Influence of age and dietary fish oil on plasma soluble adhesion molecule concentrations

Elizabeth A. MILES*, Frank THIES†1, Fiona A. WALLACE*, Jonathan R. POWELL‡, Tina L. HURST‡, Eric A. NEWSHOLME† and Philip C. CALDER*

*Institute of Human Nutrition, University of Southampton, Bassett Crescent East, Southampton SO16 7PX, U.K., †Department of Biochemistry, University of Oxford, South Parks Road, Oxford OX1 3QU, U.K., and ‡Unilever Research Colworth Laboratory, Colworth House, Sharnbrook, Bedford MK44 1LQ, U.K.

**ABSTRACT**

Soluble forms of intercellular adhesion molecule-1, vascular cell adhesion molecule-1 and E-selectin (termed sICAM-1, sVCAM-1 and sE-selectin respectively) are found in the plasma, and are elevated during inflammatory conditions in which there is increased expression of the cellular forms of the molecules on endothelial and other cells. sICAM-1, sVCAM-1 and sE-selectin concentrations were measured in the plasma of 140 healthy Caucasian subjects aged between 18 and 75 years (100 males/40 females). sICAM-1 concentrations varied between 59.9 and 299.7 ng/ml (median 150 ng/ml), sVCAM-1 concentrations varied between 222.8 and 1672.9 ng/ml (median 662 ng/ml) and sE-selectin concentrations varied between 12.4 and 90.3 ng/ml (median 45.5 ng/ml). There were significant positive linear correlations between age and the plasma concentrations of sICAM-1 ($r = 0.580; P < 0.001$) and sVCAM-1 ($r = 0.392; P < 0.001$), which were retained when the effects of gender, body mass index and fasting plasma triacylglycerol and total cholesterol concentrations were controlled for. The significant positive linear correlation between age and the plasma concentration of sE-selectin ($r = 0.234; P = 0.027$) was lost when other variables were controlled for. Male subjects < 40 years of age had significantly lower plasma concentrations of both sICAM-1 and sVCAM-1 than males > 55 years of age (both $P < 0.001$), but the difference in plasma sE-selectin concentrations between the age groups did not reach significance ($P = 0.073$). Subgroups of 16 males aged < 40 years and 12 elderly subjects (> 55 years of age) participated in a double-blind, placebo-controlled study of fish oil supplementation over 12 weeks. The level of eicosapentaenoic acid in plasma phospholipids did not change with placebo supplementation, but was significantly increased with fish oil supplementation in both young male and elderly subjects (median increase 200%). sICAM-1, sVCAM-1 and sE-selectin concentrations were unaffected by supplementation with placebo in either young male or elderly subjects. sICAM-1 concentrations were unaffected by fish oil supplementation. sE-selectin concentrations were significantly increased by fish oil supplementation in young males ($P = 0.043$; median increase 38%), but fish oil tended to decrease plasma sE-selectin concentrations in the elderly subjects ($P = 0.075$), with a median decrease of 11%. sVCAM-1 concentrations were unaffected by fish oil supplementation in young males. Fish oil supplementation significantly decreased plasma sVCAM-1 concentrations in the elderly subjects ($P = 0.043$), with a median decrease of 20% (range 16–60%). These observations suggest that fish oil decreases endothelial activation in elderly subjects.

Key words: adhesion molecules, fish oil, n−3 fatty acids.

Abbreviations: BMI, body mass index; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; FO, fish oil; ICAM-1, intercellular adhesion molecule-1; NFκB, nuclear factor κB; PUFA, polyunsaturated fatty acids; TAG, triacylglycerol; TBARS, thiobarbituric acid-reactive substances; VCAM-1, vascular cell adhesion molecule-1; the prefix s denotes soluble.

1Present address: Institute of Human Nutrition, University of Southampton, Bassett Crescent East, Southampton SO16 7PX, U.K.

Correspondence: Dr Philip Calder (e-mail pcc@soton.ac.uk).
INTRODUCTION

Adhesion molecules are cell surface proteins involved in the cell-to-cell communication which contributes to the movement of leucocytes between body compartments. Such interactions involve adhesion molecules on the vascular endothelial cell surface and their ligand adhesion molecules on the leucocyte surface. These molecules serve to bind circulating leucocytes and to promote their movement into the subendothelial space. While this movement of leucocytes is important in mounting appropriate immune and inflammatory responses and in the homing of leucocytes to lymphoid organs, it also appears to play an important role in the development of atherosclerosis and chronic inflammatory diseases [1,2]. Among the key adhesion molecules on the vascular endothelial surface are vascular cell adhesion molecule-1 (VCAM-1), E-selectin and intercellular adhesion molecule-1 (ICAM-1). These molecules play important roles in the firm attachment and trans-endothelial migration of leucocytes [3,4], and have been identified in atherosclerotic plaques [5–11]. Endothelial VCAM-1, E-selectin and ICAM-1 expression increases if experimental animals are fed atherogenic diets [12,13], and a deficiency of ICAM-1 reduces the development of atherosclerosis in such animals [14]. Thus endothelial expression of ICAM-1, VCAM-1 and E-selectin appears play a central role in the development of atherosclerosis and other chronic inflammatory diseases.

Soluble forms of ICAM-1, VCAM-1 and E-selectin (term sICAM-1, sVCAM-1 and sE-selectin respectively) are found in the plasma [15], probably as a result of shedding from the surface of activated endothelial cells [16]. The plasma concentrations of sICAM-1, sVCAM-1 and sE-selectin are elevated during inflammatory conditions in which there is increased expression of the cellular forms of the molecules on endothelial cells and other cells [17]. Plasma concentrations of sICAM-1, sVCAM-1 and sE-selectin are higher in individuals with cardiovascular disease than in controls [18–21], and there is a positive correlation between the extent of atherosclerosis and the plasma concentrations of sICAM-1 [22] and sVCAM-1 [22–24]. Recently it was reported that the sICAM-1 concentration predicts future myocardial infarction [25] and represents a molecular marker of atherosclerosis and of other forms of coronary heart disease which is independent of other risk factors [26].

Fish oil (FO) is rich in the long-chain n–3 polyunsaturated fatty acids (PUFA) eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). FO prevents the development of atherosclerosis in experimental animals fed high-fat diets [27,28]. Epidemiological studies show a low incidence of coronary heart disease in human populations which consume large amounts of long-chain n–3 PUFA [29,30]. This effect is certainly due in part to the ability of EPA to lower plasma triacylglycerol (TAG) concentrations, to lower blood pressure, to inhibit platelet aggregation and to prevent thrombosis [31]. However, more recently it has emerged that EPA and DHA potentially have other effects which might also contribute to the protective effect of FO consumption against atherosclerosis and coronary heart disease. Among these effects is the regulation of adhesion molecule expression. EPA and/or DHA have been shown to inhibit the cytokine-induced up-regulation of expression of ICAM-1, VCAM-1 and E-selectin by cultured human endothelial cells [32–35] and of ICAM-1 by cultured human monocytes [36]. Feeding rats an FO-rich diet significantly reduced the expression of ICAM-1 on the surface of lymphocytes [37–39], while supplementation of the human diet with FO lowered levels of ICAM-1 expression on peripheral blood monocytes [40]. These studies demonstrate the potential for an FO-induced decrease in adhesion molecule expression, and so in the pathological events that follow from inappropriate movement of leucocytes to the subendothelial space. Whether such effects would translate into altered plasma concentrations of sICAM-1, sVCAM-1 and sE-selectin is unclear. Therefore, in the present study we investigated the effects of FO supplementation of the diet of healthy young and elderly subjects on plasma concentrations of sICAM-1, sVCAM-1 and sE-selectin; the study was placebo-controlled and double-blind.

MATERIALS AND METHODS

Subjects and study design

All volunteers for the study completed a health and lifestyle questionnaire before entering the study, and doctor’s consent for inclusion into the study was obtained. Volunteers were excluded if they took any prescribed medication, were being treated for a hyperlipidaemia, had diagnosed coronary heart disease or diabetes or a chronic inflammatory disease, took aspirin regularly, smoked > 10 cigarettes per day, were vegetarian, or consumed FO or other oil or vitamin capsules. Subjects were aged between 18 and 75 years, with a median age of 52 years. All subjects were Caucasian and were free living. The characteristics of the subjects are shown in Table 1. Subjects fasted overnight before giving blood into heparinized vacutainer tubes. Blood was taken between 08.30 and 10.00 hours, and subjects who were smokers did not smoke in the morning before giving blood. Plasma was prepared by centrifugation of the blood (3000 g, 10 min) within 1 h of collection; plasma was frozen at −20 °C until analysis.

Subsets of < 40 years of age and 12 elderly subjects (six males and six females; aged > 55 years) participated in a doubled-blind, placebo-controlled study of FO supplementation. Approval was obtained from the appropriate ethical committees, and all...
Table 1  Characteristics of subjects studied
Values shown are medians, with 25th and 75th quartiles given in parentheses. All smokers smoked < 10 cigarettes per day.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>All subjects (n = 140)</th>
<th>Males (n = 100)</th>
<th>Females (n = 40)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI (kg/m²)</td>
<td>24.5 (22.4, 27.3)</td>
<td>24.5 (22.3, 27.1)</td>
<td>24.9 (22.6, 28.3)</td>
</tr>
<tr>
<td>Plasma TAG (mmol/l)</td>
<td>1.15 (0.82, 1.69)</td>
<td>1.26 (0.82, 1.72)</td>
<td>1.03 (0.78, 1.64)</td>
</tr>
<tr>
<td>Plasma cholesterol (mmol/l)</td>
<td>5.03 (3.78, 6.35)</td>
<td>4.38 (3.48, 5.80)</td>
<td>6.35 (5.27, 7.20)</td>
</tr>
<tr>
<td>Smokers (number)</td>
<td>14</td>
<td>11</td>
<td>3</td>
</tr>
</tbody>
</table>

Table 2  Characteristics of subjects in the placebo and FO groups
Values shown are medians, with 25th and 75th quartiles given in parentheses. All smokers smoked < 10 cigarettes per day.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Young males</th>
<th>Elderly subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total number</td>
<td>8</td>
<td>6</td>
</tr>
<tr>
<td>Males</td>
<td>8</td>
<td>3</td>
</tr>
<tr>
<td>Age (years)</td>
<td>24.6 (19.9, 29.5)</td>
<td>21.6 (20.6, 30.8)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>24.1 (21.2, 24.5)</td>
<td>21.0 (20.6, 24.4)</td>
</tr>
<tr>
<td>Smokers</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

subjects gave written consent to participate. Subjects were randomly allocated to receive gelatine-coated capsules containing a placebo oil [an 80:20 (w/w) mix of palm oil and soybean oil; the fatty acid composition of this mix mimics that of the average U.K. diet] or FO. Details of the subjects who completed this study are shown in Table 2. Within the different age groups, those receiving the placebo and FO did not differ according to age or body mass index (BMI) (Table 2). Subjects consumed nine capsules per day (three capsules three times daily, immediately before breakfast, lunch and dinner) for 12 weeks. Subjects in the FO groups consumed 1.2 g of EPA  DHA per day from the capsules. Fasting blood was collected into heparinized vacutainer tubes at baseline and after 12 weeks of supplementation. Blood was taken between 08.30 and 10.00 hours, and subjects who were smokers did not smoke in the morning before giving blood. Plasma was prepared as described above. Compliance was assessed by a self-reporting questionnaire and, biochemically, by determining the plasma phospholipid fatty acid composition.

Biochemical analyses
Plasma TAG and total cholesterol concentrations were measured by enzymic procedures using commercially available kits (procedure nos. 337 and 352 respectively; Sigma Chemical Co., Poole, Dorset, U.K.). Plasma thiobarbituric acid-reactive substances (TBARS) were measured by a modification of the original procedure [41]. Briefly, 100 μl of plasma was incubated with 1.2 ml of 3.35 g/l thiobarbituric acid in 100 g/l trichloroacetic acid for 15 min at 95 °C, and the absorbance was recorded at 535 nm after cooling; TBARS were calculated using a molar absorption coefficient of 1.56 × 10² mM⁻¹ cm⁻¹. Plasma sICAM-1 and sVCAM-1 concentrations were measured using commercially available ELISA kits (Biosource Europe SA, Fleurus, Belgium). Plasma sE-selectin concentrations were measured using a commercially available ELISA kit (R&D Systems Europe Ltd, Abingdon, Oxon., U.K.). Inter- and intra-assay coefficients of variation were both < 5% for both the sICAM and sVCAM-1 assay kits; the inter-assay coefficient of variation for the sE-selectin assay kit was < 10%, while the intra-assay coefficient of variation was < 5%. Minimum detectable concentrations were 0.04 ng/ml for sICAM-1, 0.5 ng/ml for sVCAM-1 and 0.1 ng/ml for sE-selectin.

Determination of the fatty acid composition of plasma phospholipids
Lipid was extracted from plasma with chloroform/methanol (2:1, v/v) and phospholipids were isolated by TLC using a mixture of hexane/diethyl ether/acetic acid (90:30:1, by vol.) as the elution phase [42]. Fatty acid methyl esters were prepared by incubation with 14% boron trifluoride in methanol at 80 °C for 60 min [43]. Fatty acid methyl esters were isolated by solvent extraction, dried and separated by gas chromatography in a Hewlett-Packard 6890 gas chromatograph (Hewlett Packard, Avondale, PA, U.S.A.) fitted with a 30 m × 0.32 mm BPX70 capillary column (film thickness 0.25 μm). Helium at 1.0 ml/min was used as the carrier gas, and the split/splitless injector was used with a

© 2001 The Biochemical Society and the Medical Research Society
split/splitless ratio of 20:1. Injector and detector temperatures were 275 °C. The column oven temperature was maintained at 170 °C for 12 min after sample injection, and was programmed to then increase from 170 to 210 °C at 5 °C/min, before being maintained at 210 °C for 15 min. The separation was recorded with HP GC Chem Station software (Hewlett Packard). Fatty acid methyl esters were identified by comparison with standards purchased from Sigma.

**Statistical analyses**

The relationships between concentrations of soluble adhesion molecules and age, gender, body mass index (BMI), plasma TAG concentration and plasma total cholesterol concentration were determined as Spearman linear correlations ($r$). Partial correlation coefficients for the relationships between soluble adhesion molecule concentrations and age were determined by controlling for other variables that were found to be significantly related to soluble adhesion molecule concentrations (see the Results section). Differences in soluble adhesion molecule concentrations between genders and between different age groups were determined by the Mann–Whitney $U$ test. Comparisons between placebo oil and FO supplementation groups were made using the Mann–Whitney $U$ test. The effects of placebo oil or FO supplementation within a single treatment group were determined by the Wilcoxon matched pairs test. In all cases, a value for $P$ of $<0.05$ was taken to indicate a statistically significant effect. All statistical analyses were performed using SPSS Version 7 for Windows (SPSS Inc., Chicago, IL, U.S.A.).

### RESULTS

**Relationship between age and plasma concentrations of sICAM-1, sVCAM-1 and sE-selectin**

sICAM-1 concentrations varied between 59.9 and 299.7 ng/ml (median 150 ng/ml), sVCAM-1 concentrations varied between 222.8 and 1672.9 ng/ml (median 662 ng/ml), and sE-selectin concentrations varied between 12.4 and 90.3 ng/ml (median 45.9 ng/ml).

There were significant positive linear relationships between the plasma concentration of sICAM-1 and age, gender, BMI, fasting plasma TAG concentration and fasting plasma cholesterol concentration (Table 3). The partial correlation coefficient for the relationship between plasma sICAM-1 concentration and age, after controlling for BMI, gender and fasting plasma TAG and cholesterol concentrations, was significant ($r = 0.355; P < 0.001$).

There were significant positive linear relationships between the plasma concentration of sVCAM-1 and age, BMI and fasting plasma TAG concentration (Table 3). The partial correlation coefficient for the relationship between plasma sVCAM-1 concentration and age, after controlling for BMI and fasting plasma TAG concentration, was significant ($r = 0.402; P < 0.001$).

There were significant positive linear relationships between the plasma concentration of sE-selectin and age, BMI, fasting plasma TAG concentration and fasting plasma cholesterol concentration (Table 3). The partial correlation coefficient for the relationship between plasma sE-selectin concentration and age, after controlling for BMI and fasting plasma TAG and cholesterol concentrations, was not significant.

Male subjects were divided into those $<40$ years and those $\geq 55$ years of age; the plasma concentrations of both sICAM-1 and sVCAM-1 were significantly higher ($P < 0.001$) in the older age group (Table 4). Although the plasma concentration of sE-selectin was higher in the older subjects (Table 4), the difference did not reach statistical significance ($P = 0.073$).

| Table 3 | Relationships between concentrations of soluble adhesion molecules and other subject characteristics |
|-----------------------|-----------------------|-----------------------|-----------------------|
|                      | Relationship with sICAM-1 | Relationship with sVCAM-1 | Relationship with sE-selectin |
| Parameter             | $r$ | $P$     | $r$ | $P$     | $r$ | $P$     |
| Age                   | 0.580 | $< 0.001$ | 0.392 | $< 0.001$ | 0.234 | 0.027 |
| Gender                | 0.381 | $< 0.001$ | 0.168 | NS | 0.168 | NS |
| BMI                   | 0.326 | $< 0.001$ | 0.172 | NS | 0.303 | 0.004 |
| Plasma TAG            | 0.321 | $< 0.001$ | 0.177 | 0.048 | 0.225 | 0.041 |
| Plasma cholesterol    | 0.531 | $< 0.001$ | 0.118 | NS | 0.219 | 0.046 |

**Effects of FO on the EPA content of plasma phospholipids and on the plasma TBARS concentration**

The level of EPA in plasma phospholipids was not different between young male and elderly subjects in either the placebo or FO groups before supplementation (Table 5). The level of EPA in plasma phospholipids did
Fish oil and soluble adhesion molecules

Table 4  sICAM-1, sVCAM-1 and sE-selectin concentrations in young and elderly males
Values shown are medians, with 25th and 75th quartiles given in parentheses. Significance of differences: *P < 0.001 compared with subjects < 40 years old (Mann–Whitney U test). The difference in sE-selectin concentrations between the groups was not significant (P = 0.073).

<table>
<thead>
<tr>
<th>Molecule</th>
<th>Subjects &lt; 40 years (n = 52)</th>
<th>Subjects ≥ 55 years (n = 38)</th>
</tr>
</thead>
<tbody>
<tr>
<td>sICAM-1</td>
<td>107.9 (94.1, 133.3)</td>
<td>173.9* (133.9, 204.6)</td>
</tr>
<tr>
<td>sVCAM-1</td>
<td>600.5 (495.6, 738.9)</td>
<td>830.3* (650.2, 1050.0)</td>
</tr>
<tr>
<td>sE-selectin</td>
<td>43.2 (29.3, 49.7)</td>
<td>49.3 (31.3, 63.1)</td>
</tr>
</tbody>
</table>

Table 5  Effects of supplementation with placebo oil or FO on the EPA content of plasma phospholipids
Values shown are medians, with 25th and 75th quartiles given in parentheses. Significance of differences: *P = 0.007, **P = 0.043 compared with level before supplementation (Wilcoxon matched pairs test); †P = 0.025, ††P = 0.004 compared with placebo group (Mann–Whitney U test).

<table>
<thead>
<tr>
<th>EPA concentration (g/100 g total fatty acids)</th>
<th>Young males</th>
<th>Elderly subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Placebo (n = 8)</td>
<td>FO (n = 8)</td>
</tr>
<tr>
<td>Before supplementation</td>
<td>0.85 (0.15, 1.63)</td>
<td>0.47 (0.25, 1.34)</td>
</tr>
<tr>
<td>After supplementation</td>
<td>0.67 (0.1, 1.55)</td>
<td>1.85* (0.95, 2.51)</td>
</tr>
<tr>
<td>Change</td>
<td>−0.19 (−0.48, 0.43)</td>
<td>1.35† (0.44, 1.84)</td>
</tr>
<tr>
<td>Change (%)</td>
<td>−7.9 (−62.1, 0.8)</td>
<td>194.4† (19.3, 521.9)</td>
</tr>
</tbody>
</table>

not change with placebo supplementation (Table 5). However, FO supplementation significantly increased the concentration of EPA in plasma phospholipids in both young male and elderly subjects (Table 5). The median increase in EPA in both young and elderly subjects was approx. 200% (Table 5).

Plasma TBARS concentrations (approx. 4.5 μmol/l) were not different between the young males and the elderly subjects, and were not affected by either placebo oil or FO supplementation (results not shown).

Effects of FO on plasma sICAM-1, sVCAM-1 and sE-selectin concentrations
sICAM-1 concentrations were unaffected by either placebo or FO in both the young males and the elderly subjects (Table 6).

sVCAM-1 concentrations in the young males in the elderly subjects were not affected by placebo treatment (Table 6). Likewise, FO supplementation in the young males did not alter sVCAM-1 concentrations (Table 6). In contrast, FO supplementation significantly decreased plasma sVCAM-1 concentrations in the elderly subjects (Table 6). The concentration of sVCAM-1 was decreased in every elderly subject in the FO group. The minimum and maximum decreases were 16% and 60% respectively, with a median decrease of 20%; this change from baseline was significantly different from the median percentage change from baseline in the placebo group (Table 6). The minimum and maximum absolute changes in sVCAM-1 concentration in the FO group were −161.6 and −469.7 ng/ml respectively, with a median change of −188 ng/ml; this absolute change from baseline was significantly different from the change from baseline in the placebo group (Table 6). The sVCAM-1 concentration in the elderly subjects before FO supplementation was significantly higher than in the young males before supplementation (P = 0.012; Mann–Whitney U test); however, there was no significant difference in sVCAM-1 concentration between the elderly FO group after FO supplementation and the young males before supplementation (P = 0.77).

sE-selectin concentrations were not affected by the placebo treatment in either the young males or the elderly subjects (Table 6). In contrast, FO supplementation in young males led to a significant increase (P = 0.043) in sE-selectin concentrations (Table 6). The concentration of sE-selectin increased in six out of eight of the young males in the FO group. The median increase was almost 12 ng/ml, representing a 38% increase (Table 6). FO supplementation decreased plasma sE-selectin concentrations in the elderly subjects (Table 6), although this
The concentration of sE-selectin was decreased in five out of six elderly subjects in the FO group. The median change across all six subjects (−4.7 ng/ml) represented a median decrease of 11% (Table 6).

**DISCUSSION**

The origin and physiological/pathological role of soluble adhesion molecules are not well understood. VCAM-1 and E-selectin are expressed almost exclusively by activated endothelial cells. Thus the most likely origin of sVCAM-1 and sE-selectin is from activated endothelial cells, and this is believed to occur as a result of proteolytic ‘shedding’ from the endothelial cell surface [17]. ICAM-1 is expressed at basal levels on monocytes and endothelial cells, and its expression can be strongly induced on a variety of cell types, including endothelial cells, monocytes and macrophages, T and B lymphocytes, dendritic cells and smooth muscle cells, by stimuli such as cytokines. As for sVCAM-1 and sE-selectin, the appearance of sICAM-1 is believed to be as a result of shedding from the cell surface [17]. Thus increased concentrations of sICAM-1, sVCAM-1 and sE-selectin in the circulation are considered to reflect up-regulation of the molecules on the cell surface. The cell-associated forms of these molecules have been identified in atherosclerotic plaques and other chronic inflammatory lesions [1–14], where they are believed to play a role in the inappropriate movement of leucocytes to these sites. If the soluble forms of ICAM-1, VCAM-1 and E-selectin do reflect the activation-induced up-regulation of the cellular forms of the molecules, then they may be useful biomarkers of the development of atherosclerosis and other inflammatory conditions [17–26]. Therefore interventions that lower concentrations of soluble adhesion molecules, indicating decreased endothelial activation, may be beneficial and may slow the progress of atherosclerosis.

Table 6  Effects of supplementation with placebo oil or FO on plasma sICAM-1, sVCAM-1 and sE-selectin concentrations

<table>
<thead>
<tr>
<th></th>
<th>Young males</th>
<th>Elderly subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Placebo (n = 8)</td>
<td>FO (n = 8)</td>
</tr>
<tr>
<td>sICAM-1 (ng/ml)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before</td>
<td>104.2 (93.7, 113.1)</td>
<td>107.1 (87.8, 115.6)</td>
</tr>
<tr>
<td>After</td>
<td>99.5 (93.8, 108.4)</td>
<td>102.0 (91.7, 122.6)</td>
</tr>
<tr>
<td>Change</td>
<td>−1.8 (−12.3, 15.7)</td>
<td>−3.2 (−13.3, 9.9)</td>
</tr>
<tr>
<td>Change (%)</td>
<td>−2.2 (−10.9, 16.8)</td>
<td>−2.8 (−11.8, 11.8)</td>
</tr>
<tr>
<td>sVCAM-1 (ng/ml)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before</td>
<td>518.0 (465.8, 698.2)</td>
<td>634.2 (462.5, 899.7)</td>
</tr>
<tr>
<td>After</td>
<td>593.1 (494.4, 703.2)</td>
<td>579.5 (497.4, 1059.3)</td>
</tr>
<tr>
<td>Change</td>
<td>−11.4 (−33.7, 116.7)</td>
<td>61.5 (18.9, 176.3)</td>
</tr>
<tr>
<td>Change (%)</td>
<td>−3.9 (−12.5, 15.9)</td>
<td>12.5 (3.8, 20.5)</td>
</tr>
<tr>
<td>sE-selectin (ng/ml)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before</td>
<td>40.1 (34.5, 58.1)</td>
<td>30.9 (28.8, 49.9)</td>
</tr>
<tr>
<td>After</td>
<td>42.2 (39.2, 47.8)</td>
<td>43.6* (42.2, 50.4)</td>
</tr>
<tr>
<td>Change</td>
<td>1.4 (−11.9, 7.6)</td>
<td>11.7 (0.4, 13.4)</td>
</tr>
<tr>
<td>Change (%)</td>
<td>2.2 (−28.6, 21.2)</td>
<td>37.9 (0.8, 46.5)</td>
</tr>
</tbody>
</table>

Effect did not reach statistical significance (P = 0.075). The concentration of sE-selectin was decreased in five out of six elderly subjects in the FO group. The median change across all six subjects (−4.7 ng/ml) represented a median decrease of 11% (Table 6).
been reported, while a mean sVCAM-1 concentration of 664 ng/ml in males and females aged > 50 years was measured [22]. The ranges of concentrations of sICAM-1 and sVCAM-1 among the subjects in the present study were similar to those reported in the studies in Japanese [20] and American [21,22,25,26,44] subjects.

A positive correlation between sVCAM-1 concentration and age has been reported in Japanese [20] and American [26] subjects. A similar relationship is shown for the first time in the present study in free-living, healthy subjects (both males and females) in the U.K. A positive correlation between sICAM-1 concentration and age was reported in Japanese subjects [20]. One study in American subjects did not show a relationship between sICAM-1 or sE-selectin concentration and age [26], but a recent study identified a positive correlation between sICAM-1 concentration and age in American men [44].

The present study found a positive correlation between sICAM-1 concentration and age in free-living, healthy subjects (males but not females) in the U.K. The correlations between concentrations of soluble adhesion molecules and age were stronger in the present study and in the Japanese study [20] than in the American studies [26,44]. This is most probably because the present study and the Japanese study [20] studied a wider age range (18–75 years in the present study and 20–85 years in [20]) than that studied by Hwang et al. [45–64 years] [26] and probably by Rohde et al. [44] (the ages of subjects whose sICAM-1 concentrations were reported by Rohde et al. were not given, but they are referred to as middle-aged). This might also explain why we and Morisaki et al. [20] found a correlation between sICAM-1 concentration and age, but Hwang et al. [26] did not. Both the VCAM-1 and ICAM-1 genes have regulatory sites for the transcription factors AP-1 (activator protein-1) and nuclear factor κB (NFκB) [45,46]; these transcription factors are activated by cellular oxidative stress [47,48]. Several studies suggest that oxidative stress increases with aging [49–51]. Thus the significant positive correlations identified between concentrations of soluble adhesion molecules and age may reflect the cumulative effects of oxidative stress.

Supplementation of the diet with a moderate level of FO (providing 1.2 g of EPA+DHA per day) for 12 weeks was found to significantly decrease the concentration of sVCAM-1 in elderly subjects. FO did not affect the concentration of sVCAM-1 in young males or that of sICAM-1 in either young males or elderly subjects. The effects of FO on sE-selectin concentrations were different in the young males and the elderly subjects: FO led to an increase in E-selectin concentration in young males, but caused a non-significant decrease in the concentration of sE-selectin in elderly subjects. The median decreases in the concentrations of sVCAM-1 and sE-selectin in elderly subjects given FO were 20% and 11% respectively. This is the first time that an increase in the level of n-3 PUFA in the diet has been shown to decrease plasma sVCAM-1 concentrations.

Three studies have previously examined the influence of FO on concentrations of soluble adhesion molecules [52–54]. None of these studies reported an FO-induced decrease in the sVCAM-1 concentration. This might be explained by the types of subjects studied, by the levels of n-3 PUFA provided or by the nature of the experimental protocols used. Seljeflot et al. [52] provided 4.8 g of EPA+DHA per day for 6 weeks to hyperlipidaemic subjects aged 41–57 years who habitually smoked > 10 cigarettes per day. Fasting blood was taken 90 min after smoking two or three cigarettes, and it was found that sVCAM-1 and sE-selectin concentrations were increased by 8% and 22% respectively in the subjects who had consumed n-3 PUFA. Clearly these conditions are very different from those used in the present study with FO, in which subjects were not hypertriglyceridaemic, did not habitually smoke > 10 cigarettes per day and did not smoke in the period before giving blood. In addition, the dose of EPA+DHA used was much higher in [52] than in the present study. It may be that the combination of the high dose of n-3 PUFA and smoking imposed significant oxidative stress, which would be expected to increase concentrations of soluble adhesion molecules. It is possible that such oxidative stress does not occur in non- and light smokers given a lower dose of n-3 PUFA. Abe et al. [53] also used a high dose of n-3 PUFA (approx. 3.3 g of EPA+DHA per day for 7 months) in hypertriglyceridaemic subjects (mean fasting plasma TAG concentration of 9.9 mmol/l). After 6 weeks, plasma sICAM-1 and sVCAM-1 concentrations were unchanged, while the concentration of sE-selection was increased significantly by 11%. After 7 months of treatment with FO, the concentrations of sICAM-1 and sE-selectin had decreased significantly, by 9% and 16% respectively, while that of sVCAM-1 had not changed significantly. Again, the different subject group and the higher level of n-3 PUFA used might explain the differences in the findings between [53] and the present study. Most recently, Johansen et al. [54] provided 5.1 g of EPA+DHA to patients with coronary heart disease for 4 weeks, and found that sVCAM-1 and sE-selectin concentrations increased by 25 and 20% respectively. That study also addressed the potential oxidative stress induced by the high level of n-3 PUFA provided, and found that plasma vitamin E concentrations decreased and plasma TBARS concentrations increased 3-fold [54]. These observations suggested that marked oxidative stress was occurring in the subjects. In contrast, plasma TBARS were not affected by FO supplementation in the present study; the plasma TBARS concentrations observed are in accordance with values reported elsewhere (e.g. 4.2 μmol/l [55]). This lack of effect of FO supplementation on plasma TBARS concentrations in the present study is most likely due to the more moderate
supplementation level compared with that in [54] (and also [52,53], although these latter studies did not measure markers of oxidative stress). Taken together, these studies suggest that providing a moderate level of \( \kappa - 3 \) PUFA (e.g. 1.2 g of EPA + DHA per day) to normotriglyceridaemic non-smokers can decrease some markers of endothelial activation in some subjects (e.g. those > 55 years of age), whereas giving a higher level of \( \kappa - 3 \) PUFA (e.g. > 3.3 g of EPA + DHA per day) to hyperlipidaemic subjects, especially those who smoke, induces oxidative stress which might contribute to further endothelial activation.

The difference in the efficacy of FO intervention between young and elderly subjects in the present study does not reflect differences in compliance or in incorporation of EPA: the level of EPA in plasma phospholipids increased in all subjects taking FO, irrespective of age; the median increase in both age groups was approx. 200%. It is possible that the concentration of sVCAM-1 found in young subjects reflects that which results from basal shedding of VCAM-1 during normal physiological processes, while the elevated concentration of sVCAM-1 found in elderly subjects reflects that which results from basal shedding of VCAM-1 during normal physiological processes plus the shedding that occurs as a result of up-regulation of VCAM-1 due to the processes associated with aging. If this is so, then the reduction in sVCAM-1 concentrations in the elderly subjects may reflect the specific inhibition of the age-related up-regulation of VCAM-1 expression. In contrast, FO does not appear to affect the age-related up-regulation of ICAM-1 expression, as determined by sICAM-1 concentrations. These observations suggest that VCAM-1 and ICAM-1 are up-regulated by different mechanisms that are not equally sensitive to FO intervention. This is supported by cell culture observations, which showed that cytokine-induced up-regulation of VCAM-1 expression on human endothelial cells is markedly inhibited by culture in the presence of EPA or DHA [32–35], whereas up-regulation of ICAM-1 is less affected [32,35] or not affected at all [33,34] by \( \kappa - 3 \) PUFA. The effect of EPA and DHA is exerted at the level of VCAM-1 mRNA [32,34,35], indicating that the fatty acids can down-regulate expression of the VCAM-1 gene. This may be via inhibition of the activation of NFkB; Weber et al. [34] showed that DHA inhibited the activation of NFkB in cytokine-stimulated human endothelial cells. More recently, EPA has been shown to prevent the degradation of IkB, the inhibitory subunit of NFkB [56], thereby providing a mechanism by which activation of NFkB might be prevented in cells enriched with EPA.

Whatever its mechanism of action, the results of the present study suggest a novel anti-inflammatory and anti-atherosclerotic mechanism of action of FO. Feeding animals a diet containing FO decreases the development of atherosclerosis [27,28]; it is possible that this effect is due in part to lowered adhesion molecule expression, leading to decreased infiltration of leukocytes into the vessel wall. This same effect might be partly responsible for the protective effect of FO against the development of human coronary heart disease [29,30].

ACKNOWLEDGMENTS

This research was supported by grants from the Ministry of Agriculture, Fisheries and Food, Unilever Research Colworth Laboratory and Nutricia Research under the Agri-Food Quality LINK Programme (grant no. AFQ51), and from the Biotechnology and Biological Sciences Research Council (grant no. 51/F05696). sE-selectin concentration measurements were funded separately by Unilever Research Colworth Laboratory. We thank Dr Paul Quinlan (Unilever Research Colworth Laboratory), Dr Gert Meijer (Unilever Vlaardingen Laboratory) and Dr Jacques Bindels and Dr Monique Al [Nutricia (now Numico) Research]. F.A.W. was supported by a Rank Prize Fund Studentship.

REFERENCES


Received 14 July 2000; 31 August 2000; accepted 29 September 2000