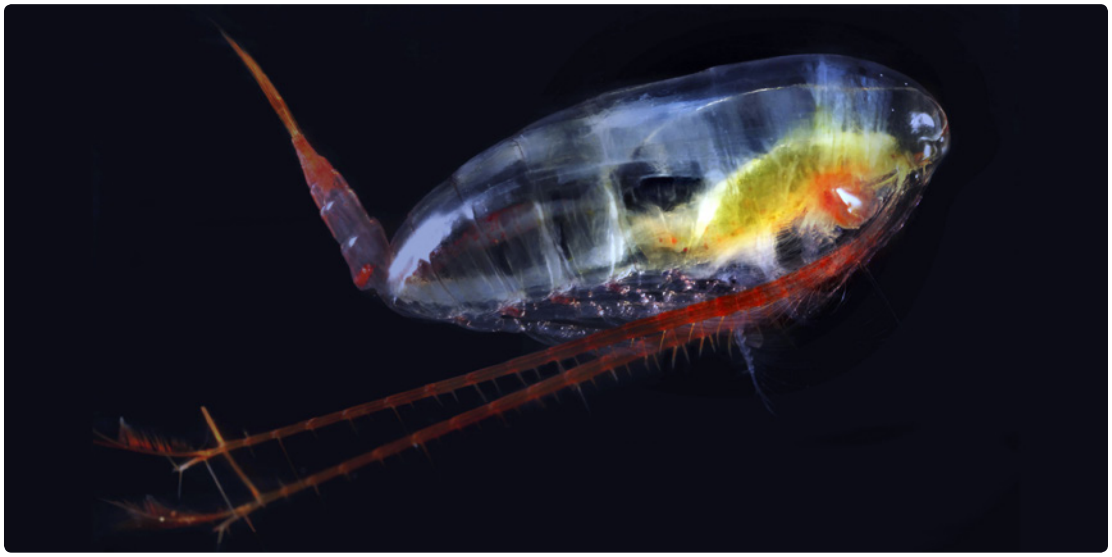
CALANUS®

WHITE PAPER

The new lipids from the Arctic

by Alice Marie Pedersen, PhD



Calanus® Oil provides benefits not seen with conventional omega-3

Calanus® Oil is a novel marine oil, providing a new and highly bioactive form of marine fatty acids with additional features compared to conventional omega-3. Calanus® Oil's solid scientific documentation shows that it counteracts insulin resistance and other obesity-induced metabolic disorders and exhibit a potent anti-inflammatory effect. Calanus® Oil is the natural lipid extract from *Calanus finmarchicus*, the small copepod that is the engine of the North Atlantic ecosystem. These precious lipids are finally available as a source of nutrients for humans.

Background

The dietary fat intake in the Western world and more recent "Westernized" countries has shifted from omega-3 towards omega-6 during the last decades. This shift coincides with the rise in prevalence of obesity, which is skyrocketing worldwide and constitutes a major health problem.

Excess fat around the intra-abdominal organs, referred to as visceral or intra-abdominal obesity, represents a greater risk factor for morbidity and mortality in humans than general obesity (Kuk *et al*. 2006). Visceral obesity is associated with elevated levels of systemic inflammation and metabolic abnormalities, with increased risk of developing insulin resistance, type 2 diabetes, stroke and cardiovascular disease.

Lifestyle interventions, such as increased exercise and calorie-reduced diets are undoubtedly effective in reducing obesity, however the long-term success rate is often very poor. Interest in omega-3 polyunsaturated fatty acids (PUFA) has escalated in recent years because of their various roles in promotion of heart and brain health, in addition to being important in fetal development.

The popularity of omega-3 is reflected in an increased global demand as consumers all over the world are choosing supplements or foods with additional health-promoting benefits. At the same time, the traditional sources of omega-3 fatty acids through fish and fish oils are limited. The future sustainability of the global fisheries stocks is uncertain, and many fisheries are already fully or over-exploited (FAO, 2018). Consequently, scientists and food researchers are continuously searching for new and sustainable sources of these much sought-after nutrients.

Calanus® Oil offers the same benefits as conventional omega-3, along with additional health-promoting features.

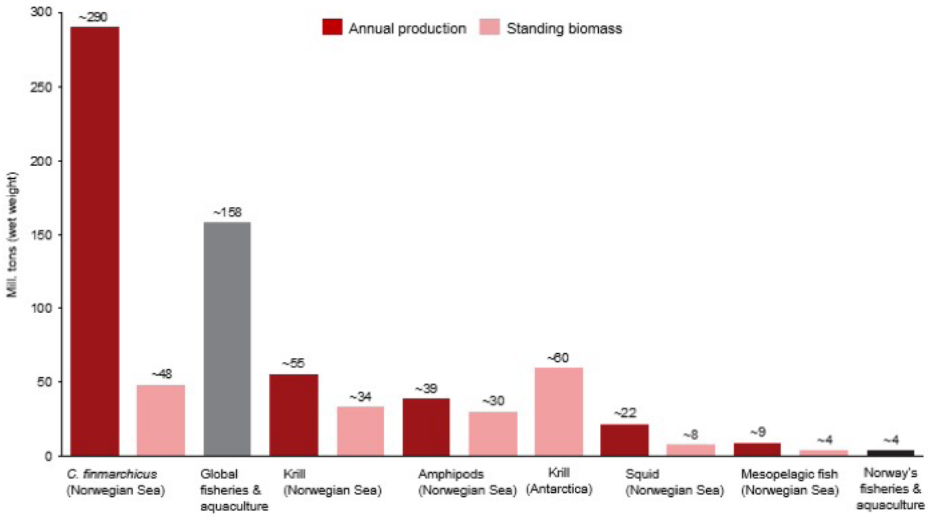


Figure 1: Biomass estimates for selected marine species and areas.

Figure 2: *Calanus finmarchicus* is harvested with proprietary equipment

The resource *Calanus finmarchicus*

Calanus® Oil is naturally potent and originates from the new resource *Calanus finmarchicus*, a tiny copepod which is the most abundant animal species on the planet, and the engine of the Norwegian Sea ecosystem (Skjoldal, 2004). The annual biomass production of *Calanus finmarchicus* in the Norwegian Sea is many times higher than the total biomass of all fish species in the same area, including cod, herring and mackerel, and even twice the size of the world´s total fisheries and aquaculture, highlighting the magnitude of this resource that until now has not been utilized for human nutrition (Figure 1).

The 3-4 mm long *Calanus finmarchicus* is a free-living crustacean with a life cycle of one year. The largest biomass is found at the base of the food pyramid, and utilizing resources such as copepod is both ecologically efficient and bioeconomically reasonable. In the Arctic waters, the copepods efficiently exploit the short and intense period of the phytoplankton bloom, rapidly converting their vegetable diet to lipid storage. Only 10-15 % of the annual production of *Calanus finmarchicus* is being incorporated in the next trophic level of the food chain. A small fraction continues as a seeding population for next spring, while the majority dies and enters the food web at deep waters.

This rapid turnover in biomass results in a highly sustainable resource with extremely low levels of environmental pollutants.

The harvesting is done with proprietary technology especially developed for efficient harvesting and low bycatch rates (Figure 2). It takes place while the copepods are aggregating close to the ocean surface in the period from April to August. The catch is immediately frozen onboard the vessel to ensure perfect freshness of the raw material.

After the catch is brought ashore, the lipids are gently extracted, explicitly without any solvents or other hazardous processing aids. Additionally, no further refining, concentrating or other processing is necessary to obtain the pure oil fraction. Therefore, Calanus® Oil is unquestionably the most natural and "untouched" marine oil commercially available with a lipid composition that reflects the vegetable diet of copepods.

Wax esters – The New Lipids

All lipids comprise a chain of carbon atoms bound to hydrogen atoms. Types of fat differ by the number of carbon atoms in the chain and the number and location of double bonds between them. Slight differences in structure may translate into critical differences in form and function.

Fatty acids in krill oil are mostly bound to glycerol as phospholipids, whereas fatty acids in fish oils are present as triglycerides. The uniqueness of *Calanus finmarchicus* derived lipids is the chemical form of wax esters (Figure 3). Described chemically, they are fatty acids esterified to fatty alcohols (FAOH) as monoesters. Wax esters are distinctly different from fat in any other marine (or terrestrial) oils in terms of chemistry, bioactivity and health benefits.

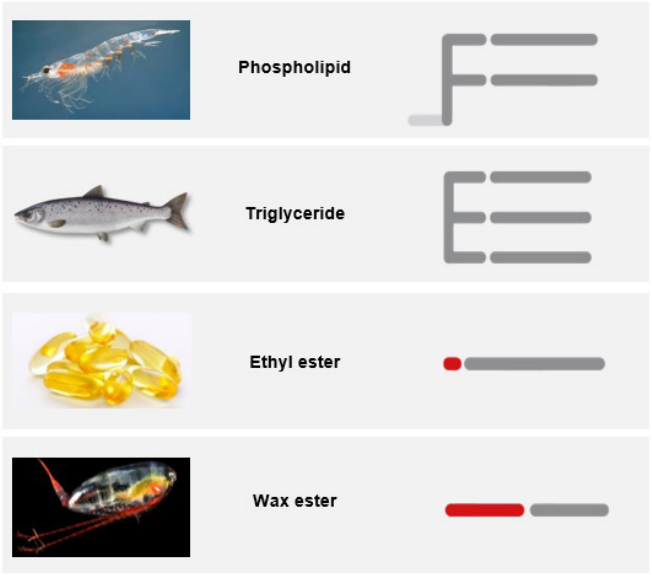


Figure 3: Graphical overview of the molecular structures of the different lipid classes present in marine oils and their corresponding origin, and how the structure of wax ester differs from the rest. The red highlighting indicates the simple alcohol chain the fatty acids are bound to, in contrast to the glycerol molecule which constitutes the backbone of phospholipids and triglycerides.

The wax esters from *Calanus finmarchicus* are rich in the health-promoting omega-3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). In addition, the lipid extract contains high amounts of the omega-3 fatty acid stearidonic acid (SDA, 18:4 n-3), which is readily converted to EPA by the human body. It also contains other important fatty acids such as gondoic acid (20:1n-9), cetoleic acid (22:1n-11) and oleic acid (18:1-n9).

Calanus® Oil appears as its name implies, like any other (poly)unsaturated nutritional oil; a slightly viscous liquid.

Digestion of wax esters and mechanism of action

Free fatty acid receptors are broadly regarded as nutrient sensors and are involved in the regulation of inflammatory and metabolic processes. One of these is the GPR120 receptor which is enriched in several tissues in the body, especially within the distal intestine and colon, as well as in adipose tissue and macrophages. This is a receptor for medium- and long-chain unsaturated fatty acids, including omega-3 fatty acids. Recent studies have shown GPR120 to play cardinal roles in metabolic disorders via modulation of gut hormone secretion and insulin sensitivity and body weight regulation (Ulven & Christiansen, 2015).

Whereas other dietary lipids claim their effects due to rapid absorption, Calanus® Oil explains it the other way around. The unique chemistry of these new lipids allows them to withstand the first line of digestive enzymes, letting the lipids reach the distal part of the digestive tract without compromising the bioavailability of the fatty acids. When the wax ester molecules ultimately are cleaved during digestion, the release of highly potent fatty acids activates GPR120 receptors present in the tissue and exert its effects. Whereas EPA and DHA are among the more potent of the common FAs on GPR120, SDA has recently shown to be one of the most potent fatty acids activating this central receptor (Ulven & Christiansen, 2015).

A strong anti-obesogenic effect, alleviating adipose tissue inflammation and improving whole body insulin sensitivity

The mechanisms of insulin resistance are multifactorial and still under investigation, but it seems clear that low-grade inflammation, especially locally in abdominal adipose tissue, together with a constant nutrient excess play the key role. These conditions are strongly associated with intra-abdominal obesity.

Because the digestion of wax esters is very slow, the fatty acids (and fatty alcohols) in Calanus® Oil will reach the distal intestine, whereas other omega-3 forms are absorbed too fast to get there. The distal intestine contains GPR120 receptors which interacts with the Calanus® Oil PUFA.

The result is reduced deposition of intra-abdominal fat, leading to reduced adipocyte (fat cell) size. Large adipocytes cause cell hypoxia, macrophage infiltration and a chronic low-grade systemic inflammation, which over time is a considerable risk factor for development of metabolic related diseases, such as insulin resistance (and in the next phase diabetes), hypertension and general inflammation in the body. Healthy adipocytes also secrete higher amounts of adiponectin, which increases insulin sensitivity and thereby counteracting development of insulin resistance.

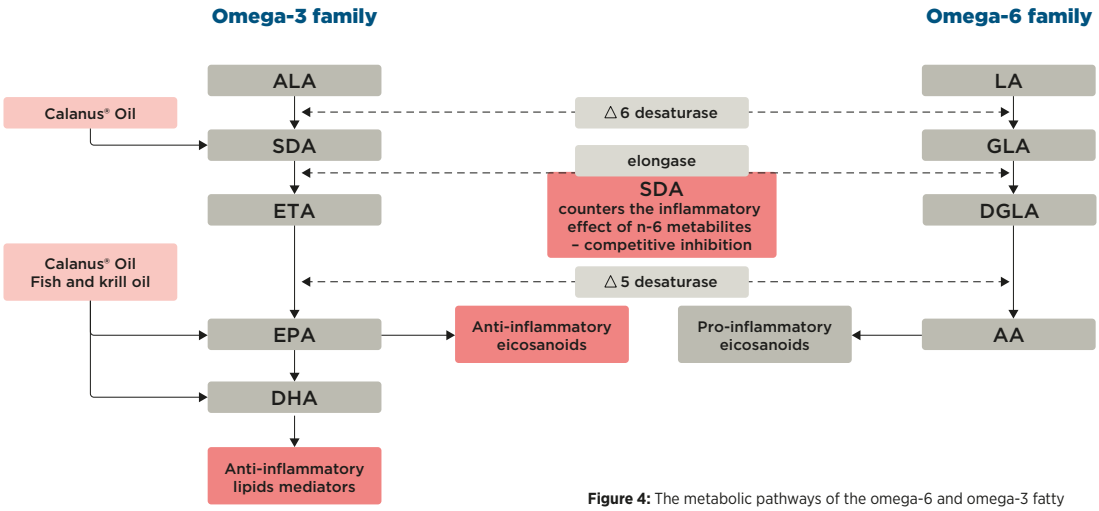


Figure 4: The metabolic pathways of the omega-6 and omega-3 fatty acids competing for the same enzyme system.

Anti-inflammatory potency

Inflammation is an important part of the pathophysiology in numerous medical conditions. Both acute and low-grade inflammation are threats to health. Whereas acute inflammation often has evident outcomes such as skin and joint problems, low-grade inflammation represents a long-term risk factor for serious conditions related to for instance cardiovascular disease, insulin sensitivity and development of diabetes (Gregor and Hotamisigil, 2011).

The consequence of increased omega-6 intake at the expense of omega-3, is that omega-6 metabolites form in larger quantities than those from omega-3 fatty acids. Simplified, omega-6 derived metabolites are regarded as pro-inflammatory. In contrast, the omega-3 derivatives mostly promote anti-inflammatory effects. Furthermore, resolvins, protectins and maresins are relatively newly discovered families of highly potent mediators with inflammation-resolving properties derived from omega-3 PUFA, adding to the insights of the important and diverse biological roles of these fatty acids (Serhan & Levy, 2018).

Calanus® Oil has the same anti-inflammatory benefits as other omega-3 sources, however offering additional potency by the reduction of low-grade inflammation through reduced deposition of intra-abdominal fat, reduction of liver steatosis as well as improvement of glucose tolerance. Stearidonic acid (SDA) also contributes to the anti-inflammatory potency by competing for the same enzymes, which is involved also in transformation of omega-6 fatty acids into pro-inflammatory arachidonic acid (Figure 4).

In summary, triglycerides and phospholipids act mainly as building blocks for anti-inflammatory eicosanoids, whereas the wax ester bound omega-3 fatty acids of Calanus® Oil exert a three-angle approach to anti-inflammatory action by:

1. providing omega-3 fatty acids, which act as building blocks for anti-inflammatory mediators eicosanoids.

2. providing stearidonic acid (SDA). SDA competes for the elongase enzyme which also converts omega-6 fatty acids, thereby lowering the output of omega-6 based pro-inflammatory mediators (Figure 4).

3. providing fatty acids as wax esters (WE). WE enhance fat metabolism, leading to a reduction in fat cell size. Enlarged fat cells are often hypoxic, which in turn strongly activates inflammatory responses, leaking pro-inflammatory mediators systemically. Therefore, healthy fat cells are important contributors to lower whole body inflammation.

Preclinical trials have confirmed that there are good reasons to believe that Calanus® Oil exerts a more potent anti-inflammatory action than other omega-3 products, and this will be further studied in both preclinical and human clinical trials.

Other important constituents of Calanus® Oil

The special combination of the three omega-3 polyunsaturated fatty acids SDA, EPA and DHA are due to the phytoplankton fatty acids being incorporated into the copepods storage lipids. Therefore, the fatty acids found in Calanus® Oil are largely reflected by the fatty acid composition of the phytoplankton. The high content of the long-chain monounsaturated fatty acids and their corresponding long-chain fatty alcohols in Calanus® Oil are the results of the copepods' own synthesis.

As Calanus® Oil is a lipid extract from a natural source, it contains a considerable number of fat soluble constituents such as astaxanthin, plant sterols, fatty alcohols and more than 15 different fatty acids, as illustrated in figure 5.

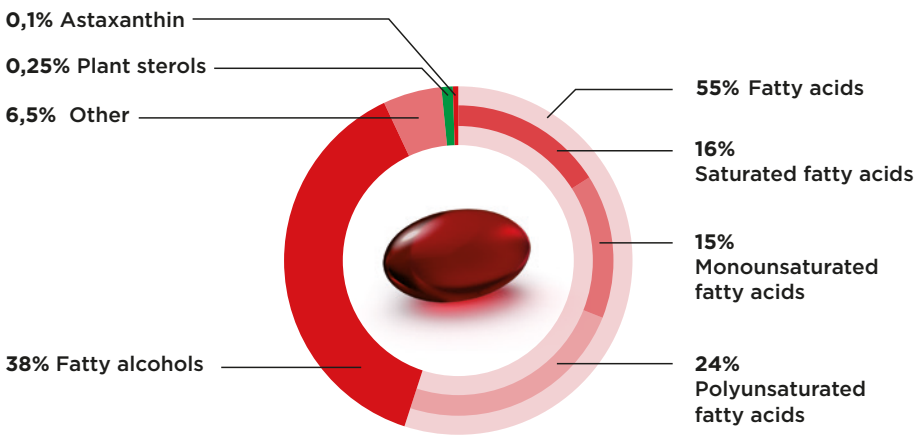


Figure 5: Graphic chemical composition of Calanus Oil

High content of EPA precursor Stearidonic acid, boosting EPA levels

Decades of research support that higher intake of EPA and DHA is associated with reduced risk of mortality from coronary heart disease and sudden cardiac death (Narayan *et al*, 2006). In addition to EPA and DHA, Calanus® Oil is especially rich in stearidonic acid. This is the first metabolite formed directly from alpha-linolenic acid (ALA, 18:3 n-3)(Figure 4), which is poorly converted to EPA and DHA in the body. SDA is by far more efficiently converted further to EPA than ALA and has been referred to as being a 'pro-EPA' fatty acid (Whelan, 2009). This has led to the suggestion of a direct intake of SDA as another strategy to increase tissue EPA levels. Consequently, SDA has recently gained more attention. Several studies show beneficial effects exerted by the fatty acids as an independent entity, and not only as an effective precursor to EPA. Stearidonic acid favorably compares with dietary EPA in side-by-side experiments in a limited number of studies (Whelan, 2009).

Monounsaturated fatty acids

The Seven Countries Study in the 1960s led to the discovery that long-chain monounsaturated fatty acids (LC-MUFA) could exert positive health effects (Keys *et al*, 1966). This finding increased the interest in olive oil and the Mediterranean diet, a diet still regarded as a healthy choice. Researchers have proposed an important role of MUFA for cardiovascular health, as they are reported to have favorable effects on metabolic syndrome and markers of cardiovascular disease risk such as blood pressure, blood lipids and insulin sensitivity, as well as the potential to ameliorate obesity risk.

Most MUFA-studies related to health effects have exclusively focused on oleic acid (C18:1 n-9), which is abundant in the Mediterranean diet. Limited information is available regarding the effect of other dietary MUFAs, such as long-chain MUFAs with carbon chains longer than 18. Although not found in most dietary sources, gondoic acid (20:1 n-9) and cetoleic acid (22:1 n-11), are abundantly found in Calanus® Oil, along with oleic acid.

When the recommendation of omega-3 consumption is combined with MUFA intake, additional improvements in cardiovascular disease risk have been reported (Yang *et al*, 2016).

Long-chain fatty alcohols

The wax esters in Calanus® Oil contain long chain fatty alcohols that are mainly monounsaturated. The fatty alcohols eicosenol (20:1 n-9) and docosenol (22:1 n-11) constitute the major part of the alcohol fraction. The fatty alcohols derive from de novo biosynthesis of the corresponding fatty acids and subsequently the reduction of the fatty acids to fatty alcohols.

Long-chain alcohols from terrestrial sources such as rice bran wax and sugar cane wax have been shown to increase physical performance and exert anti-inflammatory effects. It has been suggested that the long-chain fatty alcohols enhance physical performance through sparing of muscle glycogen stores by increasing the lipid oxidation (Taylor *et al*, 2013). However, there are few studies on the effects of marine fatty alcohols and further investigation is thus required.

Astaxanthin – the naturally occurring antioxidant

Calanus® Oil is easily recognized by its ruby red colour, which is due to the content of the lipid soluble carotenoid astaxanthin. The copepods utilize carotene from their phytoplankton diet as a precursor for astaxanthin synthesis. It has been proposed that one of the central functions of astaxanthin in calanoid copepods is to improve the antioxidative protection of the valuable storage lipids. Also, astaxanthin in copepods have been suggested to take part in lipid metabolism and serve as both photoprotection, as well as camouflage.

Oxidation plays a fundamental role in the reduction of the quality of lipids. It deteriorates the sensory quality and the nutritive value, and may ultimately lead to the production of toxic compounds. Marine oils and products with a high content of long chain polyunsaturated fatty acids are particularly susceptible to oxidation. To improve oxidative stability, natural or synthetic antioxidants are often added to crude and refined oils. In Calanus® Oil however, the addition of further antioxidants is not necessary, as the natural presence of astaxanthin strongly protects the lipids from oxidation.

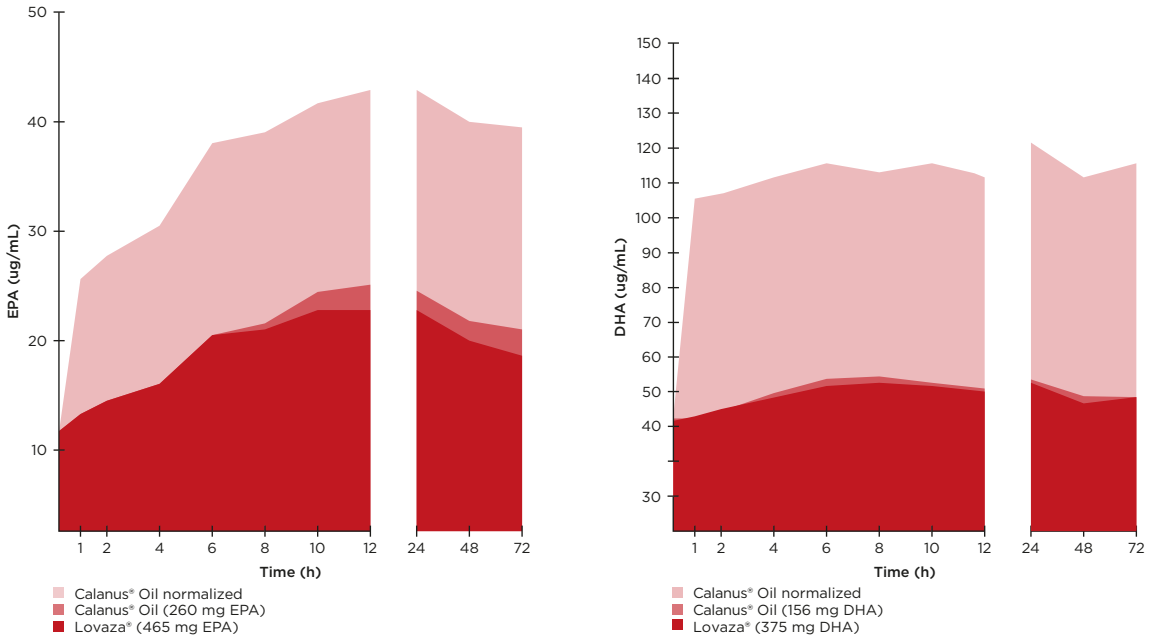


Figure 6: A 72-hour time course of plasma EPA and DHA in response to a single serving of wax ester oil or comparator fish oil.

Strong scientific foundation

Clinical and preclinical studies so far have been focusing on screening for effects and mechanisms in humans to document safety and uptake. Since the beginning of the scientific exploration, the volume of scientific publications and theses are continuously growing. Until now, four doctoral candidates have defended their work and 11 peer-reviewed papers have been produced in the areas of biomedical and biochemical science. The findings so far have attracted much attention.

Preclinical data show that Calanus® Oil has positive effect on blood sugar stabilization and weight gain reduction, or more precisely on reduced deposition of intra-abdominal and liver fat. These effects are specific for Calanus® Oil and are not seen in other marine oils that are offered as supplements. In addition, the same studies have shown an increase in endurance and a potent anti-inflammatory action.

Safety and bioavailability have been documented in human studies. The bioavailability study showed that Calanus® Oil gives a twofold uptake compared to ethyl esters (Figure 6), confirming that the wax esters are digested and its omega-3 fatty acids absorbed (Cook *et al.* 2016).

Several doctoral students and researchers are engaged in ongoing projects related to mechanistic and preclinical studies, as well as clinical trials directly related to the impact of Calanus® Oil. Calanus AS is currently involved in several human clinical trials within the areas metabolic health in a population of elderly, antiinflammatory effects combined with exercise and a big intervention study on children with ADHD.

Future studies will become increasingly more targeted towards specific effects, and the long-term goal is to qualify for one or more approved health claims in the EU and other markets.

Summary

The tiny copepod *Calanus finmarchicus* represents the Arctic in all ways – tough, fresh and healthy. The environmentally friendly harvesting methods and solvent-free processing ensures that the Arctic freshness is transferred to Calanus® Oil.

Calanus® Oil's unique combination of polyunsaturated and monounsaturated fatty acids in combination with fatty alcohols, closely protected by the strong antioxidant astaxanthin, presents a long awaited natural dietary supplement that supports the health of people with metabolic conditions or who are in the risk group of developing such conditions. The mechanism of action is closely associated to anti-inflammatory effects. With SDA competing for the elongase and desaturase enzymes (resulting in less omega-6 conversion) and GPR120 activity (supporting adipocyte health, giving reduced low-grade inflammation and enhancing insulin sensitivity), Calanus® Oil is the most potent anti-inflammatory marine oil in the market.

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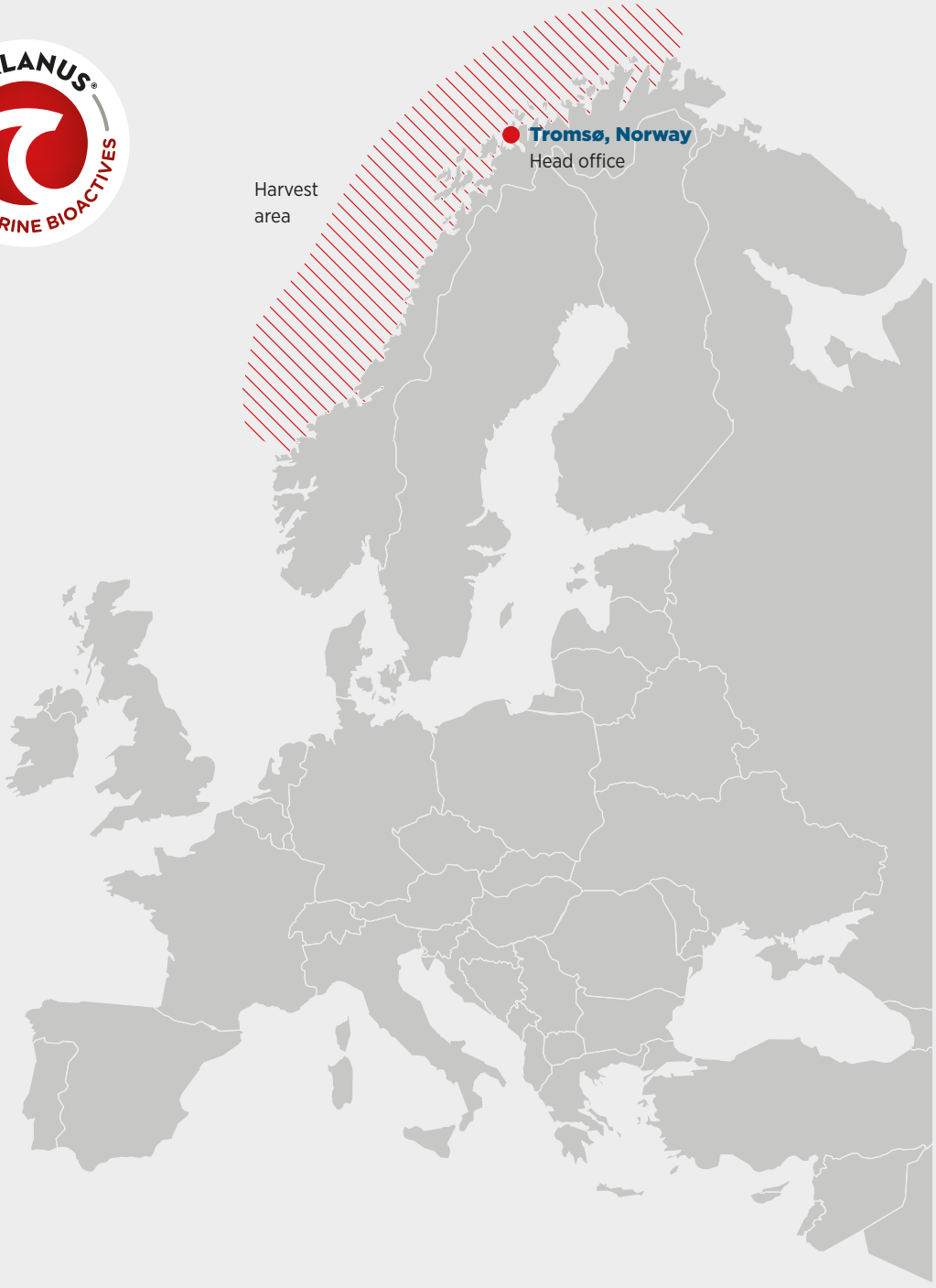
Certificates and memberships



Calanus AS is a biomarine company pioneering the utilization of the crustacean *Calanus finmarchicus*. This small pelagic zooplankter, with a life span of one year, is the most numerous animal species on the planet and represents the largest harvestable biomass on the Northern hemisphere.

Using its proprietary technology, Calanus AS has developed gentle and environmentally friendly biorefinery processes to manufacture its novel products, which includes **Calanus® Oil** and the **Calanus® Hydrolysate**, providing functional properties and health benefits to humans, domestic aquatic animals and pets.

Domiciled in Tromsø - Norway's largest seafood port and the "Gateway to the Arctic" – Calanus AS is firmly rooted in a community built on harvesting of seafood, and on research for sustainable management and utilization of marine resources.

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