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(54) Title: A COMBINATION OF SEAL OIL AND COLD-PRESSED VIRGIN OLIVE OIL

(57) Abrégé/Abstract:
A combination of oils as a supplement to, or component of, a regular diet, wherein it comprises marine oil, preferably seal oil, and cold-pressed virgin olive oil.

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(54) Title: A COMBINATION OF SEAL OIL AND COLD-PRESSED VIRGIN OLIVE OIL

(57) Abstract: A combination of oils as a supplement to, or component of, a regular diet, wherein it comprises marine oil, preferably seal oil, and cold-pressed virgin olive oil.

Combination of oils

The present invention relates to a combination of oils as a supplement to, or component of, a regular diet to counteract the development of coronary heart disease (CHD), thrombosis and other inflammatory diseases such as psoriasis and rheumatism. The combination according to the invention comprises seal oil and virgin olive oil.

Atherosclerotic lesions are formed when three cellular components of the circulation, monocytes, platelets and T-lymphocytes, react with LDL-cholesterol and two cell types in the artery wall, endothelial cells (EC) and the smooth muscle cells (SMC).

The precursor of atherogenesis is the recruitment of monocytes and lymphocytes from the peripheral blood to the intima of the vessel wall, an event that appears to depend upon the local presence of large amounts of LDL. As LDL accumulates, bound lipid and protein are oxidised and glycosylated. Cells in the vessel wall seem to interpret this change as a danger signal and call for reinforcements from the body's defence system. These processes appear to promote an up-regulation of adhesion molecules on the endothelial cells, particularly vascular cell adhesion molecule-1 (VCAM-1) and intracellular adhesion molecule-1 (ICAM-1). Thus, monocyte and lymphocyte recruitment is initiated. This leads to increased transmigration of monocytes, up-regulated exposure of adhesion molecules on the endothelium, and the production and release of chemoattractants. These are essential events for the transfer of monocytes to the intima, and the concurrent differentiation of monocytes into macrophages. Available modified LDL is also a prerequisite for the further development of macrophages into foam cells (fatty macrophages), which is the main cause of the formation of fatty streaks under the endothelium of the vessel wall. Modified forms of LDL (oxidised, glycosylated etc.) are of particular interest since the modification of LDL is associated with inflammatory reactions triggered by processes that are initiated due to the adhesion and transmigration of monocytes and lymphocytes into the intima.

As mentioned above, it is well known that monocytes play a central role in the early phase of atherogenesis. One of the first events in the atherosclerotic process is the mobilisation of monocytes into the intima. Since the recruitment of monocytes and their penetration through the endothelium are associated with the secretion of activation products such as cytokines and growth factors, it may be assumed that the functional reactivity of the circulating monocytes is very important. It is suggested that chronic infectious diseases may affect the functional reactivity by activating the monocytes and

making them more liable to produce and release harmful products such as cytokines and chemokines in response to stress.

To date, little is known as to exactly how the functional properties of circulating monocytes relate to atherogenesis. However, it is well established that hyperactive monocytes play a crucial role in the pathophysiology of rheumatism, psoriasis and other inflammatory diseases. We also know that atherogenesis is a proinflammatory disease. It may therefore be assumed that the proinflammatory function of circulating monocytes may be associated with increased risk of coronary heart disease (CHD), and that high cholesterol levels may augment production of proinflammatory products such as oxygen radicals, cytokines etc.

For many years, the inventor has observed that the reactivity of monocytes, as monitored by the production of thromboplastin (Tissue Factor = TF) and cytokines such as TNFa and IL-6 in lipopolysaccharide (LPS) stimulated blood, varies between individuals from low activity to very high activity (high responders). This property of monocytes seems to be hereditary (Østerud et al, "Blood Coagulation and Fibrinolysis" 2002; 13:399-405). The inventor has, *inter alia*, investigated *in vitro* how LPS-induced reactivity in monocytes in whole blood relates to the lipid profile in the serum of healthy individuals with a history of myocardial infarction (MI) or cancer in their close family. Of a total of 54 individuals in the myocardial infarction (MI) families, 20 had moderately high cholesterol (7.1 –10.2 mmol/l), whilst 34 had normal cholesterol. Of the individuals with normal cholesterol, 19 had hyperactive monocytes (high responders), whilst 15 had normally responding monocytes. LPS-induced thromboplastin (TF), TNFa and IL-6 were on average 3-4 times higher in the group with normal cholesterol compared with the group with moderately high cholesterol. Thus, no positive correlation between hyperactive monocytes and cholesterol level was found. All 42 individuals in the families with a history of cancer had normal cholesterol, and LPS-induced thromboplastin (TF), TNFa and IL-6 were not significantly different from the values of the group with moderately high cholesterol among the myocardial infarction (MI) families. This supports the conclusion that moderately high cholesterol is not associated with increased monocyte activation in whole blood, whilst hyperactive peripheral blood monocytes are a significant risk factor for the development of coronary heart disease.

It is probably at least as important to reduce the reactivity of monocytes, and thus the production of proinflammatory products such as cytokines, oxidative metabolites and

growth factors, as it is to reduce the cholesterol level. New studies also show that the anti-inflammatory effect of statins may be more important than their cholesterol-reducing effect (Balk et al. "Effects of statins on nonlipid serum markers associated with cardiovascular disease: a systematic review" Ann Intern Med. 2003; 139:670-82. Review).

The dietary oil according to the invention contains omega-3 fatty acids. These are known to reduce the risk of arrhythmia which can lead to sudden death. Omega-3 fatty acids are also known to reduce the risk of thrombosis which can lead to heart attack and stroke. They reduce the growth rate of atherosclerotic plaque, and thus have anti-inflammatory properties as lesion formation in the atherogenic process is mediated by proinflammatory reactions. Furthermore, omega-3 fatty acids improve endothelial function, reduce the level of triglycerides in the blood and lower the blood pressure slightly (for a brief overview, reference is made to PM Kris-Etherton, WS Harris, LJ Appell "Arterioscler Thromb Vasc Biol." 2003; 23:151-2).

In view of the properties of omega-3 fatty acids, it would be expected that a supplement of omega-3 fatty acids ought to be sufficient to prevent cardiovascular disease. However, clinical studies carried out in Norway have shown negative effects of omega-3 fatty acids (I Seljefot, O Johansen, H Arnesen, JB Eggesbo, AB Westvil, P Kierulf, "Thromb Haemost." 1999; 81:566-70; O Johansen, I Seljefot, AT Hostmark, H Arnesen "Arterioscler Thromb Vasc Biol." 1999; 19; 1681-6). Patients with cardiovascular disease who were given a supplement of omega-3 fatty acids for six months experienced a doubling of both angina and occlusions compared with controls. An increase in cytokine production was also observed, which is indicative of an increase in proinflammatory peroxidation of polyunsaturated fatty acids *in vivo* (for an overview, reference is made to H. Arnesen, "Lipids" 2001; 36 Suppl: S103-6).

The findings referred to above are in accordance with the inventor's own results with respect to diets including a supplement of omega-3 fatty acids. Thus, the anti-inflammatory effect of supplementing the diet of healthy individuals with an omega-3 fatty acid concentrate was not significant compared with a corresponding amount of omega-3 fatty acids in the form of cod-liver oil (CLO). The combination of oils according to the invention, on the other hand, gave a significant reduction in LPS-induced cytokine and eicosanoid production after the ingestion of 15 ml of oil per day for 10 weeks.

The object of modern refining processes for producing oil from fish and marine mammals is to make the oil healthier, safer, more palatable and more storage-stable. The removal of molecules that give an undesirable taste or odour in order to enhance the sensory properties may, however, destroy potent antioxidants. Nutritional quality is also affected by the quantitative content of biologically active molecules. These nutrients are affected by several factors such as environment, availability, chemical stability, the degree of processing and the form in which the nutrition is delivered. Fish oils are extracted from whole fish, fish liver (primarily cod liver) or by-products (primarily salmon). Oils from marine mammals are produced from blubber and external adipose tissue.

The processing of marine oils to render them suitable for human consumption can be problematic. Traditional extraction techniques involve heating or steam-stripping the raw material in order to release the lipids. Marine oils have a high content of unsaturated fatty acids. The use of high temperatures during the extraction process will cause undesirable effects such as the initiation of oxidation reactions, the destruction of antioxidants and the formation of molecules that give the oil an odour and taste. Detectable changes occur in lipid components during heat extraction at temperatures of more than 40°C, compared with their "virgin" state in the cells. To obtain a stable, sensorily 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 substances) is usually necessary. The conventional refining process consists of four main steps: polishing, acid washing, bleaching and deodorisation. In addition, steps such as clarification (filtration, sedimentation), mixing of different batches, winterisation and polishing filtration are also used. During the refining steps, a number of chemical reactions (hydrolysis, autooxidation, isomerisation, conjugation, polymerisation, pyrolysis and dehydration) will occur independent of the process conditions. The refining process must allow for the removal of any undesirable by-products that might be formed. The number of treatment steps is also influenced by the quality of the crude oil, including accompanying substances, amount and type of impurities, former oxidative and hydrolytic damage. Quality criteria for the dietary oil, environmental conditions, finances and a reduction in loss of material are all critical criteria when selecting the refining process.

Refining marine oils to improve their sensory properties and stability may also destroy potent antioxidants and components having potentially beneficial functional properties.

Polyunsaturated fatty acids, including omega-3 fatty acids, may be incorporated into LDL particles and render them more prone to oxidation. As oxidation of LDL particles is one of the main reactions in the early phase of atherogenesis, prevention of oxidation will prevent the formation of foam cells in intima (for an overview, reference is made to B Østerud, E Bjørklid, "Physiological Reviews", 2003, 83: 1069-112. Review). Thus, antioxidants have been shown to reduce lesion formation in animal models (M. Aviram, B. Fuhrman "Ann N Y Acad Sci." 2002; 957: 146-61. Review). Furthermore, antioxidants are also important in the down-regulation of eicosanoid metabolism. The lipoxygenase pathway, which leads to the formation of, *inter alia*, leucotrien B4, is inhibited by antioxidants with a subsequent reduction in LTB4 production. Recently, it has been shown, *inter alia*, that inhibition of the LTB4 receptor in transgenic mice which were predisposed to atherosclerosis reduced lesion formation by about 70% (RJ Aiello, PA Bourassa, S Lindsey, W Weng, A. Freeman, HJ Showell, "Aterioscler Thromb Vasc Biol." 2002; 22: 443-9).

A subject (the inventor) who has used the dietary oil according to the invention for a period of ten years has very low LTB4 generation in LPS-induced whole blood. The addition of commercial LTB4 to the subject's blood causes the LPS-induced TF (Tissue Factor) to rise by more than 70% and the blood response to LPS is once again among the highest measured (Østerud, unpublished data).

The combination of oils according to the invention combines the effect of omega-3 fatty acids and a synergistic component which gives an antioxidation effect both *in vivo* and *in vitro*. This combination has, according to the invention, given surprising advantageous properties in the form very good clinical effects, better functional properties and longer storage life. The inventors have shown that a particularly advantageous effect is obtained using a product that contains oil from marine mammals, preferably the seal, and cold-pressed virgin olive oil, both of these components being produced in a per se known way. The effect obtained may seem to be more pronounced than would be expected if each component were used alone.

Thus, the invention relates to a combination of oils as a supplement to a regular diet, characterised in that it comprises a combination of seal oil and cold-pressed virgin olive oil.

The invention also relates to the use of the combination according to the invention as a component in an oil-in-water or water-in-oil emulsion in foodstuffs.

Furthermore, the invention relates to the use of the combination according to the invention, optionally together with adjuvants, for example, for preparing a composition for counteracting the development of coronary heart disease and thromboses and to suppress psoriasis, rheumatism and other proinflammatory diseases.

Two clinical studies have been conducted on the effect of using a dietary oil according to the invention as a daily supplement for healthy individuals. In the first study there were 28 participants in both the control group and the group that received the dietary oil according to the invention, whilst there were 37 participants in a cod-liver oil (CLO) group. Each person consumed 15 ml oil per day or nothing (the control group) for 12 weeks. Although there was a higher rise in omega-3 fatty acids in the serum of the individuals in the CLO group compared with the group that received the combination of oils according to the invention, the reduction in LPS-induced TNF in whole blood was 24.0% in the group that received the combination of oils according to the invention compared with 5.0% in the CLO group. This shows, as in the previously mentioned study, that the reduction in the amount of inflammatory products in stimulated blood cells is not directly related to the content of omega-3 fatty acids (Østerud et al. 1995).

Another study produced the results given in Table 1:

Table 1. The effect of a dietary supplement of CLO or the combination of oils according to the invention compared with a control (% change compared with values obtained prior to dietary intake)

Group	*HDL-chol*	*hsCRP*	*MCP-1*	*TxB2*	*LTB4*
Control	0	+5.1	+8.5	0	+36.6
CLO	+1.4 (i.s.)	+12.5 (n.s.)	-5.2 (0.005)	-14.3 (0.05)	+8.6 (0.001)
Oil of the invention	**+8.3 (0.05)**	**-24.0 (0.001)**	**-14.3 (0.05)**	**-17.6 (0.05)**	**+8.8 (0.001)**

Table 1 shows a change in some of the most important parameters related to coronary heart disease (CHD). HDL-chol is the beneficial cholesterol and any positive change in this is a good thing. Hypersensitive C-reactive protein (hs-CRP) reflects chronic inflammatory reactions in the body. It has been shown that increased values of between

0 and 5 mmol/l are a good risk indicator for coronary heart disease, and especially when the proportion of total cholesterol over HDL cholesterol related to hs-CRP increases (Rifai N, Ridker PM "Inflammatory markers and coronary heart disease" Curr Opin Lipidol. 2002; 13: 383-9. Review). Monocyte chemotactic protein-1 (MCP-1) is a very important chemoattractant protein which plays a major role in the development of atherosclerosis in that it mobilises proinflammatory substances at the site of their production. Thus, any dietary supplement which gives a reduction in MCP-1 may be highly beneficial. LTB4 and TxB2 (a stable product of TxA2) are proinflammatory products derived from the metabolism of arachidonic acid.

The above study was carried out on respectively 23, 18 and 19 healthy people in the control group, CLO and the group that received the dietary oil of the invention. The samples were taken from fasting volunteers between 08.00 and 10.00 hours immediately prior to the start of the study and at the end after 12 weeks of supplementary ingestion of 15 grams of oil (or nothing = control). The fatty acid composition before and after was determined in serum samples.

The conclusion of the above study is that the combination of oils according to the invention has the potential to increase the beneficial HDL cholesterol, significantly reduce the important marker and the risk factor for coronary heart disease (hs-CRP) and further reduce MCP-1 more efficiently than CLO. In addition, the beneficial effect of reducing the proinflammatory products TxA2 and LTB4 is at the same level as for CLO. Overall, the dietary oil according to the invention as a dietary supplement has significantly more anti-inflammatory effects than CLO, and is superior to olive oil, which has also been used alone as a control in several studies of omega-3 fatty acids and fish oils. The effect of the edible oil according to the invention probably occurs through a synergistic combination of omega-3 fatty acids from the marine oil and strong antioxidants present in the virgin olive oil.

Patent claims

1.

A combination of oils as a supplement to, or a component of, a regular diet, characterised in that it includes seal oil and cold-pressed virgin olive oil.

2.

A combination according to claim 1, characterised in that the combination is in the form of a blend.

3.

A combination according to claim 1, characterised in that the ratio of seal oil to virgin olive oil is from 1:9 to 9:1, preferably 2:8 to 8: 2 , more preferably 3:7 to 7:3, even more preferably 4:6 to 6:4, and most preferably 1:1.

4.

The use of the combination according to claim 1 as an edible oil or as a component of an oil-in-water or water-in-oil emulsion in foodstuffs.

5.

The use of the combination according to claim 1, optionally with adjuvants, for preparing a composition to counteract the development of coronary heart disease and thromboses and to suppress psoriasis, rheumatism and other proinflammatory diseases.