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Serum levels of interleukin-18 are reduced by diet and n-3 fatty acid intervention in elderly high-risk men

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Abstract

Inflammation plays a central role in the development and progression of atherosclerosis, and inflammatory markers have been reported to predict cardiovascular events. Mediterranean-like diet and very long chain omega-3 polyunsaturated fatty acid (n-3 PUFA) supplementation have been reported to reduce the risk of cardiovascular mortality and morbidity, but the mechanisms are not fully clarified. The aims of the present study were to investigate the effect of such interventions on serum levels of inflammatory markers, and potential associations with changes in serum fatty acids and anthropometric measures. This was a randomized 2 × 2 factorial-designed trial comparing the effect of 3 years of dietary counseling, n-3 PUFA supplementation (2.4 g/d), or both on different measures of atherosclerosis in elderly high-risk men (N = 563). Levels of interleukin-18 (IL-18) were decreased by diet (−10.5% vs baseline, $P = .012$ compared with no diet) and by n-3 PUFA supplementation (−9.9% vs baseline, $P = .008$ compared with placebo). Other measured inflammatory markers were not affected. Changes in IL-18 were significantly correlated to changes in triglycerides ($r = 0.20$, $P < .001$), eicosapentaenoic acid ($r = −0.14$, $P = .030$), docosahexaenoic acid ($r = −0.14$, $P = .034$), body mass index ($r = 0.16$, $P < .001$), and waist circumference ($r = 0.12$, $P = .007$). In conclusion, levels of IL-18 were significantly reduced by Mediterranean-like diet and n-3 PUFA supplementation. However, the changes correlated only weakly to changes in triglycerides, serum fatty acids, and anthropometric measures. The cardioprotective effects of both interventions might thus in part be explained by reduced levels of IL-18, but probably beyond changes in serum fatty acids and body composition.

1. Introduction

Inflammation plays a central role in the development and progression of atherosclerosis from the fatty streak to the complex atheromatous plaque formation [1]. Circulating levels of inflammatory markers such as C-reactive protein (CRP) and interleukin-6 (IL-6) have been reported to predict cardiovascular events [2]. Recently, interleukin-18 (IL-18) has emerged as a predictor of coronary events; and a role in plaque destabilization has been suggested [3,4].

Mediterranean-like diet and very long chain polyunsaturated omega-3 fatty acid (n-3 PUFA) supplementation have been reported to reduce the risk of cardiovascular morbidity and mortality [5-8]. Still, the mechanisms behind these protective effects are not fully explained.

Diet interventions have been shown to improve endothelial function and the concentrations of serum lipoproteins [9,10]. However, the effect on inflammation by various dietary strategies has been conflicting [11]. Indeed, adipose tissue is an active endocrine organ that releases a large number of metabolically active substances known as *adipokines*, including several proinflammatory markers such as monocyte chemoattractant protein–1 (MCP-1) and tumor necrosis factor–α (TNF-α) and the anti-inflammatory marker adiponectin [12]. Thus, the interpretation of especially calorie-restricted diet interventions is complicated because weight loss itself might reduce inflammation [11].

Interventions with n-3 PUFAs have also been shown to improve several cardiovascular risk factors, including serum triglyceride levels, blood pressure, platelet reactivity, and endothelial dysfunction [13]. Furthermore, it has been speculated that some of the beneficial effects of n-3 PUFAs might be by reduced inflammation due to a more favorable fatty acid profile in membrane phospholipids of inflammatory cells [14]. However, results from previous intervention studies have been conflicting [13].

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We have previously shown beneficial effect of Mediterranean-like diet and n-3 PUFA supplementation on the degree of atherosclerosis assessed by carotid intima-media thickness (IMT) and pulse wave propagation in the present population [15]. The aims of the present study were to investigate the intervention effects on some selected inflammatory markers and adipokines and to evaluate potential effects in relation to the changes observed in serum fatty acids and anthropometric measures.

2. Material and methods

2.1. Subjects

The basis for recruitment into the present study was a follow-up of the participants from the Oslo Diet and Anti-smoking study carried out in 1972-1977, comprising 1232 men with high risk of cardiovascular disease [16]. The survivors of this population were 25 years later invited to participate in the Diet and Omega-3 Intervention Trial on Atherosclerosis [15]. Altogether, a total of 563 subjects, age 64 to 76 years, were included. The study was carried out in compliance with the Helsinki Declaration and was approved by the Regional Ethics Committee. All subjects gave their written informed consent to participate.

2.2. Study design and intervention

Study design and intervention principles have previously been described in detail [15]. In brief, the study was designed to evaluate the effect of a 3-year intervention with dietary counseling, n-3 PUFA supplementation (2.4 g/d), or both. The study had a randomized 2 × 2 factorial design and was placebo controlled for the n-3 PUFA capsules. The n-3 PUFA capsules contained about 35% eicosapentaenoic acid (EPA; C20:5n-3), 20% docosahexaenoic acid (DHA; C22:6n-3), and 3.5 mg tocopherols per gram to prevent fatty acid peroxidation. The placebo capsules contained 56% linoleic acid (18:2n-6), 32% oleic acid (18:1n-9), 10% palmitic acid (16:0), and 4 mg tocopherols per gram. The dietary counseling was undertaken on an individual basis and consisted of advice to increase the use of vegetable oil and margarines (rapeseed oil, olive oil, and sunflower oil), vegetables, fruit, and fish, and to decrease the use of meat and fat from animal sources. Special oil and margarine (VITA margarine; Norwegian food company Mills DA, Oslo, Norway) were specifically supplied to these participants at all visits. Overweight subjects were encouraged to adopt a calorie-restricted diet. As an estimate of compliance, serum levels of PUFAs were measured and food frequency questionnaires were recorded at baseline and 36 months [15].

2.3. Laboratory methods

Fasting blood samples, without intake of any medications, were collected by standard venipuncture. Serum was prepared by centrifugation within 1 hour at 2500*g* for 10 minutes for determination of CRP, TNF-α, IL-6, IL-18, and adiponectin. Monocyte chemoattractant protein−1 was determined in citrated plasma (0.129 mol/L in dilution 1:10), stored on ice and separated within 30 minutes by centrifugation at 4°C and 3000*g* for 20 minutes to obtain platelet-poor plasma. All samples were stored at −80°C until analysis.

C-reactive protein was determined by an enzyme-linked immunosorbent assay (DRG Instruments, Marburg, Germany) (detection limit, 0.1 mg/L). Interleukin-6, TNF-α, MCP-1, and adiponectin were all measured by enzyme immunoassays from R&D Systems Europe (Abingdon, Oxon, United Kingdom). Enzyme-linked immunosorbent assay method from Medical Biological Laboratories (Naku-ku Nagoya, Japan) was used for analysis of IL-18. To minimize the influence of run-to-run variability, serial samples from the same individuals were analyzed in the same run. In our laboratory, the interassay coefficients of variation were as follows: CRP, less than 5%; TNF-α, 8.5%; IL-6, 10.5%; IL-18, 6.5%; MCP-1, 9.0%; and adiponectin, 9.5%.

Serum lipids and glucose were determined by conventional enzymatic methods.

In a random subset of participants (n = 278), fatty acid composition in serum phospholipids was analyzed by gas-liquid chromatography [15]. Pooled serum samples were used as control. The interassay coefficients of variation for fatty acid peaks were as follows: arachidonic acid (AA), 20%; EPA, 18%; and DHA, 29%. The values are presented as percentage of total fatty acids in serum.

2.4. Statistics

Because the distribution of several biochemical markers was skewed, nonparametric statistics were used throughout for continuous data. Intervention effects were principally analyzed according to the 2 × 2 factorial design, and no interaction between the treatment modalities was observed. Thus, diet intervention was compared with no diet intervention; and n-3 PUFA was compared with placebo. Significant findings were further explored by comparing single-treatment groups with the control group. Intragroup changes from baseline to 36 months were evaluated by Wilcoxon test, and between-group differences in relative changes from baseline were evaluated by Mann-Whitney *U* test. Correlation analyses were performed using the Spearman method. A significance level of .05 was used. The statistical analyses were performed with SPSS software, version 15.0 (SPSS, Chicago, IL).

3. Results

After 3 years, 487 subjects completed the study and were included in the treatment effect analyses. Of the remaining, 38 had died, 29 dropped out because of disease states interfering with study follow-up, and 9 individuals were unwilling to complete the study.

Table 1
Clinical characteristics, use of medication, and fasting laboratory variables in the study population (N = 563)

Age (y)	70 (67, 73)
Myocardial infarction (%)	18
Cardiovascular disease (%)	28
Diabetes (%)	15
Treated hypertension (%)	30
Current smokers (%)	34
Use of medication	
Statins (%)	27
ACE inhibitors (%)	13
Acetylsalicylic acid (%)	26
Total cholesterol (mmol/L)	6.3 (5.7, 7.0)
HDL cholesterol (mmol/L)	1.4 (1.2, 1.6)
Triglycerides (mmol/L)	1.5 (1.1, 2.0)
Glucose (mmol/L)	5.6 (5.3, 6.2)
BMI (kg/m^2)	26.5 (24.1, 28.7)
Waist circumference (cm)	98 (92, 104)
Systolic blood pressure (mm Hg)	148 (135, 160)
Diastolic blood pressure (mm Hg)	84 (77, 91)

Data are presented as percentages or median values (25th, 75th percentiles)
ACE indicates angiotensin-converting enzyme; HDL, high-density lipoprotein.

Some of the baseline characteristics are given in Table 1. The median age of the subjects was 70 years, 28% had a history of cardiovascular disease, 34% were current smokers, 30% had treated hypertension, and 15% had diabetes. At baseline, there were no significant differences between the randomized groups for any of the studied variables, with the exception of age (69 years in the control group vs 70 years in the intervention groups, $P = .01$) and fasting glucose (6.3 mmol/L in the control group vs 5.8, 5.9, and 5.9 mmol/L in the groups receiving n-3 PUFA supplementation, diet intervention, and the combination of both interventions; $P = .01$) [15].

According to the food frequency questionnaires, the intake of saturated fat decreased and the ratio of poly-unsaturated to saturated fat increased by dietary intervention, indicating compliance to the dietary advice given [15]. The serum levels of some selected PUFAs at baseline and after 36 months are presented in Table 2. Eicosapentaenoic acid and DHA were markedly increased and the ratio of n-6 to n-3 was reduced by n-3 PUFA supplementation compared with placebo ($P < .001$ for all), indicating good compliance with the intervention [15].

Table 3 summarizes the treatment effect of diet and/or n-3 PUFA supplementation on circulating levels of inflammatory markers, triglycerides, and anthropometric measures analyzed according to the factorial design. Levels of IL-18 were decreased by diet (−10.5% vs baseline, $P = .012$ compared with no diet) and by n-3 PUFA supplementation (−9.9% vs baseline, $P = .008$ compared with placebo). As shown in Fig. 1, levels of IL-18 were also significantly reduced in each treatment group compared with the control group ($P = .021$ for both), with a potentially additive effect of the 2 intervention principles ($P < .001$). Levels of MCP-1 were less reduced in the n-3 PUFA group when compared with the

Table 2
Selected serum fatty acids at baseline and after 36 months

Fatty acids (% of total)	No diet intervention		Diet intervention		Placebo (corn oil)		n-3 PUFA		*P1*	*P2*
	Baseline (n = 138)	36 mo (n = 119)	Baseline (n = 140)	36 mo (n = 117)	Baseline (n = 139)	36 mo (n = 114)	Baseline (n = 139)	36 mo (n = 122)		
AA, 20:4n-6	4.22 (3.63, 5.05)	4.68*** (4.04, 5.41)	4.28 (3.56, 5.10)	4.58** (3.90, 5.33)	4.26 (3.70, 5.11)	4.81*** (4.06, 5.80)	4.21 (3.54, 5.03)	4.37 (3.85, 5.10)	.551	.086
EPA, 20:5n-3	1.67 (1.07, 2.93)	2.90*** (1.26, 4.83)	1.27 (0.83, 2.17)	2.70*** (1.28, 4.83)	1.47 (0.96, 2.44)	1.33 (0.77, 2.22)	1.49 (0.92, 2.52)	4.66*** (3.49, 5.66)	.010	<.001
DHA, 22:6n-3	2.97 (2.23, 3.95)	3.72*** (2.65, 4.49)	2.77 (2.10, 3.56)	3.54*** (2.79, 4.39)	2.86 (2.08, 3.62)	2.84 (2.17, 3.43)	2.87 (2.27, 3.87)	4.15*** (3.63, 4.87)	.144	<.001
Ratio n-6/n-3	6.21 (4.25, 8.56)	4.12*** (3.09, 7.33)	6.96 (5.10, 9.03)	4.75*** (3.30, 7.13)	6.85 (4.75, 9.27)	7.08 (5.35, 9.45)	6.36 (4.70, 8.37)	3.41*** (2.69, 4.28)	.129	<.001

Median values (25th, 75th percentiles) are given. *P1* refers to the between-group differences in diet vs no diet intervention from baseline to 36 months. *P2* refers to the between-group differences in n-3 PUFA vs placebo from baseline to 36 months.
**$P < .01$ and ***$P < .001$ vs baseline.

Table 3
Circulating levels of inflammatory markers, triglycerides, and anthropometric measures at baseline and after 36 months

	No diet intervention		Diet Intervention		Placebo (corn oil)		n-3 PUFA		*P1*	*P2*
	Baseline (n = 282)	36 mo (n = 240)	Baseline (n = 281)	36 mo (n = 246)	Baseline (n = 281)	36 mo (n = 239)	Baseline (n = 282)	36 mo (n = 247)		
CRP (mg/L)	3.61 (1.94, 6.01)	3.29 (1.62, 5.43)	2.97 (1.51, 5.48)	2.63* (1.26, 4.53)	3.13 (1.67, 5.90)	3.04 (1.34, 5.32)	3.58 (1.80, 5.88)	2.92* (1.59, 4.82)	.523	.482
IL-6 (pg/mL)	1.53 (0.97, 2.40)	1.32** (0.87, 2.33)	1.55 (1.04, 2.61)	1.35* (0.91, 2.56)	1.56 (1.00, 2.69)	1.48 (0.92, 2.48)	1.51 (0.98, 2.40)	1.24*** (0.85, 2.31)	.871	.144
IL-18 (pg/mL)	273 (210, 361)	253*** (197, 330)	279 (220, 347)	247*** (190, 311)	280 (222, 356)	265*** (203, 333)	274 (207, 355)	237*** (186, 300)	.012	.008
TNF-α (pg/mL)	1.16 (0.78, 2.18)	1.06*** (0.71, 1.84)	1.09 (0.79, 1.66)	0.95*** (0.69, 1.50)	1.15 (0.80, 2.14)	1.03*** (0.71, 1.80)	1.05 (0.77, 1.82)	0.94*** (0.68, 1.59)	.963	.976
MCP-1 (pg/mL)	440 (364, 525)	413*** (325, 493)	429 (368, 523)	416*** (353, 485)	442 (374, 532)	402*** (335, 479)	429 (363, 514)	420* (349, 499)	.913	.010
Adiponectin (μg/mL)	7.56 (4.85, 12.4)	7.45 (4.93, 12.4)	9.26 (5.45, 13.4)	8.54 (5.19, 13.6)	7.86 (5.18, 11.7)	7.66 (4.91, 12.4)	9.02 (5.34, 13.8)	8.30 (5.22, 13.8)	.722	.508
Triglycerides (mmol/L)	1.5 (1.1, 2.1)	1.3*** (0.9, 1.8)	1.6 (1.1, 2.0)	1.2*** (0.9, 1.7)	1.5 (1.1, 2.0)	1.4*** (1.0, 1.9)	1.6 (1.1, 2.1)	1.1*** (0.9, 1.7)	<.001	<.001
BMI (kg/m^2)	26.5 (24.3, 28.7)	26.6 (24.6, 29.3)	26.5 (24.0, 28.5)	26.2 (23.8, 28.7)	26.6 (23.9, 28.8)	26.8 (24.3, 29.3)	26.4 (24.2, 28.4)	26.3 (24.3, 28.4)	.021	.053
Waist circumference (cm)	98 (92, 103)	101*** (94, 106)	98 (93, 104)	99*** (94, 106)	98 (92, 104)	100*** (93, 107)	98 (93, 103)	100*** (94, 105)	.061	.188

Median values (25th, 75th percentiles) are given. *P1* refers to the between-group differences in diet vs no diet intervention from baseline to 36 months. *P2* refers to the between-group differences in n-3 PUFA vs placebo from baseline to 36 months.
*$P < .05$, **$P < .01$, and ***$P < .001$ vs baseline.

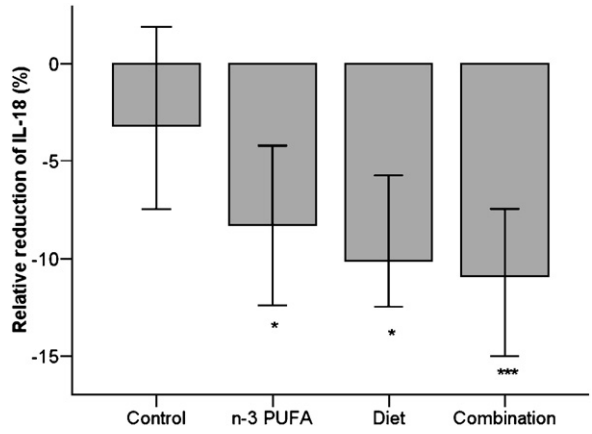

Fig. 1. Relative reduction (percentage) in serum levels of IL-18 by n-3 PUFA supplementation, Mediterranean-like diet, and the combination of both interventions. Bars represent median reduction (95% confidence interval). *$P < .05$ and ***$P < .001$ compared with the control group.

placebo group ($P = .010$). However, when comparing single-treatment groups with controls, no significant changes were observed (data not shown). C-reactive protein, IL-6, and TNF-α were reduced vs baseline in all study groups, but with no between-group differences. No changes were observed for adiponectin in any group. As previously reported [15], there was a reduction of triglycerides by diet and n-3 PUFA supplementation ($P < .001$ for both); and body mass index (BMI) was reduced by diet ($P = .021$).

As shown in Table 4, changes in IL-18 were significantly correlated to changes in triglycerides ($r = 0.20$, $P < .001$), EPA ($r = -0.14$, $P = .030$), DHA ($r = -0.14$, $P = .034$), BMI ($r = 0.16$, $P < .001$), and waist circumference ($r = 0.12$, $P = .007$) in the total population.

4. Discussion

The main finding in this study was that serum levels of IL-18 were significantly reduced by a 3-year intervention with both Mediterranean-like diet and n-3 PUFA supplementation. Reduced levels of IL-18 by diet intervention have recently been shown [11,17,18]; but to the best of our knowledge, a reduction of IL-18 by n-3 PUFA supplementation has not been previously reported.

Table 4
Correlations between changes (Δ) in serum levels of IL-18 and changes in triglycerides, fatty acid profile, and anthropometric measures in the total population

	Δ IL-18 (pg/mL)
Δ triglycerides (mmol/L)	$r = 0.20$, $P < .001$
Δ EPA (%)[a]	$r = -0.14$, $P = .030$
Δ DHA (%)[a]	$r = -0.14$, $P = .034$
Δ AA (%)[a]	$r = -0.09$, $P = .193$
Δ BMI (kg/m^2)	$r = 0.16$, $P < .001$
Δ waist circumference (cm)	$r = 0.12$, $P = .007$

[a] n = 236.

Previous studies have shown beneficial effect of fish oil supplementation on the production of various cytokines in human mononuclear cells [19,20]. A potential explanation of an anti-inflammatory effect of n-3 PUFAs might be the partial replacement of AA (20:4n-6) in membrane phospholipids of inflammatory cells. Arachidonic acid is the main substrate for eicosanoid synthesis, including the 2-series of prostaglandins [14]. Monocytes and macrophages produce large amounts of prostaglandin E2, which has several proinflammatory properties including induction of IL-6 production by macrophages [21]. In contrast, EPA (20:5n-3) gives substrate to synthesis of prostaglandin E3, which is less active in mediating an inflammatory response. Furthermore, resolvin E1, a newly described product of EPA, has been shown to have extensive anti-inflammatory properties, in part by inhibiting expression of IL-8 and TNF-α [22].

The above-mentioned mechanisms could in part explain the reduction of IL-18 by n-3 PUFA supplementation observed in our study. On the other hand, the correlations between changes in IL-18 and changes in serum fatty acids were rather weak. Furthermore, prostaglandin E2 might have both pro- and anti-inflammatory properties [23]. In fact, prostaglandin E2 has been reported to inhibit lipopolysaccharide-induced IL-18 production in monocytes [24]. Thus, other mechanisms are likely to be involved, some of which might be independent of altered eicosanoid synthesis [23].

Recent reports suggest that n-3 PUFAs might inhibit inflammatory gene expression via inhibition of the transcription factor nuclear factor–κB, an important regulator of a number of cytokines, chemokines, and adhesion molecules [23]. In contrast to most other cytokines, IL-18 is expressed constitutively in many cell types as a precursor, pro–IL-18 [25]. Pro–IL-18 is inactive until cleaved by the enzyme caspase-1, and nuclear factor–κB is probably involved in the activation of caspase-1 [26]. Thus, the nuclear factor–κB pathway might at least indirectly be involved in the reduction of IL-18 levels by n-3 PUFAs. Furthermore PUFAs, especially EPA and DHA, are natural ligands for peroxisome proliferator–activated receptors, which in activated state inhibit nuclear factor–κB and thus several inflammatory processes [23,27]. Interestingly, a very recent publication has reported inhibition of aldosterone-induced IL-18 expression by peroxisome proliferator–activated receptor agonists in cardiomyocytes [28].

Moreover, n-3 PUFAs in low to moderate doses might have beneficial effect on circulating levels of adhesion molecules, whereas higher doses might have an adverse effect [13,29,30]. We have previously shown reduced levels of intercellular adhesion molecule–1 by n-3 PUFA supplementation in a moderate dose (2.4 g/d) from the present trial [29].

The reduction in IL-18 by diet intervention may be mediated by some of the same mechanisms, but other mechanisms are probably also involved. The adipose tissue is a major source of IL-18 production, and IL-18 is primarily produced by the nonfat cells within the adipose compartment [31]. Reduced levels of IL-18 after bariatric surgery have recently been reported [32]. In line with our results, Esposito et al [17] showed that reduced levels of IL-18 were correlated with reduced BMI and waist to hip ratio in obese women after an intervention with calorie-restricted diet. Conversely, it was recently reported that reduced IL-18 after calorie-restricted diet and exercise was not correlated with reduced BMI [18]. The rather weak correlations in our study might in part be explained by a modest weight reduction. Furthermore, another recent study reported that levels of IL-18 were correlated with triglycerides both before and after bariatric surgery, in line with our results [33]. Because BMI and triglycerides were moderately decreased and EPA was moderately increased by diet in our study, it might be speculated that the Mediterranean-like diet acts through a combination of different mechanisms.

Despite reduced levels of IL-18 by both interventions, the total effect on inflammatory markers was limited. Previous studies on n-3 PUFA have reported conflicting results concerning inflammation, and various inflammatory markers might react differently to different doses of n-3 PUFA [13]. The limited effect of diet intervention is in line with a study showing no effect of Mediterranean-like diet on inflammatory markers in patients with established coronary artery disease, most taking a broad spectrum of medication [34], whereas a reduction of several inflammatory markers including IL-18 was reported from a medication-free population with the metabolic syndrome [35]. In our study, the limited effect of both interventions might thus in part be explained by a frequent use of medication that might influence inflammatory markers. A tendency toward improved diet in the group receiving no dietary counseling might also have been present, as several inflammatory markers were improved vs baseline in all groups.

Another point of discussion is whether serum levels of total IL-18 are the most relevant measure for cardiovascular disease risk. Circulating IL-18 exists in free and protein-bound form; and some data indicate that IL-18 binding protein might be protective of cardiovascular disease in older people, implicating that free rather than total IL-18 is involved in the atherosclerotic process [36,37]. Furthermore, IL-18 has been shown to be produced in the unstable atherosclerotic plaque [4]; and circulating levels do not necessarily reflect the inflammatory situation in the plaque. Still, circulating levels of total IL-18 have been shown to predict cardiovascular events in recent studies [3] and are therefore a potential and relevant target for interventions. Whether a reduction in serum levels of IL-18 translates into a reduced cardiovascular risk is another question. In the present study, we have previously shown reduced IMT of the common carotid artery by diet intervention [15]; however, there was no significant correlation between changes in IL-18 and IMT (data not shown).

Our study has some limitations, among others the heterogeneity of this older study population, with a broad

spectrum of morbidity and use of medications as mentioned above. Furthermore, the subjects consist of long-time survivors from a high-risk population, raising the possibility of survivor bias. Moreover, this study comprises a selected group of elderly white men; and different results might be obtained from other demographic groups. Our study also has several strengths, including standardized measures of compliance including serum fatty acid profile and a relatively long intervention period of 3 years.

In conclusion, levels of IL-18 were significantly reduced by both Mediterranean-like diet and n-3 PUFA supplementation. However, the changes correlated only weakly to changes in triglycerides, serum fatty acids, and anthropometric measures. The cardioprotective effects of both interventions might thus in part be explained by reduced levels of IL-18, but probably beyond changes in serum lipids, serum fatty acids, and body composition.

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