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ScienceDirect
Metabolism Clinical and Experimental 56 (2007) 547–551
Metabolism
Clinical and Experimental
www.elsevier.com/locate/metabol

Long-chain n-3 fatty acid supplementation in men increases resistance to activated protein C

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Received 7 June 2006; accepted 20 November 2006

Abstract

It has recently and controversially been demonstrated that fish oil supplementation may not be beneficial for everyone, but to date there have been no biological explanations. We suggest that resistance to the anticoagulant, activated protein C (APC), be considered as a potential mechanism, because it has been demonstrated that the type of fatty acids on phospholipids modulates function of the APC pathway. The APC ratio in plasma was decreased by 7% after fish oil supplementation in healthy men ($P < .005$; n = 35). The decrease in APC ratio equates to an increase in APC resistance. Fish oil lowered the APC ratio by (1) increasing low-density lipoprotein (LDL) cholesterol ($P < .01$) and apolipoprotein B ($P < .05$) and (2) increasing platelet microparticles ($P < .05$). In vitro, purified LDL decreased the APC ratio and increased microparticle formation. These changes affecting the anticoagulant APC could contribute toward a prothrombotic state, potentially explaining the recent observation that fish oil supplementation may not always be of benefit. These findings will need to be repeated in different disease states.

1. Introduction

The beneficial effects of fish on vascular disease are well recognized, through inhibition of procoagulant events such as platelet aggregation [1], reduction in plasma triglycerides [2,3], changes in gene expression [4], and anti-inflammatory properties [5]. However, a number of studies provide conflicting evidence on the effect of fish oil on coagulation and platelet function [6-17], and recently a systematic review suggested that fish oils did not improve the risk of mortality or cardiovascular disease [18]. Therefore, it would appear that fish oils may have multiple effects on the vascular system.

Thrombosis results from an imbalance between coagulant and anticoagulant pathways. Activated protein C (APC) acts as an anticoagulant by inhibiting the actions of coagulation factors Va and VIIIa. A mutation in factor Va (factor V Leiden) prevents inhibition by APC, causing APC resistance and venous thromboembolism [19,20]. In addition to genetic causes of APC resistance, an APC resistance phenotype exists that is associated with many of the features of the metabolic syndrome including increased triglycerides, systolic blood pressure, body mass index (BMI), and platelet activation [21,22]. An APC resistance phenotype is also associated with increasing age and cholesterol concentration, although the explanation for this finding is uncertain [21]. The causes of the nongenetic APC resistance phenotype are unclear and the role of diet, particularly fish oil supplementation, in the modulation of APC resistance has not been investigated in detail. A diet enriched in α-linolenic acid (the precursor for eicosapentaenoic acid, which is a major fatty acid in fish oil) improved anticoagulant activity via the APC ratio [23], suggesting that fish oil may alter anticoagulation. In addition, both coagulation and the APC pathway are catalyzed by phospholipids. Because the type of fatty acid present is important for the activity of coagulant and anticoagulant pathways, it is plausible that fatty acids from fish oil may modulate the rate of reaction. No previous study has examined the effect of fish oil supplementation on the anticoagulant APC.

The effects of the APC pathway on venous thrombosis are well documented; however, the APC pathway may have a role in cardiovascular disease too, as demonstrated by

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0026-0495/$ – see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.metabol.2006.11.017

associations with traditional cardiovascular risk factors [21] and both advanced atherosclerosis (stenosis) and arterial disease [24]. Recently, and perhaps controversially, it has been suggested that fish oil supplementation may not be beneficial for everyone [18,25]. The large systematic review by Hooper et al [18] demonstrated that there was no clear reduction by omega-3 fats on total mortality or combined cardiovascular events. In particular, the authors highlighted a recent randomized controlled trial by Burr et al [25], which showed that men with angina had a higher risk of cardiac death with fish oil capsule supplementation. Neither of these publications could demonstrate a mechanism for the observed effects.

The aim of our study was to test the effects of fish oil supplementation on APC resistance in healthy individuals.

2. Methods

2.1. Fish oil supplementation

Fasting blood samples from healthy men (n = 35) were collected before and after 12 weeks of fish oil supplementation (Maxepa, Seven Seas, Hull, UK; six 1-g capsules per day) after informed consent had been obtained according to local ethics committee approval. The mean age was 25.4 ± 8.3 years and the mean BMI was 23.6 ± 2.3 kg/m^2. Compliance was confirmed by measuring plasma eicosapentaenoic acid and docosahexaenoic acid by mass spectrometry [26].

2.2. APC sensitivity ratio

The APC sensitivity ratio was measured in plasma by use of Coatest APC Resistance-C kits (Chromogenix via Quadratech, Epsom, Surrey, UK) in a clot-based assay. APC ratio was calculated as the time to clot after addition of Ca and APC (activated partial thromboplastin time [APTT] in the presence of APC [APTT + APC]) divided by the time to clot after addition of Ca alone (APTT). A low APC ratio is associated with an increased prothrombotic risk. The assays were repeated in platelet-rich plasma (PRP) (250 × 10^9 platelets per liter) incubated with 7 μL of phosphate-buffered saline (PBS, pH 7.2; no agonist), collagen (19 μg/mL final concentration when incubated with PRP; Helena BioSciences, Gateshead, Tyne & Wear, UK), or adenosine diphosphate (4.7 μmol/L; Helena BioSciences) for 150 seconds before measuring APC ratio as described above. The effect of low-density lipoprotein (LDL) was examined by adding 20 μL of 200 μg/mL LDL [27] to 20 μL plasma and 20 μL APTT reagent before adding 25 μL Ca or APC + Ca. It has previously been demonstrated that in some studies, fish oil supplementation increases LDL concentration. Therefore, the effects of purified 200 μg/mL LDL were examined based on a (patho)physiological concentration. It has been suggested that 250 to 500 μg/mL protein represents physiological concentrations of LDL [28]. Therefore, adding extra LDL to plasma from young healthy men would result in concentrations that may be found in an older at-risk population. In comparison, homozygous familial hypercholesterolemia is equivalent to 3 to 5.5 mg/mL, which is more than 10 times higher than the LDL we added. A dose-response experiment using different concentrations of LDL was carried out using 3 donors (10-400 μg/mL). The samples were selected from donors with no known cardiovascular disease.

2.3. Measurement of apolipoprotein B and cholesterol

Apolipoprotein (apo) B_{48+100} (antibody recognizes both apo B_{48} and apo B_{100}) and cholesterol were measured in plasma by enzyme-linked immunosorbent assay (ELISA) and colorometric assay, respectively [27] as markers of LDL in plasma.

2.4. Platelet activation measurements

Platelet microparticles were measured using an ELISA based on a mouse monoclonal antibody against CD61 (Dako, Ely, Cambridgeshire, UK). Microparticles generated by activating platelets in PRP were diluted to give a standard curve. Results are expressed as equivalent units (EU), where 1 EU is equivalent to the number of platelet microparticles generated when 5 × 10^8 platelets were activated with 1 mmol/L A23187/0.5 mmol/L $CaCl_2$ (calcium ionophore). Low-density lipoprotein (200 μg [lipo]protein/mL) was incubated with PRP for 30 minutes at 37°C. The samples were then centrifuged at 2000*g* to remove platelets and the platelet microparticle content was measured as described. Platelet aggregation with 10 μg/mL collagen (Helena Biosciences) was performed in an APACT-4 aggregometer. Maximum aggregation was measured as percent change in light transmission.

2.5. Statistical analysis

Data was normalized by log transformation and analyzed by using paired Student *t* tests.

3. Results

APTT + APC and the APC ratio were decreased by 7% after fish oil supplementation (Table 1; P = .011 and P = .002, respectively). There was no difference in APTT. The assays were repeated in PRP because platelet activation produces an APC resistance phenotype [22]. Fish oil supplementation resulted in a decreased APTT + APC and APC ratio in PRP

Table 1
APTT, APTT + APC, and APC ratio in plasma before and after 12 weeks fish oil supplementation

	Before fish oil	After fish oil	*P*
Ca time to clot (s)	37.4 ± 0.7	37.4 ± 0.6	NS
APC time to clot (s)	107.6 ± 2.8	100.6 ± 2.4	.011
APC ratio	2.88 ± 0.04	2.69 ± 0.05	.002

Data are from duplicate measurements and are presented as mean ± SEM (n = 35). APC ratio was calculated as (APTT + APC)/APTT. NS indicates no statistically significant difference between before and after fish oil.

(2.87 ± 0.08 to 2.63 ± 0.05; P = .004), in PRP + collagen (19 μg/mL final concentration) (1.77 ± 0.03 to 1.69 ± 0.03; P = .032) and in PRP + adenosine diphosphate (4.7 μmol/L) (2.22 ± 0.06 to 2.01 ± 0.05; P = .003).

In agreement with other studies [29], fish oil supplementation increased LDL concentration in our cohort (apo B: 631 ± 80 vs 967 ± 155 μg/mL, P = .036; cholesterol: 145.0 ± 2.7 vs 152.5 ± 3.8 mg/dL, P = .008). Therefore, we examined the effect of adding purified LDL to the APC assay. LDL was associated with a decrease in APTT + APC and APC ratio compared with control PBS buffer (2.20 ± 0.09 vs 2.11 ± 0.14, n = 9, P = .037; Fig. 1A) in a dose-dependent and donor-specific manner. These results also suggest an explanatory mechanism for the observation that APC ratio was inversely associated with cholesterol concentrations in a large UK cohort [21].

Platelet microparticles support an APC resistance phenotype [22] and LDL increases platelet activation. Therefore, we hypothesized that fish oil supplementation increases microparticles in vivo. Platelet microparticles, measured by ELISA, were 12% higher in plasma samples taken after fish oil supplementation (P = .012, n = 25; Fig. 1B). We also demonstrated collagen-induced platelet aggregation was increased by 7% after fish oil supplementation (P = .029; data not shown). This suggests that platelets are more easily activated after supplementation, substantiating the platelet microparticle data. Adding LDL to platelets was associated with a 23% increase in microparticle generation (P = .023, n = 7; Fig. 1C).

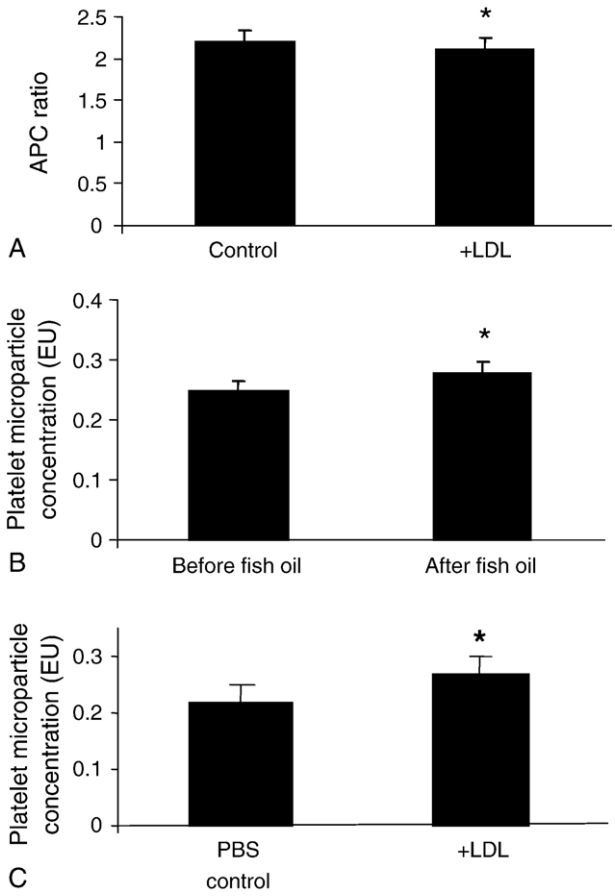

Fig. 1. A, The effect of LDL on APC ratio was examined by adding 200 μg/mL LDL to 20 μL plasma and 20 μL APTT reagent before adding 25 μL Ca or APC + Ca (n = 9). B, Platelet microparticle concentration in plasma was measured by ELISA before and after fish oil supplementation (n = 25). 1 EU is equivalent to the number of platelet microparticles generated when 5×10^8 platelets were activated with 1 mmol/L A23187/0.5 mmol/L $CaCl_2$ (calcium ionophore). C, Platelet microparticle concentration was measured after incubation of PRP with either PBS or with LDL (200 μg protein/mL; n = 7). *P < .05.

4. Discussion

These data offer support for the recent and controversial suggestion that fish oil supplementation may not always be beneficial. In this group of healthy young men, fish oil supplementation caused a small but statistically significant decrease in APC ratio that could contribute to a prothrombotic state, depending on the overall balance of coagulation, anticoagulation, and fibrinolysis in the vessel wall microenvironment. To our knowledge, this is the first report of the effects of fish oil supplementation on APC ratio. In addition to providing the original observation that fish oil supplementation modulates APC ratio, we have proposed and tested possible mechanisms that may be responsible for the changes observed. We demonstrated that LDL and platelet microparticles were increased after fish oil supplementation and that LDL had the capacity to decrease the APC ratio. We have previously demonstrated that platelet microparticles support an APC resistance phenotype [22]. However, it is also possible that other effects may contribute toward the observed changes. This is one of the first studies to demonstrate that diet modulates APC resistance, and as such it is interesting and novel because it suggests that therapeutic dietary modulation of APC resistance may be possible in the future. The only other dietary studies have been with homocysteine (no change) [30], α-linolenic acid (increases APC ratio) [23], and an atherogenic diet that induces hypercholesterolemia (varied) [31,32].

Fish oil supplementation lowers triglyceride levels; however, a number of studies have also reported that LDL cholesterol and apo B are increased by fish oil supplementation [29], although the biological consequences of these changes is uncertain. We have demonstrated that LDL directly decreased the APC ratio. Although it would have been interesting to examine longer time courses of interaction of LDL with APC, the short half-life of APC restricts such experiments, so we selected an incubation time similar to the incubation time for the phospholipids in the APTT reagent. To verify that LDL concentration had increased in our cohort after supplementation, we measured apo B and cholesterol in fasting plasma samples. Both markers of LDL increased after fish oil supplementation. These data suggest that fish oil–mediated increases in LDL in young healthy men resulted in a direct effect on

anticoagulation. These results suggest an explanatory mechanism for the observation that APC ratio was inversely associated with cholesterol concentrations in a large UK population [21]. We have no data to allow us to comment on the effect of sex or age because our study was restricted to young men. However, we suggest that it is now important to study older hyperlipidemic subjects because they are more at risk for cardiovascular and thrombotic disease. Previous publications suggest that women have more APC resistance than men and that age is associated with a lower APC ratio [21].

We have previously demonstrated that platelet microparticles support an APC resistance phenotype, ie, reduce APC ratio [22]. In our current study we have demonstrated that plasma platelet microparticles are increased after fish oil supplementation. Given that LDL activates platelets and that we have demonstrated increased markers of LDL after fish oil supplementation, we examined the effect of LDL on platelet microparticle formation. We demonstrated that adding LDL to PRP at an equivalent concentration to the increase in LDL observed after supplementation increased platelet microparticle formation. These data suggest that the increase in LDL after fish oil supplementation increased platelet microparticle formation. In this study, fish oil supplementation also increased platelet aggregation, suggesting that platelets were more likely to be activated and generate platelet microparticles in vivo. It is unlikely that the differences in APC ratio observed in this study are due to differences in fragmented platelets [33] because APC ratio was measured in fresh plasma.

We have also considered other mechanisms that have previously been demonstrated to alter the APC ratio. The increase in APC ratio was mediated by changes in APTT + APC, and not through APTT. This suggests that interaction of exogenous APC with factors V and/or VIII is altered after fish oil supplementation without modulation of coagulation or endogenous APC generation. Elevated levels of factor VIII cause an APC resistance phenotype, as exogenous APC has less effect on factor V because it is overcome with excess factor VIII. We did not measure factor VIII in this study, as there already is strong evidence that factor VIII is reduced or not altered with fish and fish oil consumption [23,34-37]. In addition, we have not measured homocysteine concentrations in this study despite work demonstrating that homocysteine inhibits inactivation of factor Va by APC [38]. Given that fish oils have previously been demonstrated to lower or have no effect on plasma homocysteine [39-42], it is thought unlikely that alterations in homocysteine levels would cause the changes in APC ratio observed in our study.

The pathophysiological relevance of the decrease in APC ratio observed in this study is not currently understood. Factors that make small differences to health outcomes in a healthy young population may have a larger deleterious effect in vulnerable populations. In a previous study, lower APC ratios were associated with increasing age, systolic blood pressure, and BMI [21]. In addition, there are a number of studies that correlate fish consumption with beneficial vascular outcomes, including original observations in the Inuit [2]. However, more recently a number of studies have been published suggesting that they are not beneficial for everyone. Hooper et al [18] suggest that differences in study design may give discrepancies due to duration of fish oil supplementation (reports suggest that fish oils give most benefit early on and may be detrimental long term due to the accumulation of methylmercury), different study groups such as angina vs heart failure, and differences in the effects of supplementation vs dietary intervention. In fact, a previous study has demonstrated that an α-linolenic acid–enriched diet results in an improved APC ratio [23]. Although these results appear contrary to our study, previous research has demonstrated that α-linolenic acid reduces cholesterol by approximately 12 mg/dL [43]. In contrast, in our study, cholesterol levels are elevated on supplementation, resulting in a decrease in APC ratio. Studies using fish oil supplementation have also produced conflicting evidence on coagulation and platelet function, some of which suggest no benefit to hemostasis [7,9-12,14,15]. Therefore, the preparation of fish oil, the way it is incorporated into the diet, the length of intervention, and the age, health, nationality, and dietary patterns of the consumer may all contribute to the effect of fish oil supplementation.

In summary, these data offer support for the recent and controversial suggestion that fish oil supplementation may not always be beneficial. In this group of healthy young men, fish oil supplementation caused a small but statistically significant decrease in APC ratio that could contribute to a prothrombotic state, depending on the overall balance of coagulation, anticoagulation, and fibrinolysis in the vessel wall microenvironment. It is now necessary to examine the effect of fish oil supplementation on APC resistance in diseased states, particularly those in which fish oil supplementation is advised and in those with altered coagulation status.

Acknowledgments

We gratefully acknowledge the Wellcome Trust, the Biotechnology and Biological Sciences Research Council and The F H Muirhead Charitable Trust for supporting this study in part. During this study, Dr Englyst was supported by a Career Track Training Fellowship awarded by the School of Medicine, University of Southampton, and by a Research into Ageing Fellowship.

The authors are grateful to Dr Gillian O'Reilly and Ms Olivera Markovic for recruitment of volunteers and collection of blood samples.

References

[1] Dyerberg J, Bang HO. Haemostatic function and platelet polyunsaturated fatty acids in Eskimos. Lancet 1979;2:433-5.

[2] Harris WS. n-3 fatty acids and lipoproteins: comparison of results from human and animal studies. Lipids 1996;31:243-52.
[3] Weber P, Raederstorff D. Triglyceride-lowering effect of omega-3 LC-polyunsaturated fatty acids—a review. Nutr Metab Cardiovasc Dis 2000;10:28-37.
[4] Deckelbaum RJ, Worgall TS, Seo T. n-3 fatty acids and gene expression. Am J Clin Nutr 2006;83:1520S-5S.
[5] Calder PC. Polyunsaturated fatty acids and inflammation. Prostaglandins Leukot Essent Fatty Acids 2006;75:197-202.
[6] Brown JE, Wahle KW. Effect of fish-oil and vitamin E supplementation on lipid peroxidation and whole-blood aggregation in man. Clin Chim Acta 1990;193:147-56.
[7] Demke DM, Peters GR, Linet OI, et al. Effects of a fish oil concentrate in patients with hypercholesterolemia. Atherosclerosis 1988;70:73-80.
[8] Donnelly SM, Ali MA, Churchill DN. Effect of n-3 fatty acids from fish oil on hemostasis, blood pressure, and lipid profile of dialysis patients. J Am Soc Nephrol 1992;2:1634-9.
[9] Haines AP, Sanders TA, Imeson JD, et al. Effects of a fish oil supplement on platelet function, haemostatic variables and albuminuria in insulin-dependent diabetics. Thromb Res 1986;43:643-55.
[10] Lox CD. The effects of dietary marine fish oils (omega-3 fatty acids) on coagulation profiles in men. Gen Pharmacol 1990;21:241-6.
[11] Mehta JL, Lopez LM, Lawson D, et al. Dietary supplementation with omega-3 polyunsaturated fatty acids in patients with stable coronary heart disease. Effects on indices of platelet and neutrophil function and exercise performance. Am J Med 1988;84:45-52.
[12] Owens MR, Cave Jr WT. Dietary fish lipids do not diminish platelet adhesion to subendothelium. Br J Haematol 1990;75:82-5.
[13] Rogers S, James KS, Butland BK, et al. Effects of a fish oil supplement on serum lipids, blood pressure, bleeding time, haemostatic and rheological variables. A double blind randomised controlled trial in healthy volunteers. Atherosclerosis 1987;63:137-43.
[14] Salonen R, Nikkari T, Seppanen K, et al. Effect of omega-3 fatty acid supplementation on platelet aggregability and platelet produced thromboxane. Thromb Haemost 1987;57:269-72.
[15] Schmidt EB, Kristensen SD, Dyerberg J. The effect of fish oil on lipids, coagulation and fibrinolysis in patients with angina pectoris. Artery 1988;15:316-29.
[16] Tilvis RS, Rasi V, Viinikka L, et al. Effects of purified fish oil on platelet lipids and function in diabetic women. Clin Chim Acta 1987;164:315-22.
[17] Zucker ML, Bilyeu DS, Helmkamp GM, et al. Effects of dietary fish oil on platelet function and plasma lipids in hyperlipoproteinemic and normal subjects. Atherosclerosis 1988;73:13-22.
[18] Hooper L, Thompson RL, Harrison RA, et al. Risks and benefits of omega 3 fats for mortality, cardiovascular disease, and cancer: systematic review. BMJ 2006:752-60.
[19] Bertina RM, Koeleman BP, Koster T, et al. Mutation in blood coagulation factor V associated with resistance to activated protein C. Nature 1994;369:64-7.
[20] Voorberg J, Roelse J, Koopman R, et al. Association of idiopathic venous thromboembolism with single point-mutation at Arg506 of factor V. Lancet 1994;343:1535-6.
[21] Lowe GD, Rumley A, Woodward M, et al. Activated protein C resistance and the FV:R506Q mutation in a random population sample—associations with cardiovascular risk factors and coagulation variables. Thromb Haemost 1999;81:918-24.
[22] Taube J, McWilliam N, Luddington R, et al. Activated protein C resistance: effect of platelet activation, platelet-derived microparticles, and atherogenic lipoproteins. Blood 1999;93:3792-7.
[23] Allman-Farinelli MA, Hall D, Kingham K, et al. Comparison of the effects of two low fat diets with different alpha-linolenic:linoleic acid ratios on coagulation and fibrinolysis. Atherosclerosis 1999;142:159-68.
[24] Kiechl S, Muigg A, Santer P, et al. Poor response to activated protein C as a prominent risk predictor of advanced atherosclerosis and arterial disease. Circulation 1999;99:614-9.
[25] Burr ML, Ashfield-Watt PA, Dunstan FD, et al. Lack of benefit of dietary advice to men with angina: results of a controlled trial. Eur J Clin Nutr 2003;57:193-200.
[26] Grimble RF, Howell WM, O'Reilly G, et al. The ability of fish oil to suppress tumor necrosis factor alpha production by peripheral blood mononuclear cells in healthy men is associated with polymorphisms in genes that influence tumor necrosis factor alpha production. Am J Clin Nutr 2002;76:454-9.
[27] Rota S, McWilliam NA, Baglin TP, et al. Atherogenic lipoproteins support assembly of the prothrombinase complex and thrombin generation: modulation by oxidation and vitamin E. Blood 1998;91:508-15.
[28] Hackeng CM, Huigsloot M, Pladet MW, et al. Low-density lipoprotein enhances platelet secretion via integrin-alphaIIbbeta3–mediated signaling. Arterioscler Thromb Vasc Biol 1999;19:239-47.
[29] Sullivan DR, Sanders TA, Trayner IM, et al. Paradoxical elevation of LDL apoprotein B levels in hypertriglyceridaemic patients and normal subjects ingesting fish oil. Atherosclerosis 1986;61:129-34.
[30] Lentz SR, Piegors DJ, Fernandez JA, et al. Effect of hyperhomocysteinemia on protein C activation and activity. Blood 2002;100:2108-12.
[31] Lentz SR, Fernandez JA, Griffin JH, et al. Impaired anticoagulant response to infusion of thrombin in atherosclerotic monkeys associated with acquired defects in the protein C system. Arterioscler Thromb Vasc Biol 1999;19:1744-50.
[32] Lentz SR, Miller Jr FJ, Piegors DJ, et al. Anticoagulant responses to thrombin are enhanced during regression of atherosclerosis in monkeys. Circulation 2002;106:842-6.
[33] Luddington R, Brown K, Baglin T. Effect of platelet phospholipid exposure on activated protein C resistance: implications for thrombophilia screening. Br J Haematol 1996;92:744-6.
[34] Archer SL, Green D, Chamberlain M, et al. Association of dietary fish and n-3 fatty acid intake with hemostatic factors in the Coronary Artery Risk Development in Young Adults (CARDIA) study. Arterioscler Thromb Vasc Biol 1998;18:1119-23.
[35] Deslypere JP. Influence of supplementation with n-3 fatty acids on different coronary risk factors in men—a placebo controlled study. Verh K Acad Geneeskd Belg 1992;54:189-216.
[36] Rylance PB, Gordge MP, Saynor R, et al. Fish oil modifies lipids and reduces platelet aggregability in haemodialysis patients. Nephron 1986;43:196-202.
[37] Sahar E, Dolsom AE, Wu KK, et al. Associations of fish intake and dietary n-3 polyunsaturated fatty acids with a hypercoagulable profile. The Atherosclerosis Risk in Communities (ARIC) Study. Arterioscler Thromb 1993;13:1205-12 [Abstract].
[38] Undas A, Williams EB, Butenas S, et al. Homocysteine inhibits inactivation of factor Va by activated protein C. J Biol Chem 2001;276:4389-97.
[39] Brude IR, Finstad HS, Seljeflot I, et al. Plasma homocysteine concentration related to diet, endothelial function and mononuclear cell gene expression among male hyperlipidaemic smokers. Eur J Clin Invest 1999;29:100-8.
[40] Grundt H, Nilsen DW, Hetland O, et al. Atherothrombogenic risk modulation by n-3 fatty acids was not associated with changes in homocysteine in subjects with combined hyperlipidaemia. Thromb Haemost 1999;81:561-5.
[41] Mezzano D, Kosiel K, Martinez C, et al. Cardiovascular risk factors in vegetarians. Normalization of hyperhomocysteinemia with vitamin B(12) and reduction of platelet aggregation with n-3 fatty acids. Thromb Res 2000;100:153-60.
[42] Olszewski AJ, McCully KS. Fish oil decreases serum homocysteine in hyperlipemic men. Coron Artery Dis 1993;4:53-60.
[43] Goyens PL, Mensink RP. The dietary alpha-linolenic acid to linoleic acid ratio does not affect the serum lipoprotein profile in humans. J Nutr 2005;135:2799-804.