INTRODUCTION

Fish-oil consumption has been consistently shown to decrease the risk of CVD in a variety of populations (1). Strong evidence from supplementation studies shows that endothelial dysfunction, an early risk marker for CVD, can be improved by fish oils, both in healthy subjects (2) and in those at increased risk of CVD (3–8). The mechanisms responsible for the effect of fish oils on endothelial function include modulation of inflammation or circulating lipids, release and stability of NO at the endothelium (12), as well as possible direct actions on endothelial smooth muscle (9).

ABSTRACT

Background: Experimental elevation of nonesterified fatty acids (NEFAs) impairs endothelial function, but the effect of NEFA composition is unknown.

Objective: The objective was to test the effect of acute elevation of NEFAs enriched with either saturated fatty acids (SFAs) or SFAs with long-chain (LC) n-3 (omega-3) PUFAs on vascular function measured via flow-mediated dilatation (FMD), laser Doppler iontophoresis (LDI), and digital volume pulse (DVP).

Design: In 59 subjects (30 men and 29 women), repeated oral fat feeding of either palm stearin (SFA) or palm stearin with DHA-rich fish oil (SFA + LC n-3 PUFA) was performed on 2 separate occasions with continuous hepatic infusion to elevate NEFAs for a duration of 60 to 240 min. Vascular function was measured at baseline and at the end of NEFA elevation; venous blood was collected for measurement of lipids and circulating markers of endothelial function.

Results: NEFA elevation during consumption of the SFA-rich drinks was associated with a marked impairment of FMD, whereas consumption of SFAs + LC n-3 PUFA improved FMD response, with a mean (±SEM) difference of 2.06 ± 0.29% (P < 0.001). Positive correlations were found with percentage weight of LC n-3 PUFAs in circulating NEFAs and change in FMD response [Spearman’s rho (r) = 0.460, P < 0.001]. LDI measures increased during both treatments (P ≤ 0.026), and there was no change in DVP indexes.

Conclusions: The composition of NEFAs can acutely affect FMD. The beneficial effect of LC n-3 PUFAs on postprandial vascular function warrants further investigation but may be mediated by nitric oxide–independent mechanisms. This trial is registered at clinicaltrials.gov as NCT01351324.

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after application of acetylcholine and SNP. These agonists can distinguish differences in response due to altered NO production (acetylcholine; endothelial dependent) as opposed to altered NO action on the smooth muscle layer (SNP; endothelial independent). This may be important in delineating the site of action of fatty acids, because one postprandial study that used fish oils showed vascular function to be improved via endothelial independent mechanisms (9). In addition, DVP was used to assess arterial stiffness of large and small vessels.

SUBJECTS AND METHODS

Subjects

Thirty men and 29 women [mean ± SD age: 27.7 ± 2.6 y; BMI (in kg/m²) 23.2 ± 3.3] were recruited from the student and local community; all were healthy nonsmokers who were not taking excessive fish-oil supplements (>1 g EPA and DHA/d) or any medication known to influence blood clotting, lipids, or blood pressure. The subjects were screened for fasting cholesterol (4.56 ± 0.80 mmol/L), triglyceride (1.06 ± 0.33 mmol/L), and glucose (5.29 ± 0.69 mmol/L), all of which were within the normal range.

Study design

The design was a single-blind crossover in which subjects attended the Hugh Sinclair Unit of Human Nutrition (University of Reading, United Kingdom) on 2 occasions separated by 4 wk for women (to help control for potential effects of the menstrual cycle on endothelial function) or ≥1 wk for men. Subjects were randomly assigned to one of the oral fat loads on each day. For 24 h before each treatment, subjects were asked to refrain from strenuous exercise and alcohol and were supplied with a low-fat (4%) ready-meal to be consumed in the evening. The protocol for acutely elevating NEFAs of specific fatty acid types was based on Beysen et al (22), and we have shown this to achieve the fatty acid elevation required to increase arterial stiffness (23). For each treatment, subjects arrived after an overnight fast and rested for 30 min in the supine position in a quiet, air-conditioned room (22–24°C). After baseline vascular function measurements, a cannula was inserted at the wrist of the operating arm for venous blood collection; a small amount of lignocaine (1%) was used as a local anesthetic. A bolus fat load was consumed at 0 min, followed by smaller volumes every 30 min for a further 240 min. At 60 min, a second cannula was inserted into the antecubital vein in the sampling arm for the infusion of heparin. A bolus of heparin (500 IU) was administered, followed by a continuous infusion of heparin (0.4 IU · kg body weight⁻¹ · min⁻¹) for the remainder of the treatment. Vascular function measurements were repeated at 240 min.

The procedures followed in the current study were in accordance with the ethical standards of the University of Reading Ethics Committee. Written informed consent was obtained from all subjects.

Test drinks

Oral fat loads were prepared (Table 1) by using palm stearin (Aarhuskarlshamn Ltd) with or without DHA-rich fish oil (77% DHA, 9% EPA; Croda Healthcare), 20 g skimmed milk powder (Premier International Foods Ltd), 10 g chocolate powder (The Spanish Chocolate Co Ltd), and 0.3 g monoglyceride emulsifier (Danisco). Water was added to achieve a final weight of 242 g, and the mixture was blended for several minutes to ensure emulsification. The test drinks were identical in protein (7.7 g) and carbohydrate (18.7 g) content. The drinks were divided into a bolus drink of 66 g given at time 0, and 8 smaller volumes of 22 g given at 30 min intervals for a period of 240 min.

Vascular measurements

Vascular function was assessed at baseline (fasting) and at the end of the 240 min treatment by FMD, LDI, and DVP. Circulating markers of endothelial function—i.e., s-CAM, ET-1, and NOx—were also measured at these time points.

FMD

FMD of the brachial artery was measured by trained researchers using an ATL Ultrasound HDI5000 broadband ultrasound system (ATL Ultrasound) and a procedure based on standard guidelines (24). The procedure used an ECG-gated trigger and image-grabbing software (Medical Imaging Applications-llc) to collect images at 0.25 frames/s. Doppler-derived velocity was measured for 1 min before commencing each FMD measurement. Baseline images were taken for 1 min, after which the blood pressure cuff was inflated to 220 mm Hg to occlude blood flow. After 5 min of occlusion, the pressure was rapidly released, allowing reactive hyperemia to occur; measurement collection continued for 5 min after release. Analysis of the images was performed by using wall-tracking software (MIA-llc). Image files were analyzed by a single researcher who was blinded to the measurement details, and peak diameter was defined as the largest diameter obtained after the occlusion was released. FMD response was calculated by using change from baseline to peak diameter divided by baseline and reported as a percentage value. Velocity analysis was performed over a minimum of 5 cardiac cycles and averaged, then converted to flow by multiplying by the cross-sectional area of the artery.

### Table 1

<table>
<thead>
<tr>
<th>Formulation of the test drinks¹</th>
<th>SFA</th>
<th>SFA + LC n–3 PUFA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Palm stearin (g/kg bw)²</td>
<td>0.52</td>
<td>0.45</td>
</tr>
<tr>
<td>Fish-oil concentrate (g/kg bw)²</td>
<td>—</td>
<td>0.07</td>
</tr>
<tr>
<td>Composition of oils (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Palmitic acid, 16:0</td>
<td>59</td>
<td>51</td>
</tr>
<tr>
<td>Stearic acid, 18:0</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>Oleic acid, 18:1n–9</td>
<td>28</td>
<td>24</td>
</tr>
<tr>
<td>Linoleic acid, 18:2n–6</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>Arachidonic acid, 20:4n–6</td>
<td>—</td>
<td>0.3</td>
</tr>
<tr>
<td>EPA, 20:5n–3</td>
<td>—</td>
<td>1.2</td>
</tr>
<tr>
<td>DPA, 22:5n–3</td>
<td>—</td>
<td>0.4</td>
</tr>
<tr>
<td>DPA, 22:5n–6</td>
<td>—</td>
<td>0.7</td>
</tr>
<tr>
<td>DHA, 22:6n–3</td>
<td>—</td>
<td>10.4</td>
</tr>
</tbody>
</table>

¹ bw, body weight; DPA, docosapentaenoic acid; LC, long-chain; SFA, saturated fatty acid.

² A 70-kg individual would receive 36.4 g palm stearin or 31.5 g palm stearin + 4.9 g fish-oil concentrate (containing 3.8 g DHA and 0.4 g EPA, equivalent to 1.5 times a standard 140-g portion of oily fish (based on 2 g EPA + DHA/100 g fish).
LDI

LDI is a validated technique that measures the vasodilatory response of the peripheral microvasculature of the forearm to vasodilator agents delivered across the skin by an electrical field (iontophoresis). Changes in blood flow are measured by using a laser Doppler imager. The vasodilatory response to acetylcholine and SNP has been shown to be lower in patients with peripheral arterial occlusive disease than in age- and sex-matched healthy controls (25). LDI was performed as previously described (26). A Moor LDl2-VR laser Doppler imager and a MIC2 i ontophoresis controller (Moor Instruments Ltd) were used to transdermally deliver 1% acetylcholine or 1% SNP, which measure endothelial dependent and independent vasodilatation, respectively. Twenty scans were taken over ~15 min, with an incremental increase in current from 0 to 20 μA. The area under the flux versus time curve during the 20 scans was calculated as an indicator of microvascular response due to acetylcholine (LDI-Ach) or SNP (LDI-SNP).

DVP

DVP records the systolic and diastolic waveforms of the pulse by measuring infrared light transmission through the finger. DVP was measured with a PulseTrace PCA 2 (Micro Medical Ltd) and used to calculate stiffness index (DVP-SI) and reflection index (DVP-RI). DVP-SI is related to large arterial stiffness and has been shown to be positively correlated with classical cardiovascular risk factors such as age, waist-to-hip ratio, blood pressure, and carotid intima-media thickness (27). DVP-RI is associated with the vascular tone of small arteries and has been shown to be markedly influenced by vasoactive drugs (28).

Biochemical measures

Venous blood samples were taken every 30 min and collected immediately into K3 EDTA (for NEFAs; ET-1) or serum tubes (triglyceride, sI-CAM, NOx) and centrifuged for 10 min at 1700 × g at 4°C. Samples were stored at −20°C until analysis. NEFAs and triglyceride were quantified by using an automated clinical chemistry analyzer (ILAB 600; Instrumentation Laboratory) with kits supplied by Alpha Laboratories and Instrumentation Laboratory, respectively. Serum sI-CAM and plasma ET-1 were measured by using commercially available colorimetric ELISA kits (R&D Systems Europe Ltd). NOx were measured by using an NO quantification kit (Actif Motif; Rixensart, Belgium).

NEFA composition analysis

Lipids were extracted from 800 μL of plasma collected at baseline (to ensure sufficient yield of plasma lipids to determine the habitual NEFA composition, the 2 baseline plasma samples were pooled before the lipid extraction) and at 240 min by using chloroform: methanol (2:1, by vol) containing butylated hydroxytoluene (50 mg/mL) and applied to a solid-phase extraction cartridge (Varian) to isolate the NEFA fraction. This fraction was saponified and methylated in methanol containing 2% (vol:vol) H2SO4 at 70°C for 1 h. FAMEs were recovered by extraction into hexane and analyzed by using a gas chromatograph as previously described (29). FAMEs were identified by comparison of retention times against a known standard, Supelco 37 component FAME mix (Supelco).

Power calculations and statistical analysis

Power calculations were performed for the primary outcome: change in FMD response. At 95% power and 5% significance, the minimum number of subjects required to allow detection of a difference of 1.5% FMD between the responses to the 2 oral fat loads was calculated to be 45; additional subjects were recruited to allow for possible dropouts due to the intensive nature of the protocol. However, only one subject failed to complete as per the protocol. Power was based on a conservative measure of intra-subject variability (SD: 2.8%); a recent trial at the Hugh Sinclair Unit reported a mean SD of 2.3%, which is within current guidelines (30).

SPSS software (version 17.0; SPSS Inc, Chicago) was used for all statistical analyses. The trapezoid rule was used to calculate the AUC, which was subtracted from the fasting value to derive iAUC. Data were tested for normality; it was necessary to log transform NEFA and triglyceride time-course data and use square-root values for FMD and LDI measures. Fatty acid composition of NEFAs and change from baseline values of DVP-SI and circulating markers of endothelial function could not be transformed; therefore, nonparametric tests were used. Two-tailed paired t tests (or nonparametric equivalent) were used to compare measures of endothelial function at baseline and at 240 min and iAUC values of NEFAs and triglyceride. For NEFAs and triglyceride time-course data, repeated-measures ANOVAs were performed using a mixed-model approach. Correlation analysis was performed by using Spearman’s rank test. Bonferroni correction was applied to control for multiple comparisons and correlations. Values of P ≤ 0.05 were taken as significant.

RESULTS

The oral fat loads were well tolerated by the subjects, and there were no significant differences in any baseline measures between treatments.

FMD

There was no significant difference in velocity, flow, or shear rate between treatments at baseline or at the end of the study period (data not shown). The effect of the 2 oral fat loads on FMD is shown in Table 2. The 2 fat regimes showed markedly different effects on FMD response. Compared with baseline, SFA-rich drinks resulted in a marked impairment of FMD (~0.94 ± 0.24%, P < 0.001), whereas SFA + LC n−3 PUFAs improved the FMD response (+1.1 ± 0.27%, P < 0.001). At 240 min, the FMD response was 2.06 ± 0.29% greater after consumption of SFA + LC n−3 PUFAs than after SFAs (P < 0.001); this difference represents 35% of the mean baseline FMD measure.

LDI and DVP

LDI and DVP measures are shown in Table 2. LDI response to both Ach (endothelial-dependent) and SNP (endothelial independent) increased after consumption of both SFA (acetylcholine P = 0.011, SNP P = 0.026) and SFA + LC n−3 PUFAs.
oral fat loads (both $P < 0.001$). There was no difference in change from baseline measures between treatments (acetylcholine $P = 0.408$, SNP $P = 0.555$).

No changes in DVP-SI or DVP-RI were observed during consumption of either SFAs (DVP-SI $P = 0.378$ and DVP-RI $P = 0.052$) or SFAs + LC n−3 PUFAs (DVP-SI $P = 0.818$ and DVP-RI $P = 0.445$). The change from baseline of either measure did not differ between treatments ($P \geq 0.322$).

**Circulating markers of endothelial function**

The effect of the test drinks on circulating markers of endothelial function is shown in Table 2. Plasma NOx decreased after consumption of both fats ($P < 0.001$), but there was no difference in change from baseline values between treatments ($P = 0.315$). No changes in sICAM or ET-1 were observed during either treatment ($P \geq 0.366$).

**Serum NEFAs and plasma triglyceride**

There was an initial decline in NEFA concentrations after consumption of the bolus test drink at 0 min, followed by a sharp increase at 60 min, which coincided with the initiation of the heparin infusion. The oral fat–heparin protocol resulted in a final 2-fold elevation of serum NEFA after consumption of both fats as compared with baseline values (Figure 1A). Repeated-measures ANOVA showed a significant effect of time ($P < 0.001$) but not fat type ($P = 0.659$); there was no time × fat type interaction ($P = 0.772$). iAUC was calculated between 60 and 240 min to represent the main increase in circulating NEFA levels during the heparin infusion, and a paired $t$ test showed that there was no difference ($P = 0.238$) between the SFAs ($76,594 \pm 7042 \text{ mmol/L} \times 180 \text{ min}$) and SFA + LC n−3 PUFA drinks ($69,196 \pm 5675 \text{ mmol/L} \times 180 \text{ min}$).

Plasma triglyceride remained at baseline concentrations until the start of the heparin infusion at 60 min when there was an initial decrease in concentration (Figure 1B). After this, triglyceride concentrations showed a slight increase throughout the oral fat–heparin protocol, returning to baseline values at 180 min for SFAs and at 240 min for SFA + LC n−3 PUFAs. From 90 to 240 min, there was a tendency for triglyceride concentrations to be lower after consumption of SFA + LC n−3 PUFAs; iAUC calculations during this time period tended to be greater during SFA ($17.8 \pm 3.0 \text{ mmol/L} \times 180 \text{ min}$) than during SFA + LC n−3 PUFA ($10.5 \pm 2.7 \text{ mmol/L} \times 180 \text{ min}$) consumption ($P = 0.063$). Repeated-measures ANOVA showed an effect of time ($P < 0.001$) and fat type ($P = 0.035$); there was no time × fat type interaction ($P = 0.106$).

**Plasma NEFA composition and correlates with vascular function measures**

The percentage weights of SFAs and LC n−3 PUFAs in the NEFA fraction of plasma collected at baseline and at 240 min are shown in Table 3. As expected, there was a significant increase in the proportion of SFAs in NEFAs during both treatments ($P < 0.001$), together with a significant increase in the proportion of LC n−3 PUFAs in the SFA + LC n−3 PUFA treatment. This consisted of a 3-fold increase in EPA and a 5-fold increase in DHA (all $P < 0.001$).

The change in FMD response relative to baseline was positively correlated with the proportion of LC n−3 PUFAs ($r_s = 0.460$, $P < 0.001$), EPA ($r_s = 0.404$, $P < 0.001$), and DHA ($r_s = 0.466$, $P < 0.001$) in NEFA at 240 min. Change from baseline of other measures of vascular function was not correlated with LC n−3 PUFAs ($P \geq 0.240$). There was a weak negative correlation with the percentage of SFA with change in FMD ($r_s = -0.213$, $P = 0.036$), NOx ($r_s = -0.262$, $P = 0.006$), and ET-1 ($r_s = -0.267$, $P = 0.007$). No significant correlations were found between iAUC NEFA$_{60-240}$ min ($r_s = -0.170$, $P = 0.080$) or iAUC triglyceride$_{90-240}$ min ($r_s = 0.004$, $P = 0.967$) with FMD or any other measure of vascular function ($P \geq 0.101$).

### Table 2: Vascular function measures at baseline (0 min) and change from baseline (Δ 240 min)

<table>
<thead>
<tr>
<th>Measure</th>
<th>SFA</th>
<th>SFA + LC n−3 PUFA</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>n 0 min</td>
<td>$5.80 \pm 0.43$</td>
<td>$5.74 \pm 0.42$</td>
<td>$1.11 \pm 0.27$** &lt;0.001</td>
</tr>
<tr>
<td>LDI-Ach AUC (perfusion units)</td>
<td>$2.18 \pm 0.21$</td>
<td>$2.32 \pm 0.25$</td>
<td>$0.84 \pm 0.28$** 0.048</td>
</tr>
<tr>
<td>LDI-SNP AUC (perfusion units)</td>
<td>$2.45 \pm 0.28$</td>
<td>$2.30 \pm 0.24$</td>
<td>$0.78 \pm 0.21$** 0.408</td>
</tr>
<tr>
<td>DVP-SI (m/s)</td>
<td>$6.09 \pm 0.22$</td>
<td>$6.02 \pm 0.16$</td>
<td>$0.11 \pm 0.16$ 0.707</td>
</tr>
<tr>
<td>DVP-RI (%)</td>
<td>$5.86 \pm 0.72$</td>
<td>$6.02 \pm 1.8$</td>
<td>$-3.2 \pm 1.6$ 0.322</td>
</tr>
<tr>
<td>Circulating markers</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NOx (µmol/L)</td>
<td>$23.0 \pm 1.0$</td>
<td>$22.7 \pm 0.7$</td>
<td>$-2.8 \pm 0.8$** 0.315</td>
</tr>
<tr>
<td>ET-1 (ng/mL)</td>
<td>$1.05 \pm 0.06$</td>
<td>$1.03 \pm 0.06$</td>
<td>$-0.04 \pm 0.04$ 0.859</td>
</tr>
<tr>
<td>sICAM (ng/mL)</td>
<td>$188.2 \pm 6.0$</td>
<td>$187.9 \pm 5.0$</td>
<td>$-0.3 \pm 3.0$ 0.647</td>
</tr>
</tbody>
</table>

*Values are presented as means ± SEMs. DVP, digital volume pulse; DVP-RI, DVP–reflection index; DVP-SI, DVP–stiffness index; ET-1, endothelin-1; FMD, flow-mediated dilatation; LDI, laser Doppler imitophoresis; LDI-Ach, LDI response to acetylcholine; LDI-SNP, LDI response to sodium nitroprusside; NOx, total nitrites; SFA, saturated fatty acid; sICAM, soluble intercellular cell adhesion molecule. There was no difference in any measure at baseline between the 2 treatments. Wilcoxon’s tests were used to compare nonnormal data that could not be transformed (DVP-SI and all circulating measures). ***With respect to within-meal changes, there was a significant difference in the actual value at 240 min compared with the corresponding baseline value: *$P < 0.05$, **$P < 0.001$.**
DISCUSSION

The main finding of this study was that acute elevation of NEFAs enriched in SFAs was associated with an impaired FMD response and could be completely reversed by a 3- to 5-fold increase in the enrichment of circulating NEFAs with EPA and DHA. The positive correlation between the LC n-3 PUFAs in NEFAs at 240 min with changes in FMD response supports the notion that NEFA composition is an important factor in relation to the effect of elevated NEFAs on vascular function.

Although our protocol differs from that of a typical postprandial study, the observation of impaired FMD after consumption of SFAs supports the findings of Borucki et al (31), who reported a reduction in FMD with cream consumption in healthy subjects. In contrast, other researchers have found no effect of high-fat, SFA-rich meals on FMD (32–34); however, many of the studies were less well powered than the present investigation. One postprandial study found that impairment of FMD by a high-fat meal could be prevented by a supplement of 0.9 g EPA and DHA (11). These findings support our conclusions that, even under conditions of elevated NEFAs, which are known to normally impair endothelial function (13–17), LC n-3 PUFAs have beneficial effects, supporting a consistent body of evidence from chronic supplementation trials (2–8).

The current study observed slightly lower concentrations of triglyceride during consumption of SFAs + LC n-3 PUFAs than during consumption of SFAs alone. Despite some postprandial studies showing the extent of lipemia to be negatively correlated with FMD in healthy subjects (35–37), triglyceride response as measured by iAUC90–240 was not correlated with change in FMD in the current study, indicating that lipemia was not a major factor in the differing FMD responses between the fats. Our conclusions are supported by Steinberg et al (13), who showed that doubling of triglyceride concentration did not alter leg blood flow in response to the endothelium-dependent vasodilator metacholine.

Mechanisms underlying the association between the NEFA composition and changes in vascular function could include modulation of the production and stability of NO, and attenuation or enhancement of NO action on smooth muscle. FMD has been shown to be primarily mediated by NO bioavailability, because reactive hyperemia caused by brachial artery occlusion induces local release of NO from the endothelium (38). LDI also measures vasodilation mediated by NO, but it measures the vasodilatory capacity of the microvasculature of the forearm rather than large arteries. Our findings indicate that adverse effects of SFAs on the larger peripheral vessels measured by FMD are not evident in the microcirculation as measured by LDI. In fact, consumption of both fats was associated with improved small vessel vasodilatory response, suggesting a generalized increase in peripheral vasodilation after meal consumption as reported by Raitakari et al (33) who showed that a high-fat meal increased forearm blood flow but did not affect FMD.

<table>
<thead>
<tr>
<th>NEFA composition</th>
<th>SFA</th>
<th>n-3 PUFA</th>
<th>EPA</th>
<th>DHA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline (amalgamation of both treatments)</td>
<td>38.7 (36.5–39.9)</td>
<td>1.4 (1.0–1.8)</td>
<td>0.2 (0.1–0.3)</td>
<td>1.2 (0.9–1.5)</td>
</tr>
<tr>
<td>After oral feeding and heparin infusion</td>
<td>45.9 (44.3–49.0)*</td>
<td>1.0 (0.7–1.5)</td>
<td>0.2 (0.1–0.2)</td>
<td>0.8 (0.6–1.3)</td>
</tr>
<tr>
<td>SFA treatment</td>
<td>43.8 (42.0–45.9)*</td>
<td>6.4 (5.5–7.3)*</td>
<td>0.6 (0.5–0.7)*</td>
<td>5.8 (4.8–6.6)*</td>
</tr>
<tr>
<td>SFA + LC n-3 PUFA treatment</td>
<td>38.7 (36.5–39.9)</td>
<td>1.4 (1.0–1.8)</td>
<td>0.2 (0.1–0.3)</td>
<td>1.2 (0.9–1.5)</td>
</tr>
</tbody>
</table>

*Significant difference from baseline by using Wilcoxon’s tests with Bonferroni correction (P < 0.008).
In addition to synthesis, the degradation of NO also affects bioavailability. This can occur through the generation of reactive oxygen species, which react with NO to form peroxynitrite. The observation that the antioxidant vitamin C can prevent lipid-induced endothelial dysfunction suggests that the harmful effect of NEFAs may be mediated by oxidative pathways (15, 16). Reactive oxygen species can be generated in endothelial cells by NADPH oxidase; in endothelial cell culture, NADPH oxidase, has been shown to be downregulated by 4 h incubation with triglyceride-rich lipoproteins isolated from subjects who consumed DHA-rich fish oil (9).

However, whereas our findings for FMD may suggest that LC n–3 PUFAs increase the bioavailability of NO and that, at least in large vessels, SFA decreases NO bioavailability, serum NOx decreased to a similar extent after consumption of both fats. A decrease in serum NOx but a paradoxical increase in FMD during consumption of LC n–3 PUFA may suggest that, contrary to our original hypotheses, the observed improvement in endothelial function occurred through a mechanism independent of NO production. Recent findings from in vitro studies have shown that CYP450 (cytochrome P450 epoxygenases) present in endothelial cells can metabolize LC n–3 PUFAs such as DHA to fatty epoxides, which cause vasodilation through activation of calcium-activated potassium channels present in smooth muscle cells (39). We could speculate that because our measurements were taken during the acute elevation of NEFAs, endothelial generation of fatty acid epoxides may have may have overridden any effect of NEFA-mediated NO production on FMD. Although the action of DHA-derived fatty epoxides is still being elucidated, CYP450 metabolites of arachidonic acid have been shown to be released in response to shear stress (40), which is induced during the measurement of FMD.

The current study found a difference of 2.06% in FMD response at 240 min between the SFA and SFA + LC n–3 PUFA protocols, a difference that represents 35% of the baseline FMD value. Consideration of the possible clinical significance of this effect may be made by comparison of a meta-analysis of clinical trials using L-arginine (an NO donor), which showed a mean value. Consideration of the possible clinical significance of this protocols, a difference that represents 35% of the baseline FMD response at 240 min between the SFA and SFA + LC n

We thank our volunteers for their participation and Agnieszka Przemska, Dafni Vasilopoulou, and Alice Turner for their help during the treatments and sample analysis. We are also grateful to Sue Todd for statistical advice.

The authors’ responsibilities were as follows—CMW: was the principal investigator; AKT, KJN and JW: carried out the studies; and KJN drafted the manuscript and had primary responsibility for final content. All authors contributed to the design of the study and the redrafting of the manuscript and approved the final manuscript. Unlever Discover, one of the supporters of this study, contributed to the study design but was not involved in data collection, analysis, or interpretation of the study findings. The authors had no conflicts of interest.

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