.5
Carbonyls (mmol/kg)	14.5 ± 1.2	4.3	ND
Vitamin E (mmol/kg)	1.33 ± 0.36	1.62	ND

ND indicates not determined.

CD42b negative. Absolute numbers of CD144-EMP were calculated using standard beads (BD Trucount; BD Biosciences) as described previously [3].

2.5. Laboratory measurements

Venous blood was collected in tubes containing dipotassium EDTA and citrate. Plasma was harvested after centrifugation of the EDTA tube at 1500*g* and 4°C, and aliquots of plasma were immediately stored at −80°C until analyzed. For most variables, all samples from an individual were measured in the same analytical run to minimize interindividual variation. Plasma HDLs were isolated in the supernatant precipitation of apolipoprotein B–containing lipoproteins with dextran sulfate and magnesium chloride [16]. Cholesterol and triglycerides (TG) in plasma and plasma fractions were measured enzymatically using an autoanalyzer (Cobas Mira) and commercial kits and calibrators (Roche, Boehringer-Mannheim, Mannheim, Germany). Fasting plasma glucose was measured using a routine automated method by the Otago Laboratory Services in Dunedin Hospital. A plasma S_f >400 fraction was isolated by overlaying EDTA plasma (2 mL) with saline (d = 1.006) in 6.5-mL ultracentrifuge tubes and centrifuging the tubes in a Beckman (Palo Alto, CA) 50.3 Ti rotor at 22 000 rpm for 30 minutes at 4°C. The S_f >400 fraction was isolated by tube slicing near the top of the tube. Lipids in the S_f >400 fraction were extracted by the Dole [17] procedure using hexane/isopropanol/sulfuric acid. Solvent was evaporated from the combined hexane fractions containing chylomicron lipids, and the residue was redissolved in cyclohexane. Absorbance of the cyclohexane solution at 234 nm was determined, and conjugated diene content was calculated using molar absorption coefficient of 29 500 L mol^{-1} cm^{-1}. Triglyceride concentration in the chylomicron fraction (0.1 mL) was determined by a manual enzymatic method using a commercial kit (Roche, Boehringer-Mannheim) and a standard glycerol solution (Boehringer-Mannheim, Mannheim, Germany). Plasma peroxides were measured by a method using horseradish peroxidase [18]. Plasma NEFA concentration was measured enzymatically using a commercial kit (Roche Diagnostics, Auckland, New Zealand). Plasma vitamin E concentration was measured as described previously [19].

Conjugated diene content of HSO and USO was determined from the absorbance of hexane solutions of the fats at 234 nm and using a molar absorption coefficient of 29 500 L mol^{-1} cm^{-1}. Cream (1 g) was extracted with hexane/isopropanol (2/1 vol/vol), and conjugated dienes were measured in the supernatant after dilution (1/100) with hexane [20]. Aldehyde content of USO and HSO was measured by the quinoidal ion method [21]. Vitamin E was estimated in HSO and USO as described previously [22].

2.6. Statistics

Data are reported as geometric mean (SD range) unless stated otherwise. Data were log-transformed and then analyzed by repeated-measures analysis of variance (ANOVA) with type of meal and time after meal as within-subject factors and order of meals as a between subject factor. Greenhouse-Giesser–corrected tests of significance were used. Because there was no significant effect of order of the meals in the data, simplified models without order terms are presented. Within-subject contrasts for time after the meals and meal × time interactions were also calculated. When a significant overall effect of time or meal × time was detected, repeated-measures ANOVA with within-subject contrasts were used to test for change with time after each meal separately. First-visit fasting glucose was also included as a covariate in ANOVA models. The 3-hour changes in CD144-EMP numbers after the meals were compared using repeated-measures ANOVA with meals as a within-subjects factor and with first-visit values of fasting glucose as a covariate. Pearson product-moment correlation analysis was used to test for relationships between variables. Two-tailed tests of significance were used, and a P value < .05 was considered to be statistically significant. The SPSS computer software (version 11; SPSS, Chicago, IL) was used to analyze the data.

3. Results

One subject dropped out from the study after the first meal because he could not tolerate the relatively high fat content of the meal. Difficulties with venipuncture resulted in incomplete data for 2 subjects. Complete data were obtained for 22 subjects. The characteristics of these subjects at baseline (first visit) are shown in Table 2. Three subjects were obese (body mass index [BMI] >30 kg/m^2), 2 subjects had impaired fasting glucose (≥6.1 mmol/L), 4 subjects had hypertriglyceridemia (>2.00 mmol/L), 3 subjects had hypercholesterolemia (>6.5 mmol/L), and 3 subjects had low plasma HDL-C (<1.00 mmol/L).

Table 3 shows the response of plasma lipids and lipoproteins to the fatty meals. Plasma TG concentration increased significantly after the CR meal, and this increase was significantly (P < .001) larger compared with the corresponding response to the other meals. The concentration of TG in the S_f >400 plasma fraction increased significantly after all the meals, with a significantly larger

Table 2
Characteristics of the participants at baseline first visit

Age (y)	40 ± 13
Sex (men/women)	13/9
Body weight (kg)	75.4 ± 12.7
BMI (kg/m^2)	25.3 ± 3.7
TC (mmol/L)	5.19 ± 1.18
TG (mmol/L)	1.16 (0.88-1.69)
HDL-C (mmol/L)	1.33 ± 0.38
Glucose (mmol/L)	5.3 ± 0.7

Values are mean ± SD or median (interquartile range). TC indicates total cholesterol.

Table 3
Effect of fatty meals on plasma lipids, lipoprotein lipids, and NEFA concentrations

	Baseline	1 h	3 h	P_{time}	P_{meal}	$P_{meal \times time}$
TG (mmol/L)						
USO	1.29 (0.76-2.18)	1.35 (0.83-2.19)	1.38 (0.85-2.23)	<.001	.03‡	<.001‡
HSO	1.23 (0.79-1.93)	1.29 (0.82-2.05)	1.33 (0.84-2.10)			
CR	1.26 (0.77-2.05)	1.54 (0.97-2.45)	1.76 (1.12-2.77)†			
S_f >400-TG (mmol/L)						
USO	0.06 (0.02-0.18)	0.14 (0.06-0.33)	0.17 (0.07-0.41)†	<.001	.02§	.03‡
HSO	0.04 (0.02-0.11)	0.11 (0.05-0.28)	0.13 (0.06-0.29)†			
CR	0.05 (0.02-0.15)	0.15 (0.06-0.39)	0.25 (0.11-0.61)†			
TC (mmol/L)						
USO	5.07 (4.07-6.32)	5.11 (4.11-6.37)	5.17 (4.17-6.42)*	<.001	.73	.07
HSO	5.10 (4.17-6.25)	5.19 (4.26-6.32)	5.25 (4.27-6.46)†			
CR	5.07 (4.08-6.31)	5.24 (4.22-6.51)	5.18 (4.18-6.42)*			
HDL-C (mmol/L)						
USO	1.30 (1.00-1.69)	1.32 (1.02-1.70)	1.35 (1.03-1.76)†	<.001	.44	.06
HSO	1.31 (1.02-1.68)	1.34 (1.02,1.75)	1.32 (1.04-1.69)*			
CR	1.28 (1.00-1.64)	1.32 (1.04-1.67)	1.30 (1.01-1.66)†			
NEFA (μmol/L)						
USO	324 (200-526)	137 (87-215)	350 (221-557)†	<.001	.48	.02‡
HSO	315 (194-509)	144 (70-297)	386 (246-607)†			
CR	258 (169-394)	159 (96-265)	310 (218-441)†			

Values are geometric mean (SD range); n = 22. S_f >400-TG indicates large VLDL plus chylomicron TG.
Significance of change with time after the meal: *$P < .05$; †$P < .01$.
Significance of contrasts: ‡$P < .05$ CR vs USO and HSO; §$P < .05$ CR vs HSO.

increase after the CR meal. This plasma fraction contains large very low-density lipoprotein (VLDL) in the fasting state and chylomicrons plus large VLDL in postprandial plasma. Plasma cholesterol and HDL-C concentrations increased significantly after all the meals. Plasma NEFA concentrations decreased significantly 1 hour after all the meals and increased significantly ($P < .05$) from baseline 3 hours after the USO and CR meals. The response of plasma NEFA to the CR meal was significantly ($P = .02$) different from the corresponding response to ingestion of USO.

Table 4 summarizes the effect of the meals on plasma concentrations of S_f >400 conjugated dienes, peroxides, and vitamin E. Plasma S_f >400 conjugated diene concentrations increased significantly after all the meals. The increase after the CR meals was significantly larger than the corresponding increases after the other meals. The postprandial content of conjugated dienes in the S_f >400 plasma fraction at 1 hour (USO, 64 [33, 125] μmol/mmol TG; HSO, 71 [40,125] μmol/mmol TG; CR, 63 [33, 123] μmol/mmol TG; $P = .57$) and 3 hours (USO, 54 [26, 113] μmol/mmol TG; HSO, 64 [32, 128] μmol/mmol TG; CR, 54 [35, 82] μmol/mmol TG; $P = .40$) was not significantly different among the meals (repeated-measures ANOVA). Plasma peroxides concentrations did not change significantly after the meals. The concentration of plasma vitamin E increased significantly after the USO and HSO meals, and these responses were significantly different compared with the corresponding response to the CR meal.

Table 4
Effect of fatty meals on plasma concentrations of S_f >400 conjugated dienes, peroxides, and vitamin E

	Baseline	1 h	3 h	P_{time}	P_{meal}	$P_{meal \times time}$
S_f >400-CD (μmol/L)						
USO	7.1 (4.6-11.0)	8.5 (5.5-13.3)	8.8 (6.8-11.3)*	<.001	.006†	<.001‡
HSO	5.9 (4.2-8.2)	7.5 (5.0-11.1)	7.8 (4.2-8.2)*			
CR	6.4 (3.9-10.6)	9.0 (5.2-15.5)	12.9 (7.7-21.6)*			
Peroxides (μmol/L)						
USO	277 (120-642)	273 (115-653)	279 (119-649)	.60	.88	.82
HSO	283 (128-625)	280 (125-630)	281 (125-625)			
CR	282 (121-660)	278 (117-663)	279 (118-658)			
Vitamin E (μmol/L)						
USO	36.3 (27.5-48.0)	36.4 (27.7-47.8)	38.3 (29.5-49.7)*	.001	.97	.006‡
HSO	36.2 (28.0-46.8)	37.1 (28.7-47.9)	37.9 (29.3-49.2)*			
CR	37.0 (28.2-48.5)	37.8 (28.6-47.9)	37.7 (28.8-49.4)			

Values are geometric mean (SD range); n = 22.
Significance of change with time after the meal: *$P < .01$.
Significance of contrasts: †$P < .01$ HSO vs USO and CR; ‡$P < .05$ CR vs USO and HSO.

Table 5
Effect of fatty meals on plasma numbers of EMP

	Baseline	1 h	3 h	P_{time}	P_{meal}	$P_{meal \times time}$
No. of CD144-EMP ($\times 10^5$/mL)						
USO	4.54 (2.46-8.39)	4.10 (2.40-7.01)	5.52 (2.73-11.19)*	.03	.29	.12
HSO	3.93 (2.48-6.24)	4.06 (2.27-7.26)	4.70 (2.49-8.87)*			
CR	4.73 (2.48-9.03)	5.13 (2.39-11.02)	5.07 (2.81-9.15)			

Values are geometric mean (SD range); n = 22.
Significance of change with time after the meal: *$P < .05$.

Table 5 shows geometric mean values of numbers of CD144-EMP in plasma immediately before and after the fatty meals. The number of CD144-EMP increased significantly 3 hours after the meals ($P = .03$) mainly because of significant increases after the USO and HSO meals. The number of CD144-EMP did not vary significantly after the CR meal ($P = .55$). The changes in CD144-EMP with time after the meals were not significantly ($P = .12$) different among the meals. The first-visit baseline data showed that some of the participants were obese (BMI >30 kg/m^2) and had abnormally high values of fasting plasma glucose, cholesterol, and TG and abnormally low values of HDL-C that might potentially influence the response of CD144-EMP to the meals. When these first-visit variables were included as covariates, there was a significant meal × time interaction ($P = .002$) and a significant meal × time × glucose interaction ($P = .004$). There was a significant meal × time interaction ($P = .002$) and a significant meal × time contrast ($P = .02$) between USO and CR meals when first-visit fasting plasma glucose was the sole covariate. Whereas baseline EMP numbers tended to be lower before the HSO meal, mean numbers of CD144-EMP immediately before the meals were not significantly different ($P = .29$, repeated-measures ANOVA). To test for a possible effect of regression to the mean on the response of EMP numbers to the meals, individual SD values were calculated for the baseline EMP numbers before the 3 meals and were used as a covariate along with baseline glucose in the ANOVA model. Regression to the mean is most likely to occur in individuals with appreciable differences in baseline values. The SD was used as an index of the variation in baseline values before the 3 meals. When individual SD values of the 3 baseline EMP numbers before the meals were included with baseline glucose as a covariate in the ANOVA model of the response of EMP numbers to the meals, a significant meal × time interaction ($P = .001$) remained, suggesting that variation in baseline values before the meals was not mainly responsible for the difference in the response of EMP numbers to the meals. Sex did not significantly ($P = .69$) affect the response of CD144-EMP to the meals.

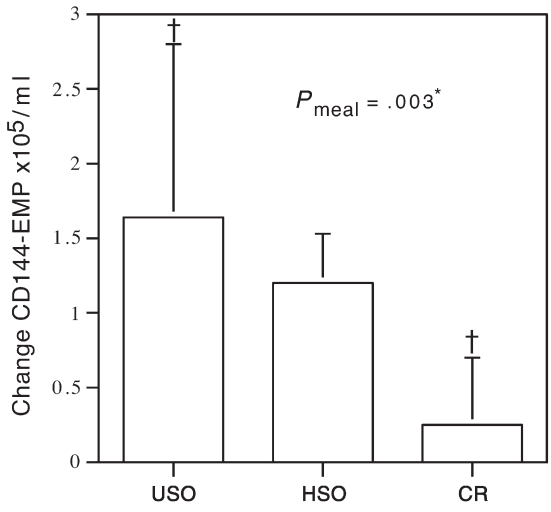

Fig. 1. Change in CD144-EMP numbers between baseline and 3 hours after the meals. Values are mean ± SE. *Repeated-measures ANOVA with first-visit fasting plasma glucose concentration as a covariate. †$P = .004$ within-subjects contrast.

Fig. 1 shows the change in numbers of CD144-EMP from baseline to 3 hours after the meals. These changes in CD144-EMP numbers were significantly different among the meals, with the increase after the USO meal significantly different from the change after the CR meal in repeated-measures ANOVA with first-visit fasting plasma glucose concentration as a covariate.

The increase in CD144-EMP numbers was correlated at a marginal level of significance with the concomitant increase in plasma NEFA concentration 3 hours after the HSO meal ($r = 0.416$, n = 22, $P = .05$). The corresponding correlation after the USO meal did not achieve statistical significance ($r = 0.236$, $P = .29$).

4. Discussion

Our data show that circulating numbers of CD144-EMP increased by approximately 20% at 3 hours after meals rich in PUFA-rich vegetable oil irrespective of whether the oil had been thermally oxidized, and did not increase appreciably after a meal rich in SAFA in healthy individuals. These findings suggest that consumption of meals rich in n-6 PUFA but not meals rich in SAFA has an acute effect on the vascular endothelium, leading to shedding of increased numbers of CD144-EMP.

A previous study has reported substantial increases in circulating numbers of EMP identified as CD31-positive/CD42-negative microparticles at 1 and 3 hours in healthy subjects after consumption of a fatty fast-food meal rich in

SAFA [11]. In the present study, however, circulating numbers of CD144-positive/CD42-negative EMP did not change appreciably in healthy subjects at 1 and 3 hours after a meal rich in SAFA in the form of cream. The factors responsible for these divergent findings are unclear. It is possible that meals rich in SAFA stimulate the release of CD31-positive/CD42-negative EMP but not CD144-positive/CD42-negative EMP. Endothelial cells release heterogenous EMP in response to different stimuli [2,23]. Furthermore, it cannot be excluded that part of the reported increase in CD31-positive/CD42-negative microparticles after a SAFA meal [11] may be due to release of CD31-positive/CD42-negative microparticles from platelets. Microparticles positive for CD31 and negative for CD42 have been produced from platelets in vitro [8]. By contrast, CD144 is a specific marker of endothelial cells and EMP [8].

The increase in CD144-EMP numbers after ingestion of USO and HSO appears to be in keeping with reported evidence that linoleic acid may increase and/or potentiate endothelial activation and injury in vitro [24,25]. Both USO and HSO are rich in linoleate, and high levels of NEFA including linoleic acid may be generated at the luminal surface of the vascular endothelium during lipolysis of linoleate-enriched chylomicrons formed after ingestion of these fats. Linoleic acid but not SAFA increased transendothelial albumin transport and intracellular cellular Ca^{2+} concentration in cultured endothelial cells, indicating cell injury [25]. An increase in intracellular Ca^{2+} can change cell membrane lipid asymmetry and protein organization, leading to the formation of membrane blebs and finally microparticles [2]. However, other in vitro studies have reported that the SAFA (stearic acid) [26] and a mixture of SAFA and monounsaturated fatty acids (palmitate/oleate) [27] also induce apoptosis in vascular endothelial cells. In the present study, the association of the 3-hour increase in CD144-EMP with the corresponding increase in plasma NEFA after the HSO meal tends to support an acute effect of polyunsaturated NEFA on the vascular endothelium. The fatty acid composition of plasma NEFA changes during the postprandial period to reflect the fatty acid composition of dietary fat [28]. An early decrease in plasma NEFA after meals is mainly due to the antilipolytic activity of elevated insulin levels in response to postprandial hyperglycemia. Elevated levels of plasma NEFA later in the postprandial period after ingestion of a fatty meal is partly or largely due to a portion of the NEFA that is generated from the intravascular lipolysis of chylomicrons and has escaped uptake into adipose tissue [29].

The postprandial response of CD144-EMP numbers did not appear to be affected by mild thermal oxidation of the ingested sunflower oil. The increase in CD144-EMP was not appreciably different after USO and HSO meals. It is possible that HSO was not sufficiently oxidized and the increased levels of lipid oxidation products in HSO did not reach the circulation in sufficient quantities to affect the endothelium. Despite the nearly 2-fold higher conjugated diene content in HSO compared with USO, the conjugated diene content of the S_f >400 plasma fraction was not appreciably higher after ingestion of HSO compared with USO. The factors responsible for this finding are unclear. It is possible that the continued presence of substantial levels of vitamin E may have attenuated the effect of lipid oxidation products in HSO on chylomicron content of conjugated diene lipid oxidation products after the HSO meal. A previous study has reported increased chylomicron content of conjugated diene lipid oxidation products in healthy subjects 2.5 hours after ingestion of vitamin E–depleted corn oil that had been thermally oxidized and was relatively high in conjugated diene lipid oxidation products [30]. The conjugated diene content was markedly higher in this oxidized corn oil compared with HSO in the present study. The level of conjugated diene lipid oxidation products in HSO was comparable with reported levels in plant oils that have been used for deep-frying in New Zealand commercial fast-food restaurants (mean, 25 [range. 12-41] mmol/kg; n = 9) [31]. Thus, our data suggest that ingestion of plant oils after use in commercial deep-frying may not necessarily enrich chylomicrons with conjugated diene lipid oxidation products in healthy subjects. The larger increase in plasma S_f >400 conjugated diene concentrations after the CR meal appeared to be due mainly to the corresponding larger increase in S_f >400 TG concentrations and not to enrichment of chylomicrons with lipid oxidation products. The postprandial S_f>400 conjugated diene content expressed in micromole per millimole TG was not appreciably different among the meals. Cream and dairy fat in general are important sources of conjugated linoleic acid that contains a conjugated diene structure but is not a lipid oxidation product.

The present increase in circulating CD144-EMP numbers in healthy subjects after USO and HSO meals may reflect endothelial activation, injury, and dysfunction in vivo. Inverse correlations between plasma numbers of CD144-EMP and brachial artery flow-mediated vasodilation and left main coronary artery response to acetylcholine have been reported previously in patients with end-stage renal failure [4] and in a combined group of patients with type 2 diabetes mellitus and healthy subjects [8]. On the other hand, there is evidence that ingestion of linoleate-rich PUFA vegetable oil does not acutely impair endothelium-dependent vasodilation in healthy subjects [12]. Thus, it is possible that the changes to the endothelium leading to an acute increase in EMP after linoleate-rich meals may not produce endothelial dysfunction in healthy subjects in vivo. Numbers of circulating CD144-EMP did not appear to correlate with endothelial function of the left main coronary artery in healthy subjects [8]. Furthermore, EMP from healthy subjects did not impair endothelial function in vitro as indicated by aortic ring dilation. By contrast, EMP from patients with end-stage renal failure [4], who have chronically and pathologically activated and injured endothelium, impaired endothelial function in vitro [4]. It has been suggested that formation of cell microparticles in response to apoptotic stimuli may be an

attempt to reverse the apoptotic process and may allow cells to escape phagocytosis by removing phagocytic signals such as phosphatidylserine [2]. Thus, an acute increase in circulating EMP after n-6 PUFA–rich meals in healthy subjects might indicate enhanced "repair" of proapoptotic cells by a linoleate-induced activation of cellular pathways that may be involved in microparticle formation [2,24,25]. Ingestion of SAFA would not be expected to enhance this repair process because CD144-EMP numbers did not increase after the CR meal in the present study. A previous study has reported that consumption of SAFA acutely impairs endothelial function in vivo by attenuating the anti-inflammatory potential of HDL in healthy subjects [12].

The number of subjects we studied was relatively small. Thus, care must be exercised in the extrapolation of these findings to other populations. The postprandial increase in plasma TG concentrations was relatively small and may have limited the effect of the USO and HSO meals on circulating CD144-EMP. On the other hand, plasma levels of TG in the S_f >400 plasma fraction increased markedly, indicating appreciable absorption of dietary fat from the gut. The magnitude of the postprandial increase in plasma TG per se did not appear to influence CD144-EMP numbers. Plasma TG increased by 40%, whereas numbers of CD144-EMP did not change appreciably, 3 hours after the CR meal.

Our data suggest that ingestion of meals rich in n-6 PUFA vegetable oils irrespective of mild thermal oxidation may induce the vascular endothelium to shed microparticles, leading to an increase in numbers of circulating CD144-EMP in healthy subjects. The physiologic and metabolic changes to the vascular endothelium responsible for this increase in EMP numbers and whether it is benign or not remain to be determined.

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