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# SERUM CONCENTRATIONS OF MATRIX METALLOPROTEINASE-9, TISSUE INHIBITOR OF MATRIX METALLOPROTEINASE-1 AND $A_2$-MACROGLOBULIN IN HEALTHY SUBJECTS AFTER SUPPLEMENTATION WITH DIFFERENT DOSES OF MARINE N-3 FATTY ACIDS

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**Abstract –** Objective: Extracellular matrix modification by matrix metalloproteinase-9 (MMP-9), tissue inhibitor of matrix metalloproteinases-1 (TIMP-1) and $\alpha_2$-macroglobulin may affect the stability of atherosclerotic plaques. Marine n-3 polyunsaturated fatty acids (n-3 PUFA) may protect against plaque rupture. The aim was to investigate the effect of marine n-3 PUFA supplementation on serum levels of MMP-9, TIMP-1, and $\alpha_2$-macroglobulin. Methods: Healthy volunteers were randomized to receive capsules contributing either 6.6 g marine n-3 PUFA/day, 2.0 g marine n-3 PUFA/day or 6.6 g of olive oil (control). Serum MMP-9, TIMP-1 and $\alpha_2$-macroglobulin was measured at baseline and after 12 weeks of supplementation. One way ANOVA or Kruskal-Wallis test was used to compare groups. Results: 60 healthy volunteers were enrolled and no subjects dropped out of the 12 week study. There were no statistically significant changes in serum levels of MMP-9, TIMP-1, and $\alpha_2$-macroglobulin in any of the three treatment groups (P=0.85, P=0.23 and P=0.87, respectively). Conclusion: Supplementation with marine n-3 PUFA had no effect on serum levels of MMP-9, TIMP-1 and $\alpha_2$-macroglobulin in healthy subjects. The possible protection offered by marine n-3 PUFA against plaque rupture is therefore unlikely to be mediated through a change in serum levels of MMP-9, TIMP-1 and $\alpha_2$-macroglobulin.

**Key words:** n-3 PUFA, n-3 polyunsaturated fatty acid, MMP-9, TIMP-1, $\alpha_2$-macroglobulin, matrix metalloproteinase-9, tissue inhibitor of matrix metalloproteinase-1, atherosclerosis

## INTRODUCTION

Atherogenesis and plaque evolution involve inflammation and extra-cellular matrix (ECM) modification by matrix metalloproteinases (MMPs) and their principal inhibitors, tissue inhibitors of matrix metalloproteinases (TIMPs) and $\alpha_2$-macroglobulin (13,26,29). Recruitment of inflammatory cells and their production of MMPs therefore represent a potentially important component in atheromatous plaque disruption and acute coronary events. Accordingly, circulating MMPs, TIMPs, and $\alpha_2$-macroglobulin are indices of vascular matrix turnover (3).

Particularly MMP-9 may have an important role in the degradation of ECM in the fibrous cap prior to plaque rupture and may predict atherosclerotic progression and future thrombotic complications(15,18). Increased circulating levels of MMP-9 have been reported in patients with coronary heart disease (CHD) and in patients with risk factors for CHD (12,19,21,39,40,47,50). On the other hand MMP-9 activity may be directly inhibited by TIMP-1 and non-specifically by $\alpha_2$-macroglobulin, thereby possibly reducing plaque instability (3).

**Abbreviations BMI,** body mass index; **CHD,** coronary heart disease; **CV,** coefficient of variation; **DHA,** docosahexaenoic acid; **ECM,** extra-cellular matrix; **ELISA,** enzyme linked immunosorbent assay; **EPA,** eicosapentaenoic acid; **g,** gram; **HDL,** high density lipoprotein; **hs-CRP,** high sensitivity C-reactive protein; **kDa,** kilo Dalton; **K-EDTA,** potassium ethylenediaminetetraacetic acid; **LDL,** low density lipoprotein; **MMP,** matrix metalloproteinase; **n-3 PUFA,** n-3 polyunsaturated fatty acid; **TIMP,** tissue inhibitors of matrix metalloproteinase; **QQ plot,** Quantile-Quantile plot.

Data suggest that marine n-3 polyunsaturated fatty acids (n-3 PUFA), in particular eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), may reduce the risk of coronary events (9,17,34,48) and may alter plaque composition making them less prone to rupture (41).

We hypothesized that marine n-3 PUFA administration would decrease serum MMP-9 levels and increase TIMP-1 and $\alpha_2$-macroglobulin levels. The objective of the present study therefore was to investigate the effect of dietary supplementation with marine n-3 PUFA in two different doses on the serum levels of MMP-9, TIMP-1, and $\alpha_2$-macroglobulin in healthy individuals.

## MATERIAL AND METHODS

### *Subjects*

Sixty healthy volunteers were recruited among the medical staff, bank employees and university students in Aalborg, Denmark. None of the participants took any medication, fish oil supplements or had any known diseases. Written informed consent was obtained from each subject and the protocol was approved by the regional Ethical Committee. Baseline characteristics of the subjects are given in Table 1.

**Table 1.** Baseline characteristics

| | Treatment group | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Control group | | | 2,0 g n-3 PUFA/day | | | 6,6 g n-3 PUFA/day | | |
| Characteristics: | Mean | SD | n | Mean | SD | n | Mean | SD | n |
| Sex: Male(n) / Female(n) | | | 12 / 8 | | | 12 / 8 | | | 11 / 9 |
| Current smokers (n) | | | 5 | | | 6 | | | 5 |
| BMI (kg/m²) | 24.0 | 2.7 | | 25.1 | 2.9 | | 24.6 | 3.7 | |
| Age (years) | 37.0 | 11.0 | | 38.0 | 10.0 | | 39.0 | 10.0 | |
| Total cholesterol (mmol/l) | 5.0 | 1.1 | | 5.0 | 0.9 | | 5.1 | 1.2 | |
| LDL cholesterol (mmol/l) | 3.0 | 1.0 | | 3.3 | 0.9 | | 3.2 | 1.1 | |
| HDL cholesterol (mmol/l) | 1.4 | 0.4 | | 1.3 | 0.2 | | 1.3 | 0.3 | |
| Triacylglycerides (mmol/l) | 1.2 | 0.7 | | 1.2 | 0.6 | | 1.3 | 1.2 | |

### *Study design*

The present work is a sub-study of a dose-response study regarding marine n-3 PUFA and heart rate variability (6). The study design was double-blind and placebo-controlled and the participants were randomized to one of three supplements by drawing an envelope signed A, B, or C.

One group received ten capsules of Pikasol® (a reesterified triacylglycerol from Pronova Biocare, Norway) delivering 6.6g marine n-3 PUFA/day (3.0 g of EPA and 2.9 g of DHA). The second group received 2.0 g of marine n-3 PUFA/day (0.9 g of EPA and 0.8 g of DHA) as three capsules of Pikasol® and seven control capsules (each contained 0.7 g of olive oil). The third group received ten capsules of olive oil per day. The subjects were randomized in blocks of ten and numbered consecutively. The subjects took three capsules in the morning and seven with the evening meal. The capsules were taken from two different boxes to assure a constant intake in the subjects randomized to the low dose of marine n-3 PUFA and to maintain blinding. The supplements were given daily for 12 weeks.

### *Blood sampling and analyses*

Blood was sampled in 1996, before and after 12 weeks of dietary supplementation after an overnight fast of at least ten hours. Blood samples were left to coagulate for 30 minutes, centrifuged for 20 minutes at 3000 x g, serum was collected and the samples were immediately frozen at -80°C

and analyzed within months, apart from MMP-9, TIMP-1 and α2-macroglobulin serum levels which were analyzed in 2008.

Commercial enzyme linked immunosorbent assay (ELISA) was used for the measurement of total serum MMP-9. The Quantikine MMP-9 immunoassay (R&D Systems Europe, Ltd. Abingdon, UK) measures total MMP-9 (92 kDa pro- and 82 kDa active forms). The intra assay CV was 2.8%.

The Quantikine TIMP-1 immunoassay (R&D Systems Europe, Ltd. Abingdon, UK) was used to measure total serum TIMP-1. The intra assay CV was 3.2%.

A turbidimetric method, using antibody and calibrator (measurements were standardized against certified reference material 470) from DAKO, Glostrup, Denmark was used to measure serum α2-macroglobulin. Analysis was performed on Advia 1650 from Bayer Diagnostics, NY, USA. The intra assay CV was 1%.

Separation of granulocytes was performed by drawing whole blood into K-EDTA and layering the blood on top of a solution of Hypaque-Ficoll (Nycomed, Oslo, Norway). After discontinuous gradient centrifugation at 200 x g for 20 minutes (10), the granulocytes were harvested into RPMI, red blood cells were haemolysed and the granulocytes washed in isotonic phosphate buffer and resuspended in 0.5 ml 0.9% sodium chloride, and the vial was filled with Nitrogen and stored at -80 °C (42).

Lipids from granulocytes were extracted into dichlormethane/methanol (11), fatty acids were dissolved in heptane and methylated using sodiummethoxide and acetic acid and analyzed using a CP-9002 gas-chromatograph (Chrompack International, Middelburg, the Netherlands) equipped with autosampler, FID detector, CP-Wax 58 capillary column (25 m, 0.25 mm ID, 0.2 µm film thickness), helium as carrier gas, temperature programming from 40 ºC - 195 ºC and constant flow. Commercially available standards (Nu-chek-Prep, Inc. Minnesota) were used to recognize the individual fatty acids. This approach permits quantification of fatty acid methyl esters with 12 to 24 carbon atoms. The area of fatty acids was calculated into % of the total fatty acid areas. No internal standard was used.

A BNII nephelometer (Dade Behring Marburg, Germany) was used to measure hs-CRP as described previously (23). Plasma lipids and lipoproteins were measured by standard methods with LDL cholesterol calculated by the Friedewald method.

### *Statistical analysis*

Paired t-test was used to evaluate differences between levels before and after supplementation within the groups. Bartlett's test was used to investigate variance homogeneity and normality was checked by visual inspection of QQ plots and histograms. Comparison of the three groups (control, moderate, and high dose n-3 PUFA) was carried out by one-way ANOVA if the conditions of variance homogeneity and normality were met. Otherwise, Kruskal-Wallis equality-of-populations rank test was applied. Between-group differences before and after supplementation were evaluated using Student's t-test. Pearson's correlation coefficient was calculated to evaluate the relationships at baseline between the serum markers, traditional risk factors, and levels of EPA and DHA. Results are given as mean values and standard deviations for baseline characteristics or, when appropriate, as median values and interquartile ranges. The STATA software package (version 10.1) was employed for all analyses. P-values < 0.05 (two-tailed) were considered statistically significant.

## RESULTS

### *Compliance*

The dietary supplements were well tolerated and no subjects dropped out of the study. Adherence to the allocated supplement regimen was assured by quantifying the content of EPA and DHA in granulocytes. Satisfactory group compliance was documented by the intimate association between the granulocyte content of EPA and DHA and the dose of marine n-3 PUFA given (Table 2).

### *Before supplementation*

The three intervention groups were comparable with no significant differences prior to supplementation regarding gender, smoking status, age (P=0.87), body mass index (BMI) (P=0.55), HDL cholesterol (P=0.11), LDL cholesterol (P=0.51), triacylglycerides (P=0.24) and total cholesterol (P=0.16) (Table 1) and with respect to granulocyte content of EPA (P=0.30) and DHA (P=0.71), serum levels of MMP-9 (P=0.07), TIMP-1 (P=0.65), and α2-macroglobulin (P=0.32) (Table 2). The groups also had similar levels of hsCRP (P=0.12).

At baseline, smokers and non-smokers had similar serum levels of MMP-9, TIMP-1, and α2-macroglobulin (P=0.08, P=0.77 and P=0.34, respectively). This also applied to men and women (P=0.19, P=0.33 and P=0.06, respectively).

An exploratory analysis was performed in addition to the primary objective of the study. Correlations between serum levels of MMP-9, TIMP-1 and α2-macroglobulin and several traditional risk factors as well as the content of EPA and DHA in granulocytes were investigated for baseline values (Table 3). Significant correlations were found between α2-macroglobulin and BMI (P<0.01), HDL cholesterol (P=0.04) and LDL cholesterol (P=0.01) and between TIMP-1 and BMI (P=0.02).

### *After supplementation*

Significant increases in granulocyte concentrations of EPA and DHA occurred in subjects receiving both 2.0 and 6.6 g marine n-3 PUFA/day with the largest increase in the 6.6 g group (Table 2).

There were no statistically significant changes in serum levels of MMP-9, TIMP-1 and α2-macroglobulin (Table 2) in any of the three

**Table 2**. Median values (25; 75 percentiles) for fasting serum EPA, DHA, MMP-9, TIMP-1, and $\alpha_2$-macroglobulin at baseline and after 12 weeks of control oil and n-3 PUFA supplementation. EPA and DHA are given as percentage (%) of total fatty acids in granulocytes.

| | Treatment group | | | | | |
|---|---|---|---|---|---|---|
| | Control Group | | 2.0 g n-3 PUFA/day | | 6.6 g n-3PUFA/day | |
| | Baseline | 12 weeks | Baseline | 12 weeks | Baseline | 12 weeks |
| EPA (%) | 0.51 | 0.44 | 0.49 | 1.70* | 0.58 | 4.26* |
| | (0.33;0.62) | (0.36;0.60) | (0.32;0.68) | (1.45;2.17) | (0.48;0.73) | (3.75;4.56) |
| DHA (%) | 1.55 | 1.42 | 1.35 | 1.69* | 1.44 | 2.22* |
| | (1.24;1.83) | (1.19;1.71) | (1.15;1.58) | (1.48;1.81) | (1.24;1.89) | (1.83;2.44) |
| MMP-9 | 311 | 394 | 325 | 479 | 233 | 370 |
| (ng/ml) | (201;448) | (323;609) | (233;511) | (323;545) | (169;306) | (277;445) |
| TIMP-1 | 177 | 173 | 171 | 176 | 183 | 176 |
| (ng/ml) | (159;192) | (152;195) | (152;188) | (162;188) | (165;202) | (147;194) |
| $\alpha_2$ –macro- | 1.52 | 1.50 | 1.39 | 1.33 | 1.48 | 1.47 |
| globulin (g/l) | (1.31;2.06) | (1.31;2.03) | (1.29;1.69) | (1.25;1.51) | (1.32,1.61) | (1.25;1.56) |

* Significantly different from before supplementation ($P < 0.01$).

**Table 3.** Correlations between MMP-9, TIMP-1, $\alpha_2$-macroglobulin and traditional risk factors, EPA and DHA content in granulocytes at baseline. Results are given as Pearson's correlation coefficient.

| | BMI | HDL | LDL | hs-CRP | EPA | DHA | EPA+DHA | $\alpha_2$macro globulin | MMP-9 | TIMP-1 |
|---|---|---|---|---|---|---|---|---|---|---|
| $\alpha_2$macro-globulin | -0.56* | 0.27* | -0.34* | -0.23 | -0.21 | 0.12 | -0.02 | 1.00 | | |
| MMP-9 | 0.07 | 0.00 | 0.10 | -0.05 | 0.17 | 0.09 | 0.15 | -0.09 | 1.00 | |
| TIMP-1 | 0.29* | 0.01 | 0.10 | 0.24 | -0.02 | 0.06 | 0.03 | -0.18 | -0.08 | 1.00 |

*Significant correlations ($P<0.05$).

treatment groups (P=0.85, P=0.23 and P=0.87 respectively).

## DISCUSSION

Supplementation with two different doses of marine n-3 PUFA for 12 weeks did not change fasting serum levels of MMP-9, TIMP-1 and α2-macroglobulin in healthy subjects in the present study. Furthermore, there were no correlations between the content of EPA and DHA in granulocytes and serum levels of MMP-9, TIMP-1 or α2-macroglobulin at baseline.

MMP-9 and TIMP-1 may operate at local tissue level but most results are consistent with the hypothesis that peripheral plasma measurements may be used as a surrogate measure and relate to outcome, although some cases of alterations of serum MMP-9 and TIMP-1 levels may reflect a systemic inflammatory state or other biological or pathological processes (3). Tayebjee et al. (38) studied serum levels of MMP-9 and TIMP-1 in healthy subjects, and our baseline values were comparable to their findings. We therefore propose that our samples were stable during the long storage period (1996-2008) although a decrease in activity could have resulted in smaller concentrations. Regardless, a hypothetical loss of protein activity presumably would affect all intervention groups equally and hence not alter the final conclusion of no effect of marine n-3 PUFA.

Our results are in line with a recent study, where 300 patients were randomized following an acute myocardial infarction to receive either 4 g/day of marine n-3 PUFA or corn oil for at least one year (1). Both supplements resulted in a non-significant decrease in serum MMP-9 with no difference between groups. In another study the effects on MMP-9 and TIMP-1 levels following 3 years of supplementation with 2.4 g/day of marine n-3 PUFA compared to corn oil were evaluated in a population of elderly men at high risk of CHD (12). MMP-9 levels were significantly reduced in both the corn oil and the n-3 PUFA group, but there were no differences between groups nor did the TIMP-1 levels change. The above mentioned studies were undertaken in intensively medicated older patients with vascular disease which may be important as several medications (20,22,45) and disease states may affect MMP-9 levels (37). The subjects in the present study were healthy, normal weight, below the age of 50, and took no medications. A potential drawback concerning our healthy subjects includes low baseline concentrations of MMP-9, TIMP-1 and α2-macroglobulin which may have made a potential effect of n-3 PUFA very hard to obtain.

The evaluation of the effect of two different doses of marine n-3 PUFA on the variables assured that a potential dose response relationship was revealed. Beneficial effects of a smaller dose of marine n-3 PUFA of approximately 1 g daily in epidemiological studies and in large scale trials have been reported (9,48) and retrospectively, 1 g of n-3 PUFA might have been a better choice in the low dose group.

To our knowledge no previous studies have investigated whether an association between the content of EPA and DHA in granulocyte membranes and serum levels of MMP-9, TIMP-1 and α2-macroglobulin exists. No correlation was found at baseline, supporting the primary result of no effect of marine n-3 PUFA on the markers.

Further exploratory analyses were carried out (Table 3) as previous studies have noted changes of MMP-9 and TIMP-1 levels as a function of various traditional risk factors for CHD. Significant correlations have been described between MMP-9 and LDL cholesterol, HDL cholesterol and hs-CRP and between TIMP-1 and LDL cholesterol and between MMP-9 and TIMP-1 (1,12,38). No significant correlations were found in the present study except for a significant positive correlation between TIMP-1 and BMI. On the contrary, significant correlations were found between α2-macroglobulin and BMI, HDL cholesterol, and LDL cholesterol. To our knowledge, no previous papers have studied those correlations.

Several compounds of olive oil have been shown to decrease inflammatory mediator production in leukocytes (25), but in the control group given olive oil, no effect was noted on serum MMP-9, TIMP-1, and α2-macroglobulin in the present study.

In conclusion, no effect could be demonstrated by either a moderate (2 g/day) or a high (6.6 g/day) dose of marine n-3 PUFA on serum levels of MMP-9, TIMP-1, and α2-macroglobulin in healthy subjects. This does not exclude an effect of marine n-3 PUFA on these variables in patients with vascular disease, but lend no support to our hypothesis that the protection offered by marine n-3 PUFA against CHD and acute coronary events is mediated through a reduction of MMP-9 or an increase in TIMP-1 and α2-macroglobulin levels.

Other articles in this theme issue include references (51-62).

## REFERENCES


1. Aarsetoy, H., Brugger-Andersen, T., Hetland, O., Grundt, H. and Nilsen, D.W., Long term influence of regular intake of high dose n-3 fatty acids on CD40-ligand, pregnancy-associated plasma protein A and matrix metalloproteinase-9 following acute myocardial infarction. *Thromb. Haemost.* 2006, **95**: 329-336.
2. Baker, A.H., Edwards, D.R. and Murphy, G., Metalloproteinase inhibitors: biological actions and therapeutic opportunities. *J. Cell. Sci.* 2002, **115**: 3719-3727.
3. Beaudeux, J.L., Giral, P., Bruckert, E., Bernard, M., Foglietti, M.J. and Chapman, M.J., Serum matrix metalloproteinase-3 and tissue inhibitor of metalloproteinases-1 as potential markers of carotid atherosclerosis in infraclinical hyperlipidemia. *Atherosclerosis* 2003, **169**: 139-146.
4. Bonnema, D.D., Webb, C.S., Pennington, W.R., Stroud, R.E., Leonardi, A.E. and Clark, L.L., Effects of age on plasma matrix metalloproteinases (MMPs) and tissue inhibitor of metalloproteinases (TIMPs). *J. Card. Fail.* 2007, **13**: 530-540.
5. Calder, P.C., n-3 Fatty acids and cardiovascular disease: evidence explained and mechanisms explored. *Clin. Sci.* (Lond) 2004, **107**: 1-11.
6. Christensen, J.H., Christensen, M.S., Dyerberg, J. and Schmidt, E.B., Heart rate variability and fatty acid content of blood cell membranes: a dose-response study with n-3 fatty acids. *American Journal of Clinical Nutrition* 1999, **70**: 331-337.
7. Das UN., Beneficial effect(s) of n-3 fatty acids in cardiovascular diseases: but, why and how? *Prostaglandins Leukot. Essent. Fatty Acids* 2000, **63**: 351-362.
8. Delbosc, S., Glorian, M., Le Port, A.S., Bereziat, G., Andreani, M. and Limon, I., The benefit of docosahexanoic acid on the migration of vascular smooth muscle cells is partially dependent on Notch regulation of MMP-2/-9. *Am. J. Pathol.* 2008, **172**: 1430-1440.
9. Dietary supplementation with n-3 polyunsaturated fatty acids and vitamin E after myocardial infarction: results of the GISSI-Prevenzione trial. Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto miocardico. *Lancet* 1999, **354**: 447-455.
10. Ferrante, A., Thong, Y.H., A rapid one step procedure for purification of mononuclear and polymorphonuclear leucocytes from human blood using a modification of the hypaque-ficoll technique. *Journal of immunological methods* 1978, **24**: 389-393.
11. Frederik, J.G.M., Thomas, G.W., Gas chromatography-mass spectrometry method for determination of phospholipid peroxides; I. Transesterification to form methyl esters. *Journal of free radicals in biology and medicine* 1985, **4**: 215-225.
12. Furenes, E.B., Seljeflot, I., Solheim, S., Hjerkinn, E.M. and Arnesen, H., Long-term influence of diet and/or omega-3 fatty acids on matrix metalloproteinase-9 and pregnancy-associated plasma protein-A in men at high risk of coronary heart disease. *Scand. J. Clin. Lab. Invest.* 2008, **68**: 177-184.
13. George, S.J., Tissue inhibitors of metalloproteinases and metalloproteinases in atherosclerosis. *Curr. Opin. Lipidol.* 1998, **9**: 413-423.
14. Glowinska-Olszewska, B. and Urban, M., Elevated matrix metalloproteinase 9 and tissue inhibitor of metalloproteinase 1 in obese children and adolescents. *Metabolism* 2007, **56**: 799-805.
15. Hobeika, M.J., Thompson, R.W., Muhs, B.E., Brooks, P.C. and Gagne, P.J., Matrix metalloproteinases in peripheral vascular disease. *J. Vasc. Surg.* 2007, **45**: 849-857.
16. Hooper, L., Thompson, R.L., Harrison, R.A., Summerbell, C.D., Ness, A.R. and Moore, H.J., Risks and benefits of omega 3 fats for mortality, cardiovascular disease, and cancer: systematic review. *BMJ* 2006, **332**: 752-760.
17. Iso, H., Kobayashi, M., Ishihara, J., Sasaki, S., Okada, K. and Kita, Y., Intake of fish and n3 fatty acids and risk of coronary heart disease among Japanese: the Japan Public Health Center-Based (JPHC) Study Cohort I. *Circulation* 2006, **113**: 195-202.
18. Jiang, X., Zeng, H.S., Guo, Y., Zhou, Z.B., Tang, B.S. and Li, F.K., The expression of matrix metalloproteinases-9, transforming growth factor-beta1 and transforming growth factor-beta receptor I in human atherosclerotic plaque and their relationship with plaque stability. *Chin. Med. J.* (Engl ) 2004, **117**: 1825-1829.
19. Kai, H., Ikeda, H., Yasukawa, H., Kai, M., Seki, Y. and Kuwahara, F., Peripheral blood levels of matrix metalloproteases-2 and -9 are elevated in patients with acute coronary syndromes. *J. Am. Coll. Cardiol.* 1998, **32**: 368-372.
20. Kalela, A., Laaksonen, R., Lehtimaki, T., Koivu, T.A., Hoyhtya, M. and Janatuinen, T., Effect of pravastatin in mildly hypercholesterolemic young men on serum matrix metalloproteinases. *Am. J. Cardiol.* 2001, **88**: 173-175.
21. Laimer, M., Kaser, S., Kranebitter, M., Sandhofer, A., Muhlmann, G. and Schwelberger, H., Effect of pronounced weight loss on the nontraditional cardiovascular risk marker matrix metalloproteinase-9 in middle-aged morbidly obese women. *Int. J. Obes.* (Lond) 2005, **29**: 498-501.
22. Luu, N.T., Madden, J., Calder, P.C., Grimble, R.F., Shearman, C.P. and Chan, T., Dietary supplementation with fish oil modifies the ability of human monocytes to induce an inflammatory response. *J. Nutr.* 2007, **137**: 2769-2774.
23. Madsen, T., Christensen, J.H., Blom, M. and Schmidt, E.B., The effect of dietary n-3 fatty acids on serum concentrations of C-reactive protein: a dose-response study. *Br. J. Nutr.* 2003, **89**: 517-522.
24. Matsumoto, M., Sata, M., Fukuda, D., Tanaka, K., Soma, M. and Hirata, Y., Orally administered eicosapentaenoic acid reduces and stabilizes atherosclerotic lesions in ApoE-deficient mice. *Atherosclerosis* 2008, **197**: 524-533.
25. Miles, E.A., Zoubouli, P. and Calder, P.C., Differential anti-inflammatory effects of phenolic compounds from extra virgin olive oil identified in human whole blood cultures. *Nutrition* 2005, **21**:389-394.
26. Nagase, H., Visse, R. and Murphy, G., Structure and function of matrix metalloproteinases and TIMPs. *Cardiovasc. Res.* 2006, **69**: 562-573.
27. Newby, A.C., Do metalloproteinases destabilize vulnerable atherosclerotic plaques? *Curr. Opin. Lipidol.* 2006, **17**: 556-561.
28. Nordskog, B.K., Blixt, A.D., Morgan, W.T., Fields, W.R. and Hellmann, G.M., Matrix-degrading and pro-inflammatory changes in human vascular endothelial cells exposed to cigarette smoke condensate. *Cardiovasc. Toxicol.* 2003, **3**: 101-117.
29. Packard, RR. and Libby, P., Inflammation in atherosclerosis: from vascular biology to biomarker discovery and risk prediction. *Clin. Chem.* 2008, **54**: 24-38.
30. Raffetto, J.D. and Khalil, R.A., Matrix metalloproteinases and their inhibitors in vascular

remodeling and vascular disease. *Biochem. Pharmacol.* 2008, **75**: 346-359.
31. Schmidt, E.B., Arnesen, H., Christensen, J.H., Rasmussen, L.H., Kristensen, S.D. and De, C.R., Marine n-3 polyunsaturated fatty acids and coronary heart disease: Part II. clinical trials and recommendations. *Thromb. Res.* 2005, **115**: 257-262.
32. Schmidt, E.B., Arnesen, H., De, C.R., Rasmussen, L.H. and Kristensen, S.D., Marine n-3 polyunsaturated fatty acids and coronary heart disease. Part I. Background, epidemiology, animal data, effects on risk factors and safety. *Thromb. Res.* 2005, **115**: 163-170.
33. Schmidt, E.B., Pedersen, J.O., Varming, K., Ernst, E., Jersild, C. and Grunnet, N., n-3 fatty acids and leukocyte chemotaxis. Effects in hyperlipidemia and dose-response studies in healthy men. *Arteriosclerosis and Thrombosis* 1991, **11**: 429-435.
34. Schmidt, E.B., Rasmussen, L.H., Rasmussen, J.G., Joensen, A.M., Madsen, M.B. and Christensen, J.H., Fish, marine n-3 polyunsaturated fatty acids and coronary heart disease: a minireview with focus on clinical trial data. *Prostaglandins Leukot. Essent. Fatty Acids* 2006, **75**: 191-195.
35. Schmidt, E.B., Varming, K., Ernst, E., Madsen, P. and Dyerberg, J., Dose-response studies on the effect of n-3 polyunsaturated fatty acids on lipids and haemostasis. *Thromb. Haemost.* 1990, **63**: 1-5.
36. Seagrave, J., Barr, E.B., March, T.H. and Nikula, K.J., Effects of cigarette smoke exposure and cessation on inflammatory cells and matrix metalloproteinase activity in mice. *Exp. Lung. Res.* 2004, **30**: 1-15.
37. Sundstrom, J. and Vasan, R.S., Circulating biomarkers of extracellular matrix remodeling and risk of atherosclerotic events. *Curr. Opin. Lipidol.* 2006, **17**: 45-53.
38. Tayebjee, M.H., Lip, G.Y., Blann, A.D. and Macfadyen, R.J., Effects of age, gender, ethnicity, diurnal variation and exercise on circulating levels of matrix metalloproteinases (MMP)-2 and -9, and their inhibitors, tissue inhibitors of matrix metalloproteinases (TIMP)-1 and -2. *Thromb. Res.* 2005, **115**: 205-210.
39. Tayebjee, M.H., Lip, G.Y., Tan, K.T., Patel, J.V., Hughes, E.A. and Macfadyen, R.J., Plasma matrix metalloproteinase-9, tissue inhibitor of metalloproteinase-2, and CD40 ligand levels in patients with stable coronary artery disease. *Am. J. Cardiol.* 2005, **96**: 339-345.
40. Tayebjee, M.H., Nadar, S., Blann, A.D., Gareth, B.D., Macfadyen, R.J. and Lip, G.Y., Matrix metalloproteinase-9 and tissue inhibitor of metalloproteinase-1 in hypertension and their relationship to cardiovascular risk and treatment: a substudy of the Anglo-Scandinavian Cardiac Outcomes Trial (ASCOT). *Am. J. Hypertens.* 2004, **17**: 764-769.
41. Thies, F., Garry, J.M., Yaqoob, P., Rerkasem, K., Williams, J. and Shearman, C.P., Association of n-3 polyunsaturated fatty acids with stability of atherosclerotic plaques: a randomized controlled trial. *Lancet* 2003, **361**: 477-485.
42. Varming, K., Schmidt, E.B., Grunnet, N., Jersild, C. and Dyerberg, J., The effect of n-3 fatty acids on neutrophil chemiluminescence. *Scand. J. Clin. Lab. Invest.* 1995, **55**: 47-52.
43. Visse, R. and Nagase, H., Matrix metalloproteinases and tissue inhibitors of metalloproteinases: structure, function, and biochemistry. *Circ. Res.* 2003, **92**: 827-839.
44. Weinkouff, P.M., Koenig, W., Hagstrup, C.J. and Berg, S.E., The effect of marine n-3 fatty acids in different doses on plasma concentrations of Lp-PLA(2) in healthy adults. *Eur. J. Nutr.* 2008 Nov 21.
45. Xu, Z., Zhao, S., Zhou, H., Ye, H. and Li, J., Atorvastatin lowers plasma matrix metalloproteinase-9 in patients with acute coronary syndrome. *Clin. Chem.* 2004, **50**: 750-753.
46. Yasmin, McEniery, C.M., O'Shaughnessy, K.M., Harnett, P., Arshad, A. and Wallace, S., Variation in the human matrix metalloproteinase-9 gene is associated with arterial stiffness in healthy individuals. *Arterioscler. Thromb. Vasc. Biol.* 2006, **26**: 1799-1805.
47. Yasmin, McEniery, C.M., Wallace, S., Dakham, Z., Pulsalkar, P. and Maki-Petaja, K., Matrix metalloproteinase-9 (MMP-9), MMP-2, and serum elastase activity are associated with systolic hypertension and arterial stiffness. *Arterioscler. Thromb. Vasc. Biol.* 2005, **25**: 372.
48. Yokoyama, M., Origasa, H., Matsuzaki, M., Matsuzawa, Y., Saito, Y. and Ishikawa, Y., Effects of eicosapentaenoic acid on major coronary events in hypercholesterolaemic patients (JELIS): a randomized open-label, blinded endpoint analysis. *Lancet* 2007, **369**: 1090-1098.
49. Zampolli, A., Bysted, A., Leth, T., Mortensen, A., De, C.R. and Falk, E., Contrasting effect of fish oil supplementation on the development of atherosclerosis in murine models. *Atherosclerosis* 2006, **184**: 78-85.
50. Zeng, B., Prasan, A., Fung, K.C., Solanki, V., Bruce, D. and Freedman, SB., Elevated circulating levels of matrix metalloproteinase-9 and -2 in patients with symptomatic coronary artery disease. *Intern Med J* 2005, **35**: 331-335.
51. Andersen, V.L., Vogt, J., Obel, T., Christensen, J.H., Schmidt, E.B., The effect of n-3 fatty acids on plasma myeloperoxidase levels in healthy adults. *Cell. Mol. Biol.* 2010, **56**(1): 3-9.
52. Andreasen, J. J., Aardestrup, I. V., Eschen, R. B., Obel, T., Lundbye-Christensen, S., Schmidt, E. B., Fatty acid composition of the internal mammary artery in relation to dietary intake of marine n-3 polyunsaturated fatty acids and association with flow-mediated vasodilation. *Cell. Mol. Biol.* 2010, **56**(1): 10-17.
53. Arnesen, H., Seljeflot, I., Studies on very long chain marine n-3 fatty acids in patients with atherosclerotic heart disease with special focus on mechanisms, dosage and formulas of supplementation. *Cell. Mol. Biol.* 2010, **56**(1): 18-27.
54. Calder, P.C., Yaqoob, P., Omega-3 (n-3) fatty acids, cardiovascular disease and stability of atherosclerotic plaques. *Cell. Mol. Biol.* 2010, **56**(1): 28-37.
55. Petersen, M.M., Eschen, R.B., Aardestrup, I., Obel, T., Schmidt, E.B., Flow-mediated vasodilation and dietary intake of n-3 polyunsaturated acids in healthy subjects. *Cell. Mol. Biol.* 2010, **56**(1): 38-44.
56. Eschen, O., Christensen, J.H., La rovere, M.T., Romano, P., Sala, P., Schmidt, E.B., Effects of marine n-3 fatty acids on circulating levels of soluble adhesion molecules in patients with chronic heart failure. *Cell. Mol. Biol.* 2010, **56**(1): 45-51.
57. Isherwood, C., Wong, M., Jones, W.S., Davies, I.G., Griffin, B.A., lack of effect of cold water prawns on plasma cholesterol and lipoproteins in normo-lipidaemic men. *Cell. Mol. Biol.* 2010, **56**(1): 52-58.
58. Massaro, M., Scoditti, E., Carluccio, M. A., Campana, M. C., De caterina, R., Omega-3 fatty acids, inflammation and angiogenesis: basic mechanisms behind the cardioprotective effects of fish and fish oils. *Cell. Mol. Biol.* 2010, **56**(1): 59-82.
59. Mori, T. A., Omega-3 fatty acids and blood pressure. *Cell. Mol. Biol.* 2010, **56**(1): 83-92.
60. Von Schacky, C., Omega-3 fatty acids vs. cardiac disease – the contribution of the omega-3 index. *Cell. Mol. Biol.* 2010, **56**(1): 93-101.

61. Marchioli, R., Silletta, M.G., Levantesi, G., Pioggiarella, R., Tognoni, G., N-3 polyunsaturated fatty acids in heart failure: mechanisms and recent clinical evidence. *Cell. Mol. Biol.* 2010, **56**(1): 110-130.

62. Christensen, J.H., Svensson, M., Strandhave, C., Madsen, T., Schmidt, E.B., N-3 fatty acids and cardiac autonomic function in humans. *Cell. Mol. Biol.* 2010, **56**(1): 131-139.