Weight Loss, but Not Fish Oil Consumption, Improves Fasting and Postprandial Serum Lipids, Markers of Endothelial Function, and Inflammatory Signatures in Moderately Obese Men1–3

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Abstract

Overweight persons are at risk for cardiovascular diseases, which may relate to a disturbed endothelial function and pro-inflammatory serum profiles. Indeed, weight loss lowers cardiovascular disease risk, but weight maintenance is difficult. Therefore, dietary supplements such as fish oil, which improve endothelial function, are useful. In this study, we evaluated effects of fish oil and moderate weight loss in the same population. For this, 11 normolipidemic healthy, moderately obese men (BMI 30–35 kg/m2) received in random order 1.1 g/d eicosapentanoic acid (EPA) + docosahexaenoic acid (DHA) or oleic acid (control) for 6 wk. In the 3rd period, 8 of the 11 subjects consumed low-energy diets (2 MJ/d) for 4 wk followed by 4 wk weight stabilization. Their body weight was reduced by 9.4 ± 2.0 kg (P < 0.05). On the final day of all 3 periods, a postprandial test was conducted. Weight loss lowered fasting and postprandial plasma triacylglycerol (TG) responses (P < 0.001), whereas fish oil reduced only postprandial TG (P = 0.006). Fish oil did not affect soluble intercellular adhesion molecule (s-ICAM), whereas weight loss reduced fasting (P = 0.009) and postprandial s-ICAM responses (P < 0.001). Fasting s-ICAM and TG correlated (r = 0.68; P = 0.029), as did changes in fasting s-ICAM and TG during weight loss (r = 0.80; P = 0.029) and fish oil treatment (r = 0.76; P = 0.009). Fasting (P = 0.027) and postprandial (P < 0.001) serum C-reactive protein were lowered by weight loss. The postprandial monocyte chemoattractant protein-1 response was lowered by fish oil and after weight loss (P < 0.001). This indicates that 1.1 g/d EPA+DHA supplied for 6 wk, in contrast to ∼10 kg weight loss, does not improve markers of endothelial function and inflammation. J. Nutr. 137: 2635–2640, 2007.

Introduction

A low-grade chronic inflammation of the vessel wall contributes to the development of atherosclerotic plaques. This is accompanied by an increased expression of cellular adhesion molecules (CAM)4 to which leucocytes attach and consequently migrate into the subendothelial space. In support of this mechanism is the finding that in vitro, cultured endothelial cells increase CAM expression after treatment with pro-inflammatory stimuli (1,2). Soluble shedded forms of these CAM (s-CAM) are found in the circulation. Although their precise role is unclear, their concentrations may be considered markers of endothelial activation (3). Raised plasma s-CAM concentrations have been reported in patients with hyperlipidemia (4), diabetes (5), obesity (6), and atherosclerosis (7). More importantly, high s-CAM concentrations are strong predictors of future coronary events (8,9) and elevated concentrations of monocyte chemoattractant protein (MCP-1) predict future coronary heart disease (CHD) risk (10).

Obese and overweight persons are at increased risk for cardiovascular diseases for which a cluster of metabolic abnormalities is responsible (11). Important characteristics of increased body weight are the elevated levels of circulating acute phase proteins, pro-inflammatory cytokines, chemokines, and s-CAM (6,12–14). Therefore, weight loss is an important strategy to lower cardiovascular risk. In fact, even moderate weight loss (∼10 kg) significantly lowered TNFα, IL-6, s-CAM, C-reactive protein (CRP) (13,15), and MCP-1 (14) concentrations. These intervention studies suggest that at least a part of the cardioprotective effects of weight loss can be attributed to a lower pro-inflammatory profile, whereas the reductions in s-CAM and MCP-1 may imply an improved endothelial function.
Although weight loss can be achieved fairly quickly, weight maintenance is much more difficult. Therefore, it would be valuable if certain dietary supplements would have comparable beneficial effects as weight loss. In this respect, (n-3) PUFA have often been suggested as an effective antiinflammatory and antiatherogenic intervention. Evidence for this assumption arises from epidemiological as well as clinical studies (16). How effects of fish oil compare to those of weight loss has not been studied systematically. In this study, we therefore evaluated the effects of both interventions in the same population of moderately obese men on an inflammatory signature in plasma of fasting subjects using an antibody array as well as on individual markers in plasma reflecting endothelial activation [i.e. soluble intercellular adhesion molecule (s-ICAM), E-selectin, MCP-1, and hs-CRP]. For this, all 11 men first participated in a placebo-controlled intervention study with a crossover design comparing the effects of fish oil vs. placebo. In a stage 2, 8 of these men participated in a weight loss study that enabled us to compare the effects of fish oil consumption and weight loss in these men side-by-side. Because insulin sensitivity and s-CAM concentrations may be related (17,18), in combination with the finding that high-fat meals trigger postprandial endothelial activation (19), we also decided to evaluate changes in s-ICAM, E-selectin MCP-1, and hs-CRP concentrations during the postprandial phase.

Methods

Subjects

After screening, 11 men who met all of our inclusion criteria completed the first part of the study, fish oil vs. placebo, successfully. These participants were 59±9 y of age (mean ± SD), were not hyperlipidemic [plasma total cholesterol, 5.72±0.93 mmol/L; HDL cholesterol, 1.00±0.19 mmol/L; LDL cholesterol, 4.42±0.85 mmol/L; and triacylglycerol (TG), 1.53±0.60 mmol/L], had a BMI between 30 and 35 kg/m², and did not use any medications known to influence the variables of interest. Two of the participants smoked; however, excluding the smokers in a post hoc analysis did not essentially change the outcome of the study. After the first part of the study, 8 of the volunteers were willing to participate in another intervention trial in which they consumed a low-energy diet. The entire study was approved by the medical ethical committee of Maastricht University and all subjects gave their written informed consent before the start of the study.

Diets and design

The fish oil supplementation part of the study had a double-blind, placebo-controlled crossover design. Each subject received 2 different types of capsules for 6 wk in random order with a wash-out period of 2 wk between the diet periods. All participants were instructed by a dietician to consume a diet according the Dutch dietary guidelines throughout the study. During the study, the subjects were asked to consume 6 capsules (Marinol C45, Loders Croklaan) that provided either 1.1 g fish oil [0.6 g eicosapentanoic acid (EPA) + 0.5 g docosahexanoic acid (DHA)] or a high-oleic acid (80%) sunflower oil (control). The daily intake of 1.1 g EPA + DHA corresponds to 50–75 g fatty fish/d. Subjects were not allowed to consume fatty fish during both intervention periods. During the study, subjects recorded in diaries any symptoms of illness, visits to physicians, medication use, alcohol use, and deviations from the protocol. Body weight was recorded weekly. At the end of both periods, energy and nutrient intakes were estimated by filling in food frequency lists. Also at the end of both periods, all subjects participated in a postprandial test (see below).

Part 2 of the study (weight loss) started 2 wk after the final test day of the fish oil study. In this part, 8 subjects consumed a commercially available low-energy diet (Modifast Nutrition) for 4 wk providing 2 MJ/d. Subjects were allowed to consume energy-free beverages ad libitum throughout the day and were allowed to consume 250 g vegetables or fruits (except bananas) per day. For the following 2 wk (wk 5 and 6), subjects consumed a mixed solid energy-restricted diet providing ~4.2 MJ/d. For the next 2 wk (wk 7 and 8), subjects consumed a diet matching their energy requirements to maintain their newly achieved body weights. At the last day of this period, subjects again participated in a postprandial test, as described below.

Postprandial test

For the postprandial test, subjects arrived early at the university after an overnight fast. After blood sampling, subjects consumed within 15 min a milkshake containing 3028 kJ (724 kcal) energy provided by 50.1 g fat [62.3 energy percent (en%), 24.9 en% as SFA, 25.2 en% as monounsaturated fatty acids, and 12.2 as PUFA], 9.3 g protein (5.1 en%), and 58.8 g carbohydrates (32.6 en%)]. For the next few hours, blood was sampled regularly. During this postprandial period, subjects were not allowed to eat or drink anything except water.

Blood sampling and analyses

Blood sampling. Subjects arrived early in the morning after an overnight fast and after abstaining from drinking alcohol the preceding day and smoking on the morning before blood sampling. Three blood samples from fasting were taken with 15-min intervals before the start of the postprandial test. Before analysis, we pooled these 3 fasting blood samples and regarded them as 1 fasting sample (t = 0 h). Next, at various time points (Fig. 1) up to 5 h after the postprandial test was started, blood was sampled regularly. All blood samples were taken via a canula placed into the vein draining the hand. Blood was sampled into vacutainers containing EDTA (final concentration 1 g/L EDTA). Plasma was obtained by centrifugation at 2000 × g; 30 min at 4°C directly after sampling. All samples were immediately stored in aliquots, snap-frozen, and stored at −80°C until analysis.

Plasma lipids and lipoproteins. Fasting and postprandial total cholesterol (CHOD/PAP method, Roche Diagnostics), HDL cholesterol (after precipitation of apo-B-containing lipoproteins; precipitation method, mononest cholesterol, Boehringer), and TG (GPO-trinder; Sigma Diagnostics) concentrations were analyzed in plasma enzymatically. All samples from 1 subject were analyzed in 1 run at the end of the study.

Inflammatory signatures. To simultaneously detect expression patterns of multiple cytokines, chemokines, and growth factors, we used the human cytokine antibody array III (Ray Biotech). For this, fasting plasma samples from 8 volunteers participating in both intervention trials were pooled at the end of each of the 3 periods before analysis. This means that we analyzed 3 arrays (end of the fish oil period, control period, and weight loss period) essentially as described earlier (20). For both the fish oil intervention as well as the weight loss period, responses were calculated as the percentage change vs. the control period.

FIGURE 1 Plasma TG concentrations in moderately obese men before the milkshake (t = 0) and 2 and 4 h postprandial at the end of the control period, fish oil period, and weight loss period. Values are means of the 8 subjects participating in all 3 tests. Changes over time during the postprandial periods were examined by ANOVA with diet and time as factors. *P < 0.01 vs. control period; #P < 0.01 vs. fish oil period.
s-ICAM and sE-selectin, MCP-1, and hs-CRP plasma concentrations. s-ICAM and sE-selectin concentrations were analyzed in EDTA plasma by ELISA (provided by Prof. W. Buurman, Department of General Surgery, Maastricht University) as described (1,21). MCP-1 concentrations were measured with a commercially available ELISA (R&D Systems Europe), and hs-CRP concentrations by an immunoturbidimetric assay (Kamiya Biomedical). All samples from 1 subject were always analyzed in 1 run at the end of the study.

Plasma glucose and insulin, homeostasis model assessment of insulin resistance, and plasma phospholipid fatty acid composition. Blood glucose and insulin concentrations were analyzed in plasma as described (22). In addition, we used the homeostasis model assessment of insulin resistance as the index to measure insulin resistance (23). To assess dietary compliance, plasma phospholipid fatty acid composition was analyzed (24).

Statistics. We compared differences in fasting levels of the variables between interventions with a paired t test (α = 0.05). Using the results from the control period, Pearson correlation coefficients were calculated between fasting concentrations of the markers reflecting endothelial function (s-ICAM, sE-selectin, and MCP-1) and the inflammatory marker hs-CRP with TG, insulin, and glucose concentrations, and percentage of body fat. In addition, Pearson correlation coefficients were calculated between changes in fasting concentrations of s-CAMS or hs-CRP during the 2 interventions (fish oil or weight loss compared with the control period) with changes in fasting TG, insulin, or glucose. Changes over time of variables measured during the postprandial periods were examined by ANOVA with diet and time as factors. To compare the effects of fish oil with changes in fasting TG, insulin, or glucose. Changes over time of the markers reflecting endothelial function or low-grade systemic inflammation, whereas weight loss did (Table 1). However, weight loss did not differ between the control and fish oil periods (data not shown).

At the end of the 8-wk, low-energy diet period, fasting serum TG, LDL cholesterol, and insulin concentrations were lowered by 0.60 mmol/L (P = 0.028), 0.28 mmol/L (P = 0.022), and 14.8 pmol/L (P = 0.030), respectively (Table 1). Fasting glucose and total cholesterol concentrations were not changed by weight loss (Table 1). Further, TG concentrations remained lower and showed a less steep rise (Fig. 1) during the entire postprandial period at the end of the weight loss period, both compared with the control (P < 0.001) and the fish oil (P < 0.001) periods.

Inflammatory signatures. Expression of multiple cytokines, chemokines, and growth factors was quantified in fasting plasma samples by using a cytokine antibody array approach. An inflammatory signature of the pooled fasting plasma samples at the end of the control period (absolute values in arbitrary units) is shown in Supplemental Figure S1. Relative changes of the fish oil and weight loss intervention compared with the control period are shown in Supplemental Figure S2. As already reported, based on analysis in individual samples, we observed a weight loss-induced reduction in fasting TNFα concentrations compared with the control and fish oil periods (22). This was also confirmed in the arrays. The reductions in insulin-like growth factor-1 and leptin at the end of the weight loss period compared with control and/or fish oil periods can be regarded as positive controls for the array approach. We conclude that fish oil did not improve markers reflecting endothelial function or low-grade systemic inflammation, whereas weight loss did (Table 1). However, weight loss and fish oil consumption had comparable effects (Supplemental Fig. S2). There were only a few differences between weight loss and fish oil consumption, which were most consistent for the chemokines. Weight loss, for example, lowered the expression of MCP-1, MCP-2, MCP-3, regulated upon activation, normal T-cell expressed and secreted (RANTES) and macrophage inflammatory protein 16 compared with the effect of fish oil.

### Table 1 Effects of fish oil and weight loss in moderately obese men

<table>
<thead>
<tr>
<th></th>
<th>Control period</th>
<th>Fish oil</th>
<th>Weight loss</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>11</td>
<td>11</td>
<td>8</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>31.3 ± 2.1</td>
<td>31.3 ± 1.8</td>
<td>27.9 ± 1.5***</td>
</tr>
<tr>
<td>Waist to hip</td>
<td>1.05 ± 0.04</td>
<td>1.05 ± 0.03</td>
<td>1.02 ± 0.04****</td>
</tr>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>5.10 ± 0.71</td>
<td>5.18 ± 0.96</td>
<td>5.45 ± 0.48</td>
</tr>
<tr>
<td>HDL cholesterol, mmol/L</td>
<td>1.08 ± 0.22</td>
<td>1.13 ± 0.24</td>
<td>1.10 ± 0.24</td>
</tr>
<tr>
<td>LDL cholesterol, mmol/L</td>
<td>3.77 ± 0.70</td>
<td>3.83 ± 0.96</td>
<td>3.31 ± 0.48****</td>
</tr>
<tr>
<td>TG, mmol/L</td>
<td>1.24 ± 0.72</td>
<td>1.11 ± 0.47</td>
<td>0.70 ± 0.47****</td>
</tr>
<tr>
<td>Glucose, mmol/L</td>
<td>6.26 ± 1.40</td>
<td>6.21 ± 1.13</td>
<td>5.92 ± 0.80</td>
</tr>
<tr>
<td>Insulin, pmol/L</td>
<td>52.4 ± 27.5</td>
<td>48.6 ± 21.3</td>
<td>40.2 ± 21.6*</td>
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<tr>
<td>s-ICAM, ng/L</td>
<td>106.8 ± 32.9</td>
<td>102.5 ± 32.4</td>
<td>80.9 ± 24.1****</td>
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<tr>
<td>sE-selectin, ng/L</td>
<td>18.8 ± 13.5</td>
<td>18.4 ± 10.2</td>
<td>16.8 ± 8.2</td>
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<td>MCP-1, ng/L</td>
<td>150.8 ± 43.2</td>
<td>150.4 ± 38.4</td>
<td>145.7 ± 51.2</td>
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<td>hs-CRP, ng/L</td>
<td>2.55 ± 1.57</td>
<td>2.41 ± 1.73</td>
<td>2.10 ± 1.25*</td>
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<tr>
<td>sICAM, ng/L</td>
<td>12.2 ± 0.96</td>
<td>12.0 ± 0.93</td>
<td>11.8 ± 0.88</td>
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<tr>
<td>sE-selectin, ng/L</td>
<td>4.3 ± 0.65</td>
<td>4.2 ± 0.61</td>
<td>4.0 ± 0.57</td>
</tr>
<tr>
<td>MCP-1, ng/L</td>
<td>1.13 ± 0.04</td>
<td>1.10 ± 0.04</td>
<td>0.96 ± 0.04**</td>
</tr>
</tbody>
</table>

1 Values are means ± SD. *P < 0.05 vs. control period; **P < 0.01 vs. control period; ***P < 0.001 vs. fish oil period.
2 EPA and DHA content were analyzed in plasma phospholipids. All other variables were analyzed in plasma.
consumption, whereas expression of T-lymphocyte-secreted protein 1309, epidermal growth factor, and IL-15 was selectively elevated by weight loss.

**s-ICAM, sE-selectin, MCP-1, and hs-CRP.** Compared with the control period, 6-wk fish oil consumption did not significantly change s-ICAM concentrations (\( P = 0.285; 95\% \text{ CI}, −12.8 \text{ to } 4.2 \text{ ng/L} \); Table 1). In contrast, weight loss significantly reduced s-ICAM concentrations by \( 15 \pm 10.2\% \) (\( P = 0.009; 95\% \text{ CI}, −25.6 \text{ to } −5.3 \text{ ng/L} \)). In addition, s-ICAM was also lower after weight loss compared with the fish oil period (\( P = 0.036; 95\% \text{ CI}, −20.5 \text{ to } −0.9 \text{ ng/L} \)). Both fish oil and weight loss did not change fasting sE-selectin or MCP-1 concentrations (Table 1). Fasting hs-CRP concentrations were lowered after weight loss (\( P = 0.027; 95\% \text{ CI}, −1.54 \text{ to } −0.13 \text{ mg/L} \)) but not after fish oil consumption.

The postprandial changes in s-ICAM, sE-selectin, MCP-1, and hs-CRP concentrations are shown in **Figure 2**. The lower fasting concentration of s-ICAM after weight loss was maintained in the postprandial state. Therefore, the postprandial s-ICAM curve after the weight loss period differed from the control and fish oil periods (\( P < 0.001 \)). There was no effect on postprandial sE-selectin concentrations. Both fish oil consumption as well as weight loss resulted in significantly different postprandial MCP-1 curves compared with the control period. The decrease was more pronounced after the weight loss period than after fish oil supplementation (\( P < 0.01 \)). As for s-ICAM, the lower fasting hs-CRP concentration after weight loss was maintained throughout the entire postprandial state (\( P < 0.001 \) vs. the control and fish oil periods).

At the end of the control period, fasting s-ICAM and TG concentrations were positively correlated (\( r = 0.68; P = 0.029 \)). In addition, changes in fasting s-ICAM concentrations correlated positively with those in fasting TG during the weight loss period (\( r = 0.80; P = 0.029 \)) and during the fish oil period (\( r = 0.76; P = 0.009 \)). These associations with TG concentrations were not found for sE-selectin, whereas fasting TG concentrations were associated with fasting MCP-1 concentrations (\( r = 0.64; P = 0.033 \)). Fasting MCP-1 concentrations were also positively associated with fasting insulin (\( r = 0.64; P = 0.034 \)) and glucose (\( r = 0.73, P = 0.009 \)) concentrations as well as with the homeostasis model assessment of insulin resistance index (\( r = 0.74; P = 0.007 \)).

**Discussion**

In this study, we found that 1.1 g/d EPA + DHA [as part of 1.35 g (n-3) fatty acids in total as provided by the capsules] supplemented for a period of 6 wk, in contrast to moderate weight loss, did not improve circulating concentrations of markers reflecting endothelial dysfunction and low-grade inflammation associated with CHD.

(n-3) Fatty acids and fish oil in particular have often been suggested as effective antiatherogenic dietary compounds (16). Indeed, the GISSI-Prevention Study (25) showed a significant benefit in terms of fatal cardiovascular events by (n-3) EPA and DHA fatty acids. However, the pathways underlying these effects are not known. One potential explanation might relate to the antiinflammatory effects of fish oil (26) as has already been demonstrated in various inflammatory disorders (27,28). Studies on the effects of fish oils on adhesion molecules, which did not change in our study, are not consistent. De Caterina et al. (29) have shown that in vitro the (n-3) fatty acid DHA lowered CAM expression in cultured endothelial cells after pro-inflammatory stimulation. Also in vivo, reductions in s-VCAM, s-ICAM, and/or sE-selectin concentrations have been described after consumption of fish oil for 6 wk to 3 yr. These studies were conducted in various populations (30–33). In contrast, others found in CHD and hyperlipidemic patients increased sE-selectin and s-VCAM after 6–26 wk fish oil consumption (34,35). Daily intakes of fish oils ranged from 1 to 3.4 g in the first 4 positive in vivo studies (30–33) and was ~5 g/d in the latter 2 negative studies (34,35). This indicates that the absence of protective effects on endothelial function in our study cannot be explained by the moderate daily fish oil intake of 1.1 g/d or study duration. Not only effects of fish oil on adhesion molecule expression but also on markers reflecting low-grade systemic inflammation are conflictive (36,37). Madson et al. (38) found no significant effects on hs-CRP concentrations of 2 different doses of fish oils (2.0 and 6.6 g/d) in healthy volunteers. This finding was confirmed by studies with healthy middle-aged subjects consuming 1.3 g EPA + DHA for 12 wk (39) and type II diabetic patients consuming 4.0 g purified EPA + DHA for 6 wk (40). Also, patients with CHD did not benefit from 6-wk consumption of 2.9 g/d fish oil in terms of hs-CRP reductions (31). In contrast to these studies using doses up to 6.6 g/d fish oil without effects, megadoses of fish oil lowered hs-CRP in healthy women, in which 7 g/d fish oil was more effective compared with 14 g/d (41). As for markers reflecting endothelial function, this suggests that the absence of protective effects on hs-CRP by fish oil in our study cannot be explained by the moderate daily fish oil intake, by study duration, or by the absence of (inflammatory) disease from the participants.

Effects of fish oil on circulating concentrations of the chemokine MCP-1 have hardly been studied. Only in vitro experiments have shown that both EPA and DHA lowered LPS-induced MCP-1 protein concentrations in culture medium of tubular HK-2 cells (42). It is, however, not known if a similar response can be expected in endothelial cells. Our antibody array data suggest that fish oil, in contrast to moderate weight loss, does not lower fasting MCP-1 or concentrations of other chemokines. Confirming
this result by ELISA showed no effects on fasting MCP-1 both by fish oil or moderate weight loss. However, the pattern of a lowered expression after weight loss compared with both the control and fish oil periods is in agreement with that seen on the antibody array.

Altogether, placing the results from our study in the perspective of the literature available can only lead to the conclusion that a protective effect of increased fish intake on the risk for CHD (16) cannot be explained conclusively via their effects on endothelial function and low-grade systemic inflammation.

In contrast to fish oil consumption, moderate weight loss (10 kg) significantly improved markers reflecting endothelial dysfunction and low-grade systemic inflammation. More interestingly, these reductions were not only evident in the fasting state but also during the 4-h postprandial period. This latter finding is highly relevant, because humans are in the postprandial state most of the day and it is increasingly acknowledged that disturbances in the postprandial phase increase atherosclerotic risk.

Consistent with this, weight loss also lowered both fasting and postprandial TG concentrations. Interestingly, we found a strong, positive correlation between fasting concentrations of TG and s-ICAM and MCP-1. Also the changes in s-ICAM and the changes in TG concentrations during weight loss and during fish oil consumption were correlated. Apparently, a reduction in serum TG has a protective effect on the endothelium, which results in reduced dysfunction. This finding is consistent with higher s-CAM levels reported in hyperterglycemic patients compared with healthy controls (33).

In addition to examining effects of both interventions on individual markers of endothelial activation and inflammation, we simultaneously evaluated changes in inflammatory signatures of plasma from fasted subjects using an antibody array approach. This knowledge can be used to gain insight into the effects of both interventions on a broader scale and to obtain new leads for mechanisms underlying the observed differences on s-ICAM and hs-CRP levels by fish oil and the weight loss intervention. Particularly, the finding that numerous chemokines show the same pattern (i.e., a lowered expression after weight loss compared with fish oil intervention) makes the moderate changes in individual chemokines an interesting finding. MCP-1 is an endothelium-derived chemokine, predominantly excreted in response to endothelial damage, which can therefore be regarded as a marker for endothelial activation. Moreover, a higher expression of MCP-1 may be an independent risk marker for future coronary events (10,43). Similarly, RANTES, which was also lowered by weight loss compared with fish oil consumption, probably plays a causal role in atherosclerosis (44). Based on these findings, we postulate that the main difference between fish oil and weight loss intervention that may explain the observed improvement in markers reflecting endothelial function during weight loss is the reduction in chemokines in general. These results should be regarded as hypothesis generating and need to be confirmed in especially-forthis-purpose designed studies.

In conclusion, we have shown in a population of healthy slightly overweight men that in contrast to moderate weight loss, daily intake of a realistic amount of the fish oils EPA and DHA (1.1 g/d) does not have an effect on s-ICAM, MCP-1, and hs-CRP concentrations. This indicates that the existent protective effect of fish oil on the occurrence of coronary events is probably not mediated by the same mechanism as assumed for weight loss. Finally, based on changes in inflammatory expression profiles between weight loss and fish oil consumption, we postulate a prominent role for the chemokines in the protective effect of weight loss on endothelial dysfunction. This assumption deserves, however, further study.

**Acknowledgments**

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