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The combination of virgin olive oils and refined marine oils. Beneficial effects

Progress in Nutrition
Vol. 10, N. 4, 230-231, 2008

Titolo
La combinazione di oli vergini di oliva e oli marini raffinati. Effetti benefici

Key words
Olive oils, marine oils, antioxidants, beneficial effects

Parole chiave
Olio di oliva, oli marini, antiossidanti, effetti benefici

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Summary
The traditional extraction technique of marine oils from marine products involves heating or steam stripping of the raw material in order to release the lipids. In addition, marine oils are refined. Removal of molecules to improve sensory attributes or safety of the marine oil may destroy potent antioxidants and may as well remove components with potential beneficial effects. Indeed, in several clinical studies performed on marine oils we have observed that this is associated with loss of anti-inflammatory effects probably mediated by removal of beneficial antioxidants. Based on these findings a clinical study with combined cold pressed olive oil with refined seal oil or fish oil was performed. Healthy subjects were given 15 ml/day of the combined oils for 10-14 weeks. These recombined oils appeared to regain properties and behave more like the cold pressed marine oils. The anti-inflammatory effects were seen in reduction of MCP-1 (monocyte chomotactic protein-1), C-reactive protein (CRP), as well as thromboxane B2 and leukotriene B4 in the seal oil combined with olive oil, and reduction in cytokines in the fish oil combined with olive oil. Similar changes were not seen in the marine oils without the presence of olive oil or olive oil alone. In conclusion, cold pressed olive oil has the ability to regain beneficial effects lost during refinement of marine oils.

Riassunto
La tradizionale tecnica di estrazione degli oli marini dai prodotti del mare coinvolge il riscaldamento o lo stripping a vapore dalle materie prime al fine di rilasciare i lipidi. Inoltre, gli oli marini vengono raffinati. La rimozione di molecole dall'olio marino per migliorarne gli attributi sensoriali o la sicurezza possono distruggere gli antiossidanti e rimuovere anche i componenti con potenziali effetti benefici. Infatti, in diversi studi clinici effettuati sugli oli marini abbiamo osservato che questo è associato con la perdita degli effetti anti-infiammatori probabilmente a causa della rimozione degli antiossidanti. Sulla base di queste evidenze è stato allestito uno studio clinico utilizzando la combinazione di olio di oliva pressato a freddo con olio di foca o di pesce raffinato. Soggetti in salute hanno assunto 15 ml/die dell'olio combinato per 10-14 settimane. Quest'olio combinato sembra recuperare le proprietà e comportarsi come l'olio marino pressato a freddo. Gli

effetti anti-infiammatori sono stati osservati come riduzione della MCP-1 (proteina chemotattica dei monociti-1), della proteina C-reattiva (PCR), come pure del trombossano B2 e del leucotriene B4 nell'olio di foca in combinazione con l'olio d'oliva, e come riduzione delle citochine nell'olio di pesce in combinazione con l'olio d'oliva. Simili cambiamenti non sono stati osservati negli oli marini senza la presenza di olio d'oliva o di olio d'oliva da solo. In conclusione, l'olio d'oliva pressato a freddo ha la capacità di riacquistare gli effetti benefici persi durante la raffinazione degli oli marini.

Cardio-protective properties of seafood – lost due to processing?

The groundbreaking observations of reduced mortality due to CVD in Greenland Eskimos with a traditional diet consisting substantial amounts of meat and fat (blubber) from seal and whales, suggest that high intakes of n–3 fatty acids from fish and sea mammals prevent CVD (1). This is in contrast with the high frequency of cardiovascular disease in Western populations, who have low fish intakes and high intakes of cholesterol and saturated fat. Consumption of fish and marine PUFAs is associated with a reduced risk of cardiovascular events and mortality, in part caused by a decreased risk of sudden death (2-5). We have previously performed various studies of the effects of intake of fish, seal and whale oils, fish fillet, and fish paste enriched with taurine and the n-3 PUFAs EPA and DHA in healthy human volunteers. In all these studies, healthy human subjects have been given various dietary supplements, and the endpoints of our studies has been measurements of serum fatty acids, lipids and lipoproteins and various parameters associated with cardiovascular/thrombotic diseases (6-13). We have investigated various questions related to these marine products, such as: Which marine oil/oil combinations have the most beneficial effects? Does processing of the marine oils influence the health benefits? Are there additional benefits from eating whole fish compared to fish oils? Are fish oils more efficiently taken up from complete fish compared to fish oil capsules? Indeed, in a recent study, we found that the long-chain n-3 PUFAs were almost 3-times more efficiently taken up from complete fish than from cod liver oil (13). The main task of modern food refining processes is to make edible and stable products. Refining procedures to improve sensory attributes or safety of marine oils may for instance destroy potent antioxidants or remove other components with potential beneficial effects. Modern meal preparing techniques may also lower the content of biologically active components, and losses of low molecular weight compounds, due to preparing techniques are well known. Previous studies in our laboratories indicate (1) up to 70% loss of biological active components when preparing traditional (Norwegian) fish products (14), and (2) that there are protective substances, the effect of which disappears when products are subjected to rough processing conditions such as cooking or refining (7, 9).

Marine oils – Cardiovascular risk protection?

The objectives of our experiments have been, by mimicking parts of the traditional Eskimo diet, to explore the beneficial effects of raw or less processed food items on parameters related to development of CHD. In our studies, healthy volunteers ingested cold pressed "virgin" versus refined marine oils (seal, whale, cod liver). These studies have revealed that there may be additional to n-3 fatty acids, beneficial components of relevance for cardiovascular diseases in marine oils. Non-refined (cold pressed or "virgin") marine oil had additional positive effects on parameters related to development of CHD despite a lower content of n-3 fatty acids (7, 9, 12).

In a recent investigation, one hundred and thirty one healthy subjects were randomly divided into 7groups receiving 15 ml/day for 8 weeks cold pressed or refined whale- seal- or fish oils and one group which did not receive any oil (control group) [unpublished]. A multivariate analysis showed that intake of particularly whale oil was associated with a strong positive correlation between changes in EPA and changes in HDL-cholesterol (r=0.63 for refined and 0.40 for cold-pressed whale oils). This was less pronounced in the CLO groups. Changes in EPA in the cold-pressed CLO group were negatively correlated to changes in WBC, platelets, cytokines and proinflammatory eicosanoids, whereas in the refined CLO group changes in EPA was negatively correlated to changes in blood cells but not to changes in LPS-induced products. Our investigations seem to indicate that there may be protective substances, relevant for the development of CHD, in seafood and marine oils, the effect of which disappears when these products are subjected to rough processing conditions such as blanching, cooking or refining. This supports our hypothesis that n-3 fatty acids are not the only products of the Eskimo diet preventing cardiovascular diseases.

Oil processing/refining

The main task of modern processes for making cod liver/fish oil products is to make them edible and stable. Removal of molecules that causes off- flavours or taste to improve sensory attributes may, for instance, destroy potent antioxidants. Processing (including preparation) makes food healthier, safer, tastier and more shelf-stable. While the benefits are numerous, processing can also be detrimental, affecting the nutritional quality. The content of biological active molecules of foods subjected to processing or preparing is influenced by several factors. The chemical stability of such molecules in the food is of importance as well as the extent of processing, environmental factors and form in which the food is delivered. The nutritional quality of the food depends on quantity as well as availability of such molecules. The time and temperature of processing, product composition and storage are all factors that substantially impact the status of our foods.

Fish oils are extracted from whole fish, fish liver (mainly cod liver) or by-products from the fisheries industry (mainly salmon). Marine mammal oils are produced from blubber, external adipose tissue. The traditional extraction technique involves heating or steam stripping of the raw material in order to release the lipids. Marine oils are highly unsaturated and the application of high temperatures during extraction may cause undesired effects like; initiation of oxidation reactions, destruction of antioxidants and extraction of molecules that causes taste and smell in the oil fraction. It is inevitable that during heat extraction of the oil, detectable changes occur in the different lipid component, as compared with their "virgin" state in the cells (8). Marine oils for human consumption are normally subjected to additional refining procedures. The main objectives of this process are to remove pesticides and to make an edible and stable product. To achieve a stable, sensory acceptable and safe product the removal of a number of components (proteins, peptides, amino acids, free fatty acids, phospholipids, pigments, sterols, transformation products,

metals and possible toxic agents) are normally necessary.
In conclusion, removal of molecules to improve sensory attributes or safety of the oil may destroy potent antioxidants and may as well remove components with potential beneficial effects.

Virgin olive oil

Virgin olive oils produced by direct-press or centrifugation methods have higher phenolic content. Phenolic compounds in olive oil show strong antioxidant properties and at the same time oxidized low-density lipoprotein (LDL) is damaging to the arterial wall.
Improvements in endothelial dysfunction and the lipid profile have been reported for dietary polyphenols and the Mediterranean diets health benefits are suggested to be due to a synergism of phytochemicals and fatty acids (15-18). Olive oil (rich in oleic acid fatty acid), is the main fat of the Mediterranean diet and most of the protective effect of olive oil has been attributed to its high monounsaturated fatty acid content. However, the minor components, particularly the phenolic compounds, in olive oil are recently documented to contribute to the health benefits derived from the Mediterranean diet (15-18).
The effect of consuming virgin olive oil (high in polyphenols), refined olive oil (low in polyphenols), and a mixture of the 2 oils in equal parts were studied by Covas et al. (16). Two hundred healthy young men consumed 25 mL of an olive oil daily for 3 weeks followed by the other olive oils in a randomly assigned sequence. Olive oils with greater polyphenol content led to a slight increased high density lipoprotein (HDL) cholesterol levels and decrease in serum markers of oxidation. A linear decrease in total cholesterol-HDL cholesterol ratio and oxidative biomarkers (conjugated dienes, hydroxy fatty acids, and circulating oxidized LDL) in association with the phenolic content of the olive oils was observed. The greatest within-group effects on increasing HDL cholesterol levels and decreasing oxidative biomarkers were also measured after high polyphenol olive oil consumption. These authors have also concluded that virgin olive oil have greater health benefits than refined olive oil (16).

Why is it important to prevent oxidation in the vessel wall?

Recruitment of monocytes and lymphocytes from the peripheral blood to the intima of the vessel wall is a primordial event in atherogenesis, an event that appears to depend on the local presence of high amounts of LDL. As the LDL accumulates, their lipids and proteins undergo oxidation and glycation. Cells in the vessel wall seem to interpret the change as a dangerous sign, and they call for reinforcement from the body defense system. These events appear to promote upregulation of adhesion molecules on the endothelial cells (ECs), particularly vascular cell adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule-1 (ICAM-1). Thus monocyte and lymphocyte recruitment is initiated, leading to enhanced transmigration of monocytes, upregulated exposure of adhesion molecules on a variety of cells and chemoattractant production and release. These are all essential elements of the transfer of monocytes to the intima and the concurrent differentiation of these cells into macrophages. Available LDL is a prerequisite for the further conversion of macrophages into lipid loaded macrophages, major residents of the fatty streak formed just underneath the endothelium of the vessel wall. Modified forms of LDL (oxidized, acylated etc.) are of particular interest, since the modification of LDL is associated with inflammatory reactions, amplifying proinflammatory events already initiated due to the adherence and transmigration of monocytes and lymphocytes into the intima.
Polyunsaturated fatty acids, as e.g. the omega-3 fatty acids, are also incorporated in LDL-particles, i.e.

these particles are thereby more susceptible for oxidation. Since this is one of the major reactions in the early phase of atherogenesis19 (review), a prevention of the oxidation may prevent foam cell formation in the intima, the early event in atherogenesis. Thus, antioxidants have been shown to reduce lesion formation in animal models 20 (review). Furthermore, the antioxidants are also important in the downregulation of eicosanoid-metabolism. The lipoxygenase pathway leading to e.g. leukotriene B4, is well known to be inhibited by antioxidants with the resultant reduction in LTB4 production. It has been shown that inhibition of the LTB4 receptor in transgenic mice predisposed for atherosclerosis, reduced lesion formation by about 70% (21). Incidentally, one test subject who has been using the present nutrition oil for almost 10 years, was found to have very low LTB4 generation in LPS-induced whole blood. By adding commercial LTB4 to the blood of this subject, LPS-induced TF rose more than 70%, and the blood response to LPS was again among the highest we have measured.

Oxidation of various oils at 70°C

Acknowledging the very important role of antioxidants in the prevention of oxidation of PUFA'a in the vessel wall, we decided to evaluate the potential of cold pressed olive oil to prevent oxidation of our marine oils. We subjected the various oils, e.g. fish and seal oils mixed with olive oil, to heating at 70 oC under oxidation conditions in an Oxidograph. Table 1 shows the results where it can be seen that the polyunsaturated fatty acid rich oils are oxidized quite rapidly. Particularly is the concentrate of EPA and DHA oxidized extremely fast even when containing vitamin E. In contrast, the marine oils mixed with olive oil are resisting oxidation of a relatively long period, 38.8 hrs for seal oil in combination with olive oil and 23 hours for fish oil in combination with olive oil.

Table 1 - The stability of various oils when hated 70°C and exposed to oxidation

Oil	Hours before start of oxidation	"Second" reaction
OliVita	38	
Olive oil	>60	
Soya oil	1,1	
Cod liver oil (CLO)	0,5	9,6
Seal oil	2,1	12,5
Conc. of EPA+DHA	<0,1	

Clinical studies on marine oils in combination with cold pressed olive oil

Altogether 9 clinical studies have been performed on marine oils and in two studies the marine oils were combined with specially selected extra virgin olive oils. The number of healthy test subjects varied from 18 to 37 in each group in the two studies and they lasted respectively for 10 or 12 weeks. The oils were consumed as liquid (15 ml/day) as a supplement to regular diet. Blood was drawn on fasting test subjects between 08.00 am to 10.00 am. Blood samples were analyzed for a series of test parameters as fatty acids and lipids in serum, some coagulation test parameters as prothrombin fragment 1+2 (thombin generator indicator), adhesion molecules and inflammatory products as monocyte chemotactic protein -1 (MCP-1), hypersensitive CRP (hsCRP) and lipopolysaccharide (LPS)-induced cytokines. The major results are indicated in Tab. 3. In contrast to CLO, we found that intake of the combination of seal oil and olive oil gave a significant reduction in hsCRP and MCP-1 which imply that the inflammatory

Table 2 - Administration of marine oils; 10 weeks

Parameter	Control	Seal	Cod liver	Seal+CL	Whale
Serum:					
triacylglycerol (TG)	-	L*	-	-	-
total cholesterol	"	-	-	-	-
HDL cholesterol	"	-	-	H*	H***
Coagulation factors:					
Prothrombin F1+2	"	-	-	-	L*
Lipopolysaccharide stimulated (LPS) whole blood:					
tumor necrosis factor-TNF α (monocytes)	"	-	-	-	L*
tissue factor activity (TF) (monocytes)	"	-	-	L*	L*
thromboxane B2 (TXB2)	"	-	-	-	L**

Table 3 - Diet added 15 ml cod liver oil (CLO) or OliVita, changes in parameters related to coronary heart disese (CHD) before and after intake

Groups	HDL-chol	hsCRP	MCP-1	TxB2	LTB4
CLO	(+)	-	+	++	++
OliVita	++	+++	+++	++	++

reactions in the test subjects had been altered in a very positive way. Furthermore, HDL-cholesterol, the good cholesterol, was increased by about 8 percent in the group consuming seal oil/olive oil. This confirms our earlier observation that marine oils from blubber of mammalian animals has a very good effect on HDL-cholesterol 9. As expected was the production of thromboxane B2 and leukotriene B4 reduced by both CLO and seal oil/olive oil.

The major difference between fish oil in combination with olive oil as compared to seal oil and olive oil, was the latter's combined oil had a significantly higher rise in the good HDL-cholesterol compared to the combination of fish oil and olive oil.

Conclusions

Our studies suggest that by recombining refined marine oils, avoid of antioxidants and other contaminants, with extra virgin olive oils, we obtain a synergistic effect between the marine polyunsaturated fatty acids, particularly EPA and DHA, and the antioxidants and probably other components in the olive oil. This effect mimics the significant beneficial effect of cold pressed whale oil that appears to be largely lost during the refinement process. The unique effects of the combination of the refined marine oils and the olive oil are strongly dependent on the ratio between the marine oils and the olive oil in addition to the quality of the extra virgin olive oil.

References

1. Dyerberg J, Bang HO, Stoffersen E, Moncada S, Vane JR. Eicosapentanoic

Acid and prevention of thrombosis and atherosclerosis. Lancet 1978; 2: 117-9.
2. Albert CM, Campos H, Stampfer MJ, et al. Blood levels of long-chain n-3 fatty acids and the risk of sudden death. New Eng J Med 2002; 346: 1113-8.
3. Albert CM, Hennekens CH, O'Donnell CJ, et al. Fish consumption and risk of sudden cardiac death. JAMA 1998; 279: 23-8.
4. Siscovick DS, Raghunathan TE, King I, et al. Dietary intake of long-chain n-3 polyunsaturated fatty acids and the risk of primary cardiac arrest. Am J Clin Nutr 2000; 71: 208s-12s.
5. Valagussa F, Franzosi MG, Geraci E, et al. Dietary supplementation with n-3 polyunsaturated fatty acids and vitamin E after myocardial infarction: results of the GISSI-Prevenzione trial. Lancet 1999; 354: 447-55.
6. Brox J, Bjornstad E, Olaussen K, Osterud B, Almdahl S, Lochen ML. Blood lipids, fatty acids, diet and lifestyle parameters in adolescents from a region in northern Norway with a high mortality from coronary heart disease. Eur J Clin Nutr 2002; 56: 694-700.
7. Brox J, Olaussen K, Osterud B, et al. A long-term seal- and cod-liver-oil supplementation in hypercholesterolemic subjects. Lipids 2001; 36: 7-13.
8. Elvevoll EO, Jansson S. Process for separating lipids and proteins from biological material. 2000/023545. 2000
9. Osterud B, Elvevoll E, Barstad H, et al. Effect of marine oils supplementation on coagulation and cellular activation in whole-blood. Lipids 1995; 30: 1111-8.
10. Osterud B, Elvevoll EO, Brox J, et al. Haemostatic parameters related to lipids and adhesion molecules. Blood Coagulation & Fibrinolysis 1999; 10: 465-70.
11. Osterud B, Elvevoll EO, Brox J, Olsen JO. Cellular activation responses in blood in relation to lipid pattern: healthy men and women in families with myocardial infarction or cancer. Blood Coagulation & Fibrinolysis 2002; 13: 399-405.
12. Vognild E, Elvevoll EO, Brox J, et al. Effects of dietary marine oils and olive oil on fatty acid composition, platelet membrane fluidity, platelet responses, and serum lipids in healthy humans. Lipids 1998; 33: 427-36.
13. Elvevoll EO, Barstad H, Breimo ES, et al. Enhanced incorporation of n-3 fatty acids from fish compared with fish oils. Lipids 2006; 41: 1109-14.
14. Larsen R, Stormo SK, Dragnes BT, Elvevoll EO. Losses of taurine, creatine, glycine and alanine from cod (*Gadus morhua L.*) fillet during processing. Journal of Food Composition and Analysis. 2007; 20: 396-402.
15. Bondia-Pons I, Schroder H, Covas MI, et al. Moderate consumption of olive oil by healthy European men reduces systolic blood pressure in non-Mediterranean participants. J Nutr 2007; 137: 84-7.
16. Covas MI, Nyyssonen K, Poulsen HE, et al. The effect of polyphenols in olive oil on heart disease risk factors - A randomized trial. Ann Intern Med 2006; 145: 333-41.
17. Faine LA, Diniz YS, Galhardi CM, et al. Synergistic action of olive oil supplementation and dietary restriction on serum lipids and cardiac antioxidant defences. Canadian Journal of Physiology and Pharmacology 2004; 82: 969-75.
18. Tripoli E, Giammanco M, Tabacchi G, Di Majo D, Giammanco S, La Guardia M. The phenolic compounds of olive oil: structure, biological activity and beneficial effects on human health. Nutrition Research Reviews 2005; 18: 98-112.
19. Osterud B, Bjorklid E. Role of monocytes in atherogenesis. Physiological Reviews 2003; 83: 1069-112.
20. Aviram M, Fuhrman B. Wine flavonoids protect against LDL oxidation and atherosclerosis. Alcohol and Wine in Health and Disease 2002; 957: 146-61.
21. Aiello RJ, Bourassa PA, Lindsey S, Weng WF, Freeman A, Showell HJ. Leukotriene B4 receptor antagonism reduces monocytic foam cells in mice. Arteriosclerosis Thrombosis and Vascular Biology 2002; 22: 443-9.