Soluble Cell Adhesion Molecules in Hypertriglyceridemia and Potential Significance on Monocyte Adhesion

Yasunori Abe, Bassem El-Masri, Kay T. Kimball, Henry Pownall, Christopher F. Reilly, Karin Osmundsen, C. Wayne Smith, Christie M. Ballantyne

Abstract—Hypertriglyceridemia may contribute to the development of atherosclerosis by increasing expression of cell adhesion molecules (CAMs). Although the cellular expression of CAMs is difficult to assess clinically, soluble forms of CAMs (sCAMs) are present in the circulation and may serve as markers for CAMs. In this study, we examined the association between sCAMs and other risk factors occurring with hypertriglyceridemia, the effect of triglyceride reduction on sCAM levels, and the role of soluble vascular cell adhesion molecule-1 (sVCAM-1) in monocyte adhesion in vitro. Compared with normal control subjects (n=20), patients with hypertriglyceridemia and low HDL (n=39) had significantly increased levels of soluble intercellular adhesion molecule-1 (sICAM-1) (316±28.8 versus 225±16.6 ng/mL), sVCAM-1 (743±52.2 versus 522±43.6 ng/mL), and soluble E-selectin (83±5.9 versus 49±3.6 ng/mL). ANCOVA showed that the higher sCAM levels in patients occurred independently of diabetes mellitus and other risk factors. In 27 patients who received purified n-3 fatty acid (Omacor) 4 g/d for 7 months, triglyceride level was reduced by 47±4.6%, sICAM-1 level was reduced by 9±3.4% (P=.02), and soluble E-selectin level was reduced by 16±3.2% (P<.0001) with the greatest reduction in diabetic patients. These results support previous in vitro data showing that disorders in triglyceride and HDL metabolism influence CAM expression and treatment with fish oils may alter vascular cell activation. In a parallel-plate flow chamber, recombinant sVCAM-1 at the concentration seen in patients significantly inhibited adhesion of monocytes to interleukin-1–stimulated cultured endothelial cells under conditions of flow by 77.5±7.2%. Thus, elevated sCAMs may negatively regulate monocyte adhesion. (Arterioscler Thromb Vasc Biol. 1998;18:723-731.)

Key Words: triglycerides • cell adhesion molecules • monocytes • endothelial cells

One of the key initial events in the development of atherosclerosis is the adhesion of monocytes to endothelial cells, with subsequent transmigration into the vascular intima. Leukocyte and vascular CAMs such as selectins, integrins, VCAM-1, and ICAM-1 play critical roles in the adhesion of monocytes to endothelial cells.1 The expression of E-selectin, ICAM-1, and VCAM-1 is relatively low in normal vascular cells and is upregulated in response to various stimuli, including cytokines and oxidants, thus enabling monocytes to adhere to the vessel wall. In vivo animal studies and inmunohistochemical studies of human tissues have shown that these CAMs are expressed at increased levels in atherosclerotic plaques.2–5

The role that defects in triglyceride metabolism play in the development of atherosclerosis remains controversial, in contrast to the widely accepted relationship between disturbances in LDL metabolism and the development of premature atherosclerosis. Disorders in triglyceride metabolism may promote atherogenesis by increasing expression of vascular CAMs. Patients with markedly elevated triglyceride levels also have decreased HDL levels and abnormalities in fatty acid metabolism. In vitro studies have shown that low HDL and oxidized fatty acids increase the endothelial expression of CAMs in response to cytokines.6–9 Assessment of the relationship between elevated triglycerides/low HDL and the expression of CAMs in humans has been hampered by the difficulty of quantitative assessment of CAM expression in vivo.

CAMs are also present in the circulation as soluble forms, which lack membrane-spanning and cytoplasmic domains that are present in the membrane-bound forms. Although the origins, metabolism, and functional significance of sCAMs are not fully understood, quantitative assessment of the levels of sCAMs is straightforward. These levels have been noted to be elevated in certain pathological conditions such as sepsis, autoimmune diseases, and allograft rejection, in which tissue expression of the membrane-bound forms of CAMs is also known to be upregulated.10–12 Thus, the levels of sCAMs may serve as surrogate markers that reflect the cellular expression of CAMs.

We have previously shown that patients with severe elevations of either LDL or triglyceride levels have increased...
levels of sCAMs and that aggressive reductions in LDL cholesterol with 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitor therapy over 6 months in patients with high LDL significantly reduce the levels of sE-selectin without changing the levels of sCAM-1 or sVCAM-1.13 Although we noted that the hypertriglyceridemic patients had an increased frequency of other risk factors such as diabetes mellitus, the sample sizes of our previous studies were not large enough to examine the associations between sCAMs and the associated risk factors that are frequently concurrently seen with hypertriglyceridemia. Furthermore, it was unclear whether disorders of triglyceride/HDL led to increased sCAM levels or whether sCAMs were increased because of extensive occult atherosclerosis in patients with severe hypertriglyceridemia and low HDL.

We have recently shown that under flow conditions in vitro, interactions between VCAM-1 and αvβ3 integrin play a major role in adhesion of mononuclear cells to activated endothelial cells, whereas E-selectin and ICAM-1 play lesser, supporting roles.14 Increased levels of sVCAM-1 may influence the αv/β3/VCAM-1-dependent adhesion of monocytes to endothelial cells by competitive inhibition.

Studies were performed to further explore the following questions in regard to the relationship between hypertriglyceridemic dyslipidemia (high triglyceride/low HDL) and levels of sCAMs: (1) Is hypertriglyceridemic dyslipidemia independently associated with increased levels of sCAMs when other risk factors such as diabetes mellitus, sex, and hypertension are considered? (2) Does treatment of hypertriglyceridemic dyslipidemia with a purified n-3 fatty acid, Omacor, influence levels of sCAMs? (3) Do the levels of sVCAM-1 that are present in patients with hypertriglyceridemia inhibit adhesion of monocytes under flow conditions in vitro?

Methods

Subjects

Two groups of subjects (n = 59) were recruited in a protocol approved by the Institutional Review Board for Human Subjects Research, Baylor College of Medicine, Houston, Tex; all subjects gave informed consent, and the procedures followed in the study were in accordance with institutional guidelines. Hypertriglyceridemic subjects (n = 41) between the ages of 18 and 70 years were eligible for enrollment after an initial dietary phase (American Heart Association Step I Diet) that lasted for a period of 6 weeks. Inclusion criteria included mean serum triglyceride level of visits 4 and 5 (one visit every week until week 6) ≥500 mg/dL but ≤2000 mg/dL. Exclusion criteria were treatment with gemfibrozil or similar fibrates <3 months before entering the dietary phase, consumption of n-3 products or lipid-lowering fibers <4 weeks before entering the dietary phase, weekly consumption of cold-water fish, myocardial infarction or other serious disease <6 months before entering the study, serum alanine transaminase >three times the upper limit of normal, fasting serum glucose >300 mg/dL, serum creatinine >2 mg/dL, platelets <60 × 10^11/mm^3, hemoglobin <10 g/dL, pregnancy or breast feeding, alcohol or drug abuse, and insulin-dependent diabetes mellitus.15 A total of 41 patients were randomized: 21 patients to receive placebo and 20 patients to receive Omacor, a highly purified formulation of the ethyl esters of n-3 fatty acids, for 6 weeks while maintaining the Step I Diet regimen. One patient receiving Omacor was lost to follow-up, and 1 patient in the placebo group did not have specimens available for assessment of sCAMs; thus, 39 patients were included in these analyses. A medical history was taken and a physical examination was performed to determine whether patients had clinical evidence or symptoms of advanced coronary artery disease, peripheral vascular disease, or cerebrovascular disease. A risk factor score was calculated for each patient to indicate whether the patient had 0, 1, or 2 or more of the following risk factors: NIDDM, hypertension, smoking, and clinical evidence of atherosclerosis. NIDDM was defined as a fasting blood sugar level of >115 mg/dL or treatment with a hypoglycemic agent. Hypertension was defined as systolic blood pressure >140 mm Hg, diastolic blood pressure >90 mm Hg, or treatment with an antihypertensive agent. These patients were matched for age and sex against a group of 20 healthy normalolipidemic subjects without major cardiovascular risk factors and with levels of LDL cholesterol <160 mg/dL and fasting triglyceride <150 mg/dL.

After 6 weeks of the placebo-controlled study, 31 patients, including those who had been randomized to placebo, received 4 g daily of Omacor for >6 months. Of these patients, 27 had serum available after >6 months of treatment (7 months [n = 18] or 12 months [n = 9]) for measurement of sCAMs.

Measurements and Analytical Methods

Biochemistry, hematology, and analysis of serum triglyceride were performed at National Health Laboratory Inc (Houston, Tex). Levels of triglyceride were measured by glycerol phosphate oxidase reaction. Analyses of plasma total cholesterol and LDL cholesterol were determined using enzymatic kits from Boehringer Mannheim Diagnostics. HDL cholesterol was measured at baseline and 6 weeks of treatment after ultracentrifugation16 and at long-term follow-up after precipitation of apolipoprotein B–containing particles by National Health Laboratories Inc. Levels of sICAM-1, sVCAM-1, and sE-selectin were determined by using monoclonal antibody–based enzyme-linked immunosorbent assay (ELISA) (R & D Systems) on frozen serum collected at baseline and after treatment from the patients who received medications (placebo and Omacor). Assays of all samples and controls were performed in duplicate. Concentrations of samples were determined by analyzing standards with known concentrations of recombinant adhesion molecules coincident with samples and plotting of signal versus concentration.

Expression and Purification of rsVCAM-1

To prepare rsVCAM-1, full-length VCAM-1 cDNA (British Biotechnology, Oxford, UK) was modified by removing the 5’ noncoding sequence and introducing a translational stop codon at amino acid 716 to terminate translation at the amino acid before the start of the transmembrane region. The modified VCAM-1 cDNA was transferred into the baculovirus expression vector PVL1392 as a Pst 1–Not 1 fragment. Sf21 insect cells were subsequently cotransfected with the PVL1392/rsVCAM-1 expression vector and Baculo-Gold viral DNA. Recombinant viruses were plaque purified and used to infect sf21 cells at a multiplicity of infection of 1. Supernatants were harvested 48 hours after infection. ELISA (VCAM-1 ELISA, British Biotechnology) results indicated that the rsVCAM-1 protein was expressed at ~15 mg/mL. rsVCAM-1 was purified by Concanavalin A chromatography followed by affinity chromatography on 1G11-
Sepharose. 1G11, provided by Dr Dorian Haskard (Hammersmith Hospital, London) is an anti–VCAM-1 monoclonal antibody that blocks VCAM-1 binding to very late activation antibody.4,5 The resultant protein was >95% pure as judged by SDS–polyacrylamide gel electrophoresis and amino terminal sequence analysis.

Cell Culture
HUVECs were harvested from 5 to 10 umbilical cords by collagenase digestion as previously described.11,12 For the adhesion assay, primary cultured cells were passaged with 0.05% trypsin and 0.53 mmol/L EDTA (Life Technology) at a split ratio of 1 to 1. Two or 3 days after passage, the medium was removed, and HUVECs were incubated with M199 (Life Technology) containing 10% fetal calf serum and 1% penicillin-streptomycin (Rockville, Md) and maintained in RPMI 1640 (Life Technology) for 24 hours.

THP-1 (TIB 202), a human acute monocytic leukemia–derived cell line, was obtained from American Type Culture Collection (Rockville, Md) and maintained in RPMI 1640 (life Technology) containing 10% fetal calf serum and 1% penicillin-streptomycin (Life Technology).

Isolation of Peripheral Blood Monocytes
Human peripheral blood monocytes were isolated by density-gradient centrifugation followed by counterflow elutriation as previously described.11,12 Briefly, mononuclear cells were isolated from a buffy coat prepared from 400 mL of anticoagulated whole blood by centrifugation on a Ficoll-Hypaque density gradient at 800g, room temperature for 40 minutes (Lymphoprep, Life Technology). Mononuclear cells were washed once; resuspended in D-PBS containing 0.1% low endotoxin BSA (Sigma), 0.1% glucose (Sigma), and 3 mmol/L EDTA (Sigma); and loaded onto a 6-mL Sanderson chamber mounted in a JE-5.0 rotor with 36M centrifuge (at 2500 rpm, Beckman Instruments) at a flow rate of 14 mL/min, 4°C. Flow rates were gradually increased by 1 mL/min. Monocytes were obtained at flow rates of 19, 20, and 21 mL/min. Monocytes thus isolated were 84% pure as determined by FACScan (Becton Dickinson). Isolated monocytes were resuspended at 10^7 cell/mL in D-PBS without Ca^2+ and Mg^2+, kept at 4°C, and used within 3 hours after isolation.

Flow Adhesion Assay
The adhesion of THP-1 or isolated peripheral blood monocytes to IL-1–stimulated HUVECs under hydrodynamic flow conditions was studied as previously described.11,12 Briefly,first-passage HUVECs stimulated with IL-1 (10 U/mL for 24 hours) in 35-mm tissue culture dishes were mounted in a parallel-plate flow system. THP-1 or peripheral blood monocytes suspended at 10^7 cell/mL in 37°C D-PBS containing Ca^{2+} and Mg^{2+}, and 11 mmol/L glucose were passed through the parallel flow chamber at 2.0 dyne/cm^2 under a phase-contrast microscope (Diaphot TMD, Nikon Inc) for 10 minutes at 37°C. At the end of the perfusion, four different fields were videotaped at 15-second intervals. Videotaped images were analyzed with Optimas image analysis software (BioScan Inc). The number of stably adherent cells was quantified as the average number of leukocytes remaining on the monolayer in the four different fields.

To determine the effects of sVCAM-1, THP-1 or monocytes resuspended at 10^7 cell/mL in perfusion buffer were incubated with rsVCAM-1 at 37°C for 10 minutes before adhesion assay. sVCAM-1 was present in the perfusion buffer during the entire adhesion assay. In some experiments, 0.3 mmol/L MnCl_2 was added to the perfusion buffer.

Statistical Methods
For the primary tests of hypotheses, groups of subjects were compared with respect to baseline levels of sCAMs and percent changes in sCAMs using ANOVA; transformation of the variables or nonparametric tests was used when assumptions of these tests were not met. The interpretation of statistical significance was based on keeping the family-wise error rate ≤0.05 for each group of primary hypotheses.

In further exploratory analyses, multiple regression analysis was used to examine the relative contributions and overlap of risk factors possibly contributing to sCAM levels and percent changes in sCAM levels. Probability values from these analyses were interpreted as descriptive only, because of the exploratory nature of the analysis. Continuous data were reported as mean ± SE. All statistical tests were two-tailed. Stata software (1997) was used for the statistical analyses.

Results
Baseline Characteristics of Patients and Normal Control Subjects
Baseline characteristics of patients with hypertriglyceridemia and normal control subjects are shown in Table 1. Although the two groups were similar in age and sex distribution, the patients with hypertriglyceridemia had more risk factors, including NIDDM, hypertension, smoking history, and clinical evidence of atherosclerosis. In addition to hypertriglyceridemia, patients had significantly lower HDL cholesterol (P < 0.0001) and LDL cholesterol (P<0.0001) and higher total cholesterol (P<0.0001), which was due to the increased cholesterol in triglyceride-rich lipoproteins compared with the control group.

Baseline Levels of sCAMs
The levels of sCAMs were increased in hypertriglyceridemic patients versus control subjects: sICAM-1 (316±28.8 versus 225±16.6 ng/mL, P = .0005), sVCAM-1 (743±52.2 versus 522±43.6 ng/mL, P = .0007), and sE-selectin (83±5.9 versus 49±3.6 ng/mL, P = .0001) (Fig 1). In exploratory analyses of factors that may have contributed to the elevation of sCAMs in the patients with elevated triglycerides, the following variables were examined: age; sex; NIDDM; history of smoking; hypertension; clinical history or evidence of atherosclerosis; risk factor score; BMI; and baseline levels of triglyceride, LDL cholesterol,

### Table 1. Baseline Characteristics of Hypertriglyceridemic Patients and Control Subjects

<table>
<thead>
<tr>
<th></th>
<th>Control Subjects (n = 20)</th>
<th>Hypertriglyceridemic Patients (n = 39)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>51±2.2 (51)</td>
<td>52±1.4 (53)</td>
</tr>
<tr>
<td>Sex, M/F</td>
<td>11/9</td>
<td>22/17</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>0</td>
<td>11</td>
</tr>
<tr>
<td>Hypertension</td>
<td>0</td>
<td>13</td>
</tr>
<tr>
<td>Smoking</td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>Clinical atherosclerosis</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>Risk factor score</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>20</td>
<td>6</td>
</tr>
<tr>
<td>1</td>
<td>0</td>
<td>15</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>18</td>
</tr>
<tr>
<td>Triglyceride, mg/dL</td>
<td>99.3±6.9 (100)</td>
<td>910.8±76.8 (756)</td>
</tr>
<tr>
<td>Total cholesterol, mg/dL</td>
<td>200.8±7.1 (202)</td>
<td>336.2±13.0 (326)</td>
</tr>
<tr>
<td>HDL cholesterol, mg/dL</td>
<td>68.5±4.9 (66)</td>
<td>19.9±1.1 (18)</td>
</tr>
<tr>
<td>LDL cholesterol, mg/dL</td>
<td>112.6±6.3 (110)</td>
<td>51.2±3.8 (44)</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SE (median).
and HDL cholesterol. In univariate analyses, risk factors for higher levels of sICAM-1 were male sex (men: 361±48.7 ng/mL, n=22; women: 257±10.9 ng/mL, n=17; P=.02), NIDDM (NIDDM: 430±91.8 ng/mL, n=11; without NIDDM: 271±11.6 ng/mL, n=28; P=.01), and higher BMI (P=.08). In multivariable analyses, joint predictors of higher sICAM-1 were NIDDM (P=.007) and male sex (P=.09).

Univariate predictors of higher baseline levels of sVCAM-1 in the patients were male sex (men: 361±48.7 ng/mL, n=22; women: 257±10.9 ng/mL, n=17; P=.02), NIDDM (NIDDM: 430±91.8 ng/mL, n=11; without NIDDM: 271±11.6 ng/mL, n=28; P=.01), and higher BMI (P=.08). In multivariable analyses, joint predictors of higher sVCAM-1 in the patients were male sex (P=.001), older age (P=.007), and higher BMI (P=.08). The significance of age in the multivariable but not in the univariate analysis is explained by the significant increase in levels of sVCAM-1 with age in female (P=.03) but not in male (P=.29) patients.

In univariate analyses, the levels of sE-selectin in the patients were higher with male sex (men: 97±9.1 ng/mL, n=22; women: 65±4.2 ng/mL; P=.004), NIDDM (NIDDM: 103±11.2 ng/mL, n=11; without NIDDM: 75±6.5 ng/mL, n=28; P=.011), higher BMI (P=.0003), and younger age (P=.056). In multivariable analyses, joint predictors of higher sE-selectin in the patients were high BMI (P=.004) and male sex (P=.07). NIDDM was not a significant predictor of sE-selectin when BMI was included in the model because the patients with NIDDM were significantly heavier than the patients without NIDDM.

In patients with high triglyceride, the levels of sICAM-1, sVCAM-1, and sE-selectin were not associated with the levels of triglyceride or LDL cholesterol. Although sVCAM-1 levels were inversely associated with HDL cholesterol levels in univariate analyses, this association disappeared when sex was included in multivariable analyses. This is explained by the higher levels of HDL cholesterol and lower levels of sVCAM-1 in women and the reverse in men; however, there was no association between the levels of HDL cholesterol and sVCAM-1 when the sexes were analyzed separately. No association was found between the levels of HDL cholesterol and the levels of sICAM-1 or sE-selectin. However, patients with elevated triglyceride had increased levels of sCAMs compared with normal control subjects. Because age and sex were matched between patients and controls, age and sex could not explain the differences in the levels of sCAMs between the two groups, although higher levels of sICAM-1 and sE-selectin among the patients were in part attributable to NIDDM. In ANCOVA adjusted for NIDDM, clinical atherosclerosis, hypertension, and smoking, the levels of sICAM-1, sVCAM-1, and sE-selectin in the patients with hypertriglyceridemia were still significantly higher than those in the controls (P=.009, P=.01, and P=.0009, respectively). Therefore, NIDDM, clinical atherosclerosis, and other risk factors alone did not account for all the differences in the levels of sCAMs seen between the patients with hypertriglyceridemia and the normal controls.

**Short-term Effects of n-3 Fatty Acids on Levels of sCAMs**

After treatment of 19 patients with 4 g daily of Omacor for 6 weeks, the mean serum levels of triglyceride were reduced by 26%; the 20 patients in the placebo group had a 4% increase in triglyceride (P=.03) (Table 2). Decreases in the serum levels of total cholesterol were 11% in the Omacor group and 1% in the placebo group (P=.09); changes in HDL cholesterol were +14% in the Omacor group and -6% in the placebo group (P=.03). The levels of sE-selectin were significantly increased in the patients treated with Omacor (+11%) compared with the placebo group (−2%, P=.01) (Table 2). In contrast, the levels of sICAM-1 and sVCAM-1 were unchanged and similar in the Omacor and placebo groups.

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**TABLE 2. Short-term Effects of Omacor on Serum Levels of Lipids and sCAMs**

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Change, %</th>
<th>Baseline</th>
<th>Change, %</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Omacor (n=19)</td>
<td>Placebo (n=20)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TG, mg/dL</td>
<td>948±136.9 (756)</td>
<td>632±82.5 (536)</td>
<td>−26.3±7.3</td>
<td>875±77.5 (725)</td>
</tr>
<tr>
<td>TC, mg/dL</td>
<td>345.2±22.5 (326)</td>
<td>294.4±12.6 (288)</td>
<td>−11.4±3.4</td>
<td>330.1±14.0 (322)</td>
</tr>
<tr>
<td>HDL-C, mg/dL</td>
<td>18.7±1.3 (17)</td>
<td>21.8±2.6 (18)</td>
<td>+13.9±6.5</td>
<td>21.0±1.7 (18)</td>
</tr>
<tr>
<td>sICAM-1, ng/mL</td>
<td>310.2±25.1 (273)</td>
<td>334.5±33.8 (287)</td>
<td>+6.9±3.9</td>
<td>321.3±51.7 (279)</td>
</tr>
<tr>
<td>sE-selectin, ng/mL</td>
<td>88.7±10.3 (74)</td>
<td>99.8±13.4 (77)</td>
<td>+11.2±3.9</td>
<td>77.3±6.3 (75)</td>
</tr>
<tr>
<td>sVCAM-1, ng/mL</td>
<td>728.8±57.6 (628)</td>
<td>731.5±72.1 (629)</td>
<td>+0.47±4.77</td>
<td>756.3±87.2 (672)</td>
</tr>
</tbody>
</table>

TG indicates triglyceride; TC, total cholesterol; HDL-C, HDL cholesterol. Values are expressed as mean±SE (median).
Long-term Effects of n-3 Fatty Acids on Levels of sCAMs

After treatment with 4 g daily of Omacor for >6 months (n=27), the levels of triglyceride and total cholesterol were decreased by 47±4.6% and 21±3.2%, respectively, and levels of HDL cholesterol were increased (Table 3). HDL cholesterol level was measured by two different methods at baseline and long-term follow-up and thus cannot be easily compared. In contrast to the short-term treatment effects of Omacor seen at 6 weeks, long-term administration led to a significant reduction in levels of sICAM-1 of 9±3.4% (P =0.02) and a reduction in sE-selectin of 16±3.2% (P<0.0001) (Fig 2). Because of the marked variability in response to therapy as shown in Fig 2, we examined whether risk factors or changes in lipids, glucose, or BMI were predictive of the change in sCAMs.

In univariate exploratory analyses of risk factors, larger decreases in levels of sICAM-1 were associated with the presence of NIDDM (NIDDM: −27±7.6%, n=6; without NIDDM: −3.2±3.1%, n=21; P =0.02) and with larger decreases in BMI (P =0.05). Changes in the levels of serum lipids and fasting blood sugar were not associated with a decrease in the levels of sICAM-1. In the multivariable analysis, changes in BMI did not contribute to the reduction in sICAM-1 after NIDDM was in the model. The levels of sVCAM-1 were decreased in patients with NIDDM (NIDDM: −8.7±4.5%; without NIDDM: +13±4.6%; P =0.02), whereas they were increased in patients without NIDDM. In both univariate and multivariable analyses, the larger decreases in sE-selectin were associated with decreases in BMI (P <0.0001) and with NIDDM (P =0.04). Although the patients with NIDDM showed more marked reductions in sE-selectin, reductions were observed in 24 of 27 patients. The percent change in sE-selectin was −32±7.9% in patients with NIDDM and −12±2.7% in patients without NIDDM. Percent changes in sCAMs were not associated with percent changes in triglyceride, total cholesterol, or HDL cholesterol.

Effects of rsVCAM-1 on THP-1 and Peripheral Blood Monocyte Adhesion to HUVECs Stimulated With IL-1 for 24 Hours Under Flow Conditions

Because of previous studies that have shown that the interaction between VCAM-1 and \( \alpha_i \) integrin plays the dominant role under conditions of flow in vitro, we examined the effects of rsVCAM-1 on the adhesion of THP-1 cells and isolated peripheral blood monocytes to HUVECs stimulated with IL-1 for 24 hours in a parallel-plate flow system under flow conditions. rsVCAM-1 at a concentration of 1.0 \( \mu g/mL \), which is comparable to the levels found in some hypertriglyceridemic patients, inhibited THP-1 cell adhesion to HUVECs stimulated with IL-1 for 24 hours by 29.4±4.9% (Fig 3A). In contrast to THP-1 cells, monocytes isolated from peripheral blood were able to transmigrate through endothelial cells after adhesion. Because transmigrated monocytes were phase dark, transmigration was easily discerned under a phase-contrast microscope. The number of adherent monocytes indicated the number of monocytes on HUVECs and those that transmigrated. VCAM-1 at 1.0 \( \mu g/mL \) inhibited monocyte adhesion to HUVECs stimulated with IL-1 for 24 hours at 2.0 dyne/cm\(^2\) by 27.5±7.2% (Fig 3B).

It is known that the affinity of \( \alpha_i \) integrin remains low in unstimulated THP-1 cells and peripheral blood leukocytes.\(^{21}\)
To investigate whether activation of α₁ integrin modifies the inhibitory effect of sVCAM-1, we examined the effects of Mn²⁺ on the inhibition of THP-1 cell adhesion by rsVCAM-1. Mn²⁺ is known to induce conformational changes in α₁ integrin and to increase its affinity for VCAM-1. After activation with Mn²⁺, the number of THP-1 cells adherent to HUVECs stimulated with IL-1 for 24 hours was increased by approximately 70%. rsVCAM-1 at 0.5 μg/mL, which corresponds to the levels seen in the normal control group, inhibited Mn²⁺-activated THP-1 cell adhesion by 38.8 ± 2.3% but inhibited untreated THP-1 cell adhesion by only 14.4 ± 3.5% (Fig 4).

Discussion

In this report, we have shown that sICAM-1, sE-selectin, and sVCAM-1 were significantly increased in the serum of patients with severe hypertriglyceridemia in comparison with normal control subjects even after adjustment for consideration of NIDDM, clinical atherosclerosis, hypertension, and smoking. In the patients with elevated triglyceride levels, other risk factors also contributed to the elevation of sCAMs. Prolonged treatment with n-3 fatty acids (>7 months) led to a significant reduction in triglycerides and total cholesterol and an increase in HDL cholesterol and was associated with reduced levels of sICAM-1 and sE-selectin but not sVCAM-1. We have also demonstrated that levels of sVCAM-1 that are present in the serum of patients with elevated triglycerides can influence adhesion of monocytes to activated HUVECs under flow conditions in vitro.

Although considerable controversy still surrounds the issue of hypertriglyceridemia as a risk factor for atherosclerosis, recent studies have shown that premature atherosclerosis can occur in patients with familial chylomicronemia caused by mutations in the lipoprotein lipase gene. Defective lipolysis, which results in marked increases in triglycerides and decreases in HDL, may increase susceptibility to atherosclerosis in humans. The results of our study, which show that patients with increased levels of triglycerides and low HDL cholesterol have increased levels of sCAMs, are consistent with previous in vitro studies showing that oxidized fatty acids increase endothelial expression of CAMs in response to cytokines, whereas high levels of HDL inhibit the endothelial expression of CAMs in response to cytokines. Omacor contains 3.3 IU/g of vitamin E, which may have prevented the enhanced oxidation of fatty acids seen in some studies with fish oils. These data suggest that increased expression of CAMs may be a mechanism by which high triglyceride/low HDL promotes atherogenesis.

Previous reports by others have shown that diabetes mellitus is associated with elevated levels of circulating sE-selectin and sICAM-1. Among the 39 patients with hypertriglyceridemia that we studied, 11 patients had NIDDM, and these patients had higher levels of sE-selectin and sICAM-1. Increased levels of triglycerides and low HDL are frequently seen in diabetes mellitus, and several epidemiological studies have suggested that elevated triglyceride and low HDL may be particularly adverse for the development of coronary artery disease in patients with diabetes mellitus. Animal models of diabetes mellitus have shown increased expression of endothelial CAMs even without hyperlipidemia, and in vitro studies have shown that hyperglycemia influences adhesion of leukocytes to endothelial cells. Thus, the combined abnormalities of high triglyceride/low HDL and abnormalities of glucose metabolism, which are part of the insulin resistance syndrome, may synergistically increase the level of CAM expression. In multivariable analysis, increased BMI, which is also associated with insulin resistance, was a predictor for higher levels of sE-selectin and sVCAM-1.

The baseline levels of all three sCAMs that we studied were significantly higher in male than in female patients. In multivariable analyses, male sex remained a strong predictor of higher sCAMs. Blann et al reported that levels of sE-selectin in normal subjects were significantly higher in males than in females, although they did not find any significant differences in levels of sICAM-1 or sVCAM-1. Estradiol has been shown by two groups to reduce IL-1–induced expression of CAMs at the mRNA level by inhibiting transcription in vitro, with conflicting results reported by a third group using different methods. Our results support the hypothesis that estrogen may suppress CAM expression in vivo, and this may partially explain why the incidence of atherosclerosis in premenopausal women is reduced compared with that in men.

Although patients with elevated triglycerides had significantly higher levels of sCAMs compared with normal control subjects, we did not find a correlation between the levels of triglycerides and sCAMs among patients with severe hypertriglyceridemia. The lack of correlation may be due to several factors. First, because of the inclusion criteria (fasting triglyceride level of >500 mg/dL but <2000 mg/dL), all of the patients had marked defects in triglyceride metabolism, with a mean fasting (>12 hours) triglyceride level of 911 ± 77 mg/dL. Unlike LDL cholesterol levels, which remain relatively constant throughout the day, triglyceride levels can increase dramatically in the postprandial state after a fat load; the magnitude and duration of postprandial hypertriglyceridemia
correlates better with atherosclerosis than does the fasting value. Therefore, single fasting values among patients in this study may have had insufficient predictive value. Also, although all patients had severely elevated triglyceride, they were heterogeneous in regard to the defect leading to hypertriglyceridemia and probably in the types of triglyceride-rich particles. The atherogenicity of triglyceride-rich lipoproteins may vary depending on the underlying metabolic defect and particle composition, as well as degree of oxidation, which could be influenced by other risk factors. Age and sex could not account for the differences in sCAM levels between patients and controls because age and sex were matched between the two groups. Even after adjustment for other risk factors—NIDDM, clinical atherosclerosis, hypertension, and smoking—the levels of sICAM-1, sVCAM-1, and sE-selectin were significantly higher in the patients with hypertriglyceridemia than in the control subjects. Thus, hypertriglyceridemia was independently associated with increased levels of sCAMs.

To obtain further evidence that the association of elevated triglyceride/low HDL to increased sCAMs was causally related, we examined whether improvement in levels of triglyceride and HDL cholesterol via pharmacological intervention with a purified form of n-3 fatty acids, Ommacor, would influence the levels of sCAMs. After treatment with n-3 fatty acids for >7 months, the levels of triglyceride were decreased by 47% and the levels of HDL cholesterol were increased. Accordingly, the levels of sICAM-1 and sE-selectin were decreased by 9% and 16%, respectively, with more impressive reductions of 27% and 32% noted in the patients with NIDDM. The reduction in the levels of sICAM-1 and sE-selectin after the treatment most probably were secondary to the improvement in dyslipidemia, which included both decreased triglycerides and increased HDL cholesterol. Docosahexaenoic acid and eicosapentaenoic acid are known to inhibit the expression of E-selectin and VCAM-1 induced by cytokines or lipopolysaccharide on cultured endothelial cells in vitro. However, we believe that most of the reduction in the levels of s-selectin and sICAM-1 was due to the improvement in dyslipidemia and not to direct effects of n-3 fatty acids, since the level of sVCAM-1 was not reduced as would have been predicted from the in vitro experiments.

We have previously postulated that increased levels of sCAMs may be related to the extent of atherosclerosis, the activity of atherosclerosis, or endothelial dysfunction. We have recently observed from a large epidemiological study, the Atherosclerosis Risk in Communities (ARIC) trial, that patients who developed coronary artery disease events or who had carotid artery disease had increased levels of sCAMs compared with cohort controls after adjustment for all measured risk factors. However, it is highly improbable that treatment of dyslipidemia with fish oils could lead to a significant reduction in the extent of atherosclerosis within 12 months based on previous clinical data. In our previous study, levels of sE-selectin but not sICAM-1 were significantly decreased after treatment of hypercholesterolemia with an HMG-CoA reductase inhibitor for 6 months. Because E-selectin is expressed exclusively by endothelial cells after activation, the levels of sE-selectin should reflect the activation state of endothelial cells. Thus, our previous and present studies suggest that levels of sE-selectin in the serum of dyslipidemic patients may be a marker of endothelial cell activation or dysfunction. On the other hand, since ICAM-1 is expressed by a wide variety of cell types, including intimal smooth muscle cells as well as endothelial cells, the interpretation of changes in levels of sICAM-1 is more complicated and may be related to the “activity” of multiple cell types in atherosclerotic lesions.

In contrast to sICAM-1 and sE-selectin, sVCAM-1 levels were not reduced by therapy with fish oils. This was somewhat surprising because previous in vitro studies had suggested that fish oil would inhibit cytokine-induced expression of VCAM-1. Although triglyceride levels were improved substantially by therapy, they remained elevated, and perhaps normalization of triglyceride would be required to reduce levels of sVCAM-1. As previously mentioned, it is unlikely that fish oil therapy for <1 year significantly changed the extent of atherosclerosis, which has been shown to correlate to the levels of VCAM-1 in one study.

In this study, we found that the levels of sE-selectin were increased after administration of n-3 fatty acids for 6 weeks and then decreased after more prolonged treatment. Although the mechanisms by which these soluble molecules are released into the circulation are not well known, Newman et al demonstrated that membrane-bound E-selectin was cleaved into the soluble form in vitro. This report suggests that levels of circulating sE-selectin may be influenced by the rate of proteolytic cleavage besides cell surface expression. Therefore, it is possible that the initial increase may be due to the enhanced cleavage of E-selectin from endothelial cell surfaces by n-3 fatty acids.

Proteolytic cleavage of CAMs may serve as a regulatory mechanism to inhibit leukocyte adhesion to the vessel by reducing expression on the cell surface. The soluble forms may be mere metabolites, or they may also influence vascular function. Immunoprecipitations of sCAMs from serum have shown that these molecules were only slightly smaller than the membrane-bound forms, suggesting that they lack membrane and cytoplasmic regions but are otherwise intact. Purified sICAM-1 from serum was reported to support $\beta_2$ integrin–dependent adhesion of lymphocytes. Koch et al have shown that sVCAM-1 and sE-selectin in the synovial fluid of patients with rheumatoid arthritis induced chemotaxis of endothelial cells and were angiogenic in rat corneas. Therefore, circulating sCAMs may influence the interaction of leukocytes with the vascular endothelium.

We have previously shown that under flow conditions, monocytoid cell line THP-1 cells utilized the VCAM-1/α4 integrin pathways for both primary (tethering) and secondary (firm) adhesion to IL-1–stimulated endothelial cells when VCAM-1, ICAM-1, and E-selectin were upregulated by cytokines. E-selectin– and CD18-dependent pathways were also involved in this system but played minor roles compared with the VCAM-1/α4 integrin pathway. Therefore, we postulated that sVCAM-1 would play the most important role of the sCAMs in the regulation of monocyte-endothelial adhesion. We found that rsVCAM-1 at 1 µg/mL significantly
inhibited unstimulated THP-1 cell and peripheral blood monocyte adhesion to IL-1-stimulated endothelial cells under flow conditions, whereas rsVCAM-1 at 0.5 μg/mL did not show significant effects. In contrast, if THP-1 cells were incubated with MnCl2, rsVCAM-1 at 0.5 μg/mL inhibited the adhesion to the same levels as 1.0 μg/mL. It is known that α4 integrin on unstimulated THP-1 cells and resting peripheral blood leukocytes is in the low-affinity state.21 Jakubowski et al20 reported that there was little binding of VCAM-1 immunoglobulin to resting peripheral T cells even at a concentration of 100 μg/mL, whereas VCAM-1 immunoglobulin at a concentration as low as 0.03 μg/mL bound to T cells stimulated with MnCl2. These findings may explain why rsVCAM-1 at lower concentrations was effective in inhibiting the binding of THP-1 cells incubated with MnCl2. Thus, our results suggest that sVCAM-1 in the serum may negatively regulate resting monocyte adhesion only at high concentrations seen in patients with hypertriglyceridemia, and if monocytes are activated, sVCAM-1 even at normal concentrations may partially inhibit monocyte adhesion.

In conclusion, the levels of sE-selectin, sICAM-1, and sVCAM-1 in the serum of patients with hypertriglyceridemia were increased, and multivariable analyses showed that this increase was independent of other risk factors. Marked reduction of triglyceride and increase of HDL cholesterol by concentrated fish oil therapy led to a reduction in the levels of sE-selectin and sICAM-1 but not sVCAM-1, which was most prominent in patients with NIDDM. These results support previous in vitro data suggesting that disorders of triglyceride and HDL metabolism may promote atherogenesis through effects on CAMs and that treatment of dyslipidemia with fish oils may alter vascular cell activation. Our in vitro study suggested that the increased sVCAM-1 in the serum may play a role in the regulation of monocyte adhesion.

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