

1. What's the mechanism of NKO on hyperlipidemia? How's the mechanism different from statin?

Neptune Krill Oil is a complex combination of multiple active ingredients with a synergistic bioactivity. Hence the exact mechanism of action for lipid lowering effects is not yet entirely clear.

- a. It is known though that due to its high content of functionalized phospholipids it bypasses the stomach and the duodenum and it is readily absorbed in the small intestine exhibiting a lipid-lowering effect on the level of the small intestine, which distinguishes Neptune Krill Oil from other known lipid-lowering principles. On the basis of the demonstration that lipid oxidation can be achieved in cell cultures and in intestinal mucosa through a reversible fatty alcohol cycle and that  $\beta$ -oxidation chain shortening of very long chain fatty acids (VLCFA) occurs in rat liver peroxisomes, it is speculated that Neptune Krill Oil -induced changes in hepatic cholesterol metabolism may be caused by the presence of VLCFA and chain-shortened secondary metabolites.
  - b. Like the non-HDL particles, nascent HDL particles are assembled in the liver and intestine and are then secreted into the circulation as particles consisting almost entirely of phospholipids and Apo A1. Because these nascent HDL particles contain little or no cholesterol (ie, they have no central lipophilic core), they appear flat, or discoid. The nascent HDL particle is thus primed to remove cholesterol that it encounters in peripheral cells as it travels through the circulation.
  - c. As these nascent HDL particles move through the circulation, Apo A1 in the HDL membrane binds with phospholipids from non-HDL particle membranes and with phospholipids that are shed from those membranes when the core triglyceride-rich lipoproteins are spilled out during hydrolyzation by LPL. Next, a free fatty acid from lecithin (phosphatidylcholine) is transferred to the unesterified cholesterol that has been absorbed into the nascent HDL particle by the action of lecithin-cholesterol acyl-transferase (LCAT) and its activating cofactor, Apo A1. This free cholesterol is then esterified (ie, combined with an alcohol and depleted of water) by plasma LCAT and, because cholesterol esters are hydrophobic, they move to the core of the lipoprotein, causing the HDL particle to assume its mature spherical configuration.
  - d. In general, inhibition of cholesterol synthesis, increased hepatic LDL uptake, increased serum LDL catabolic rates and increased HDL synthesis along with inhibition of cholesterol or bile acid absorption from the intestine play an essential role on lipoprotein production/secretion.
  - e. Statins act by competitively inhibiting HMG-CoA reductase, the first committed enzyme of the HMG-CoA reductase pathway. By reducing intracellular cholesterol levels, they cause liver cells to make more LDL receptors, leading to increased clearance of low-density lipoprotein from the bloodstream.
2. What's the mechanism of NKO on osteoarthritis and rheumatoid arthritis? How does NKO reduce CRP?

- a. Please refer to attachment.
- b. Neptune Krill Oil is a rich source of unique phospholipid carriers of omega-3 fatty acids, eicosapentanoic acid (EPA) and docosahexanoic acid (DHA), esterified on antioxidants, as astaxanthin and a novel flavonoid. Phospholipids are important in protecting membranes from toxic injury and free radical attack<sup>39</sup>. The composition

of phospholipids in Neptune Krill Oil appears to be optimal to offer such protection. The unraveling of the exact mechanism of action is a multifactorial project which is still ongoing. We speculate that it is based on the blockage of leukotriene formation by interfering at the level of the lipoxygenase pathways. The significantly dominant omega-3 to omega-6 ratio (15:1) in Neptune Krill Oil may partially explain the anti-inflammatory effects observed in this trial. The balance of polyunsaturated (essential) fatty acids in the body is critical for the maintenance of healthy cell membranes and hormone regulation. During the last decades, the American diet has shifted to much higher levels of omega-6 and less omega-3 fatty acid intake. Long-chain omega-6 such as arachidonic acid, predominating in the phospholipids of cell membranes can encourage the production of pro-inflammatory type-2 prostaglandins (PGE2), while omega-3 fatty acids promote the production of anti-inflammatory prostaglandins.<sup>1,2</sup> An additional factor is the naturally occurring astaxanthin in NKO™ which may also actively contribute in its anti-inflammatory potency. A recent study by Ohgami K. et al demonstrates that astaxanthin inhibits nitric oxide production through inhibiting the activity of inducible nitric oxide synthase (NOS), and production of PGE2 and TNF-. This study suggests that astaxanthin may have an anti-inflammatory effect and may be a promising agent for the treatment of inflammation<sup>43</sup>. (References correspond to the Deutsch study attached)

3. What's the mechanism of NKO on PMS? How's it different from EPO and GLA?
  - a. Following ovulation, there is shift of fatty acid balance in the phospholipids of the cell membranes.<sup>43</sup> Prior to menstruation, excessive amounts of arachidonic acid are released, and an increase in prostaglandins and leukotrienes (LTs) is triggered in the uterus. The inflammatory response initiated by the PGs and LTs results in vasoconstriction, myometrial contractions, and ischemia that cause pain; gastrointestinal symptoms such as nausea, vomiting, bloating; and headaches.<sup>43</sup> Supplementation with omega-3 fatty acids mediates the production of less potent PGs and LTs, resulting in a reduction in the severity of myometrial contractions and uterine vasoconstriction, a decrease in the formation of inflammatory mediators, and subsequently reduced ischemia and improved blood flow.<sup>41-43</sup> Evidence has shown that phospholipids of the brain have an especially high content of the long-chain omega-3 DHA, and that these phospholipid species are centrally involved in brain function.<sup>44-46</sup> The effectiveness of Neptune Krill Oil™ on emotional menstrual symptoms may thus be based on potential modulating effects on neurotransmitters that affect emotional and psychological symptoms. The synergistic effects of omega-3 and phospholipids are specific to Neptune Krill Oil™ since the solvent-based cold extraction process used to produce this oil maintains the integrity of the phospholipids. Processes for fish oil extraction involve conditions that irrevocably damage certain components like phospholipids. (References correspond to the Bunea study attached)
  - b. EPO is made from the seeds *Oenothera biennis* or evening primrose. The oil from this plant is rich in gamma-linolenic acid (GLA). GLA is categorized as omega-6, which don't have any conclusive clinical findings supporting their anti-inflammatory effect. In fact, as mentioned earlier, due to the shift of the American diet to much higher levels of omega-6 it might cause a worsen state of inflammation.

4. What's the main ingredient of antioxidant in NKO? Is there a difference between the astaxanthin in NKO and the astaxanthin in the market?

Astaxanthin (AX) is the main antioxidant in NKO. There main other source of AX on the market is synthetic AX. Depending on its origin, AX can be found in association with other compounds. It may be sterified in one or both hydroxyl groups with different fatty acids such as palmitic, oleic, estearic, or linoleic: it may also be found free, that is, with the hydroxyl groups without sterification. Synthetic AX is not sterified, while found in NKO, AX is a mixture of the three forms. The bioavailability of esterified astaxanthin is believed to be superior to that of the synthetic form.

5. Is there a correlation between reducing CRP and stiffness in arthritis?

Yes there is a correlation. Since the reduction of inflammation would cause a reduction in the amount of swelling and pressure in the synovial fluid and the lining of the joints which is the main cause of joint pain and stiffness.

6. Besides arthritis, can the effect of reducing CRP help to reduce inflammation and pain of other medical problems?

The effect of CRP reduction on inflammation in not targeted to one organ and is a global effect on the entire body. Therefore, yes it can help reduce the inflammation and pain of patients suffering from other medical problems.