Lena Burri, Ph.D

KRILL OIL CONCENTRATE

The phospholipid factor that sets krill oil apart

The next generation Omega-3 Phospholipid product from Antarctic krill
To effectively maintain our bodily functions and to keep cells and body in balance, we need a continuous supply of nutrients. Whereas in diseased conditions, it is also important to address the fluctuations often seen in nutrient uptake and utilization. There is excitement and growing evidence surrounding krill oil as an important nutrient based on its major components:

- Long-chain omega-3 fatty acids (EPA/DHA)
- Phospholipids and specifically its choline via its phosphatidylcholine- “lecithin” component

Krill oil is unique in that it is a combination of these naturally occurring nutrients. And when ingested, it delivers EPA/DHA, phospholipids, and choline to the body, where they work individually and in combination; i) as vital constituents in the structure and functioning of cells, ii) for assisting in the balancing of bodily functions, and iii) to address their deficiencies in health conditions related to the heart, brain, inflammation, immunity, liver, etc.

Omega-3 fatty acids are one of the most recognized and studied compounds with more than 20,000 scientific papers published. Research has shown that when omega-3 fatty acids from marine sources, such as fish and krill, are ingested, they incorporate into cells to affect cell structure and function. Unlike fish oil, krill oil not only delivers EPA and DHA, but also phospholipids into cells, where they also exert their effects on remodeling cells’ structures, fluidity, and functions. With this, and their ability to affect cholesterol metabolism, phospholipids may trigger downstream pathways and mechanisms into meaningful health benefits.

This book identifies some of the research that addresses several of these pathways and mechanisms important for conditions of e.g. the heart, brain, and liver. It details
the above-mentioned aspects of krill oil, its components, and how science and research points to its beneficial health effects while drawing comparisons to fish oil where applicable.

The latest product development from Antarctic krill is also introduced. It is a highly concentrated and purified product developed on the premise that a higher omega-3 phospholipid concentration may deliver better health benefits across various health conditions than its predecessors. Thus, the next generation omega-3 phospholipid product is a krill oil concentrate with more omega-3 fatty acids being efficiently delivered to tissues via more phospholipids high in choline.

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Summary

- Krill oil is extracted from Antarctic krill.
- Antarctic krill lives in the Southern Ocean around Antarctica.
- The Convention of the Conservation of Antarctic Marine Living Resources (CCAMLR), an international treaty, regulates krill harvesting in a sustainable way. In Area 48 in the Southern Ocean, which is the only area where krill fisheries can operate, the krill industry is allowed to catch one per cent of the estimated 60 million tons of krill.
- Krill oil is rich in omega-3 phospholipids and therefore a good source of both omega-3 fatty acids and choline from the phospholipid head group.
- What differentiates krill oil from fish oil is the molecular form, in which the omega-3 fatty acids are bound to, namely phospholipids in krill oil and triglycerides in fish oil.
- The long-chain omega-3 fatty acids EPA (eicosapentaenoic acid) and DHA (docosahexaenoic acid) promote healthy heart, brain and visual function and contribute to reducing and maintaining normal levels of inflammation, blood triglycerides, and blood pressure.
- Choline is vital for many biological functions (e.g. membrane structure, nerve signaling, methylation reactions, lipid transport, etc.) and deficiency can lead to fatty liver, muscle damage, atherosclerosis, etc.
- The typical Western diet contains inadequate amounts of omega-3 fatty acids and choline. This contributes to the approximately 90% of the American population who are deficient in omega-3 fatty acids and choline [1,2].
- Krill oil supplementation can help to increase levels of omega-3 fatty acids and choline in the body, thus reducing the deficiencies of these important nutrients.
- Several clinical studies have shown health benefits for supplementation with krill oil.
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Euphausia superba (E. superba) is the scientific name for ‘Antarctic krill’. The shrimp-like crustaceans from the Euphausiacea family are commonly referred to as ‘krill’ and consist of 86 species [3]. Euphausia superba (E. superba), also called ‘Antarctic krill’, is the most dominant krill species in the pristine oceans surrounding Antarctica [4]. Even though Antarctic krill are only about the size of your smallest finger, they are a keystone species of the Antarctic marine ecosystem. Krill is considered to be at the bottom of the food chain, since they feed on phytoplankton and are food for many marine animals, such as whales, seals, penguins, squid, and fish. Of all multi-cellular animal species on Earth, Antarctic krill is the most abundant species with one of the largest biomasses of around 500 million metric tons.

Compared with other marine life, Antarctic krill gathers in the largest groups [5] and also has the most potent known digestive enzymes on Earth [6].

These small pinkish-red transparent creatures are one of the world’s largest swarming animals. They often aggregate in large, dense swarms stretching for tens of kilometers and measuring 30 meters deep.

They tend to swarm and migrate vertically as methods of avoiding attacks from predators. They also swim up to the sea’s surface to feed and reproduce mainly during the night and then swiftly swim down into deeper waters to avoid predators, such as birds, penguins, seals, squid, fish or whales. When larger, swarm-hunting animals attack, they quickly scatter by swimming backwards (as fast as 60 cm per second) in all directions to confuse the predator. Another defense from smaller attackers is to leave their exoskeleton behind as a food distraction.

Some krill swarms may consist only of young krill or either females or males.

Krill are bioluminescent, meaning that their bodies can emit light by chemical reactions involving oxygen molecules. These reactions take place in photocytes, luminous cells assembled into light organs. Krill use muscle contraction and relaxation
for the regulation of the intensity of the light. When special closure muscles relax, the light is emitted, which is suspected to be due to more oxygenated blood reaching the photocytes.

It is believed that this light emission is important for recognizing members of the same species and keeping them together in a swarm [7]. Though suspected, but unknown, this feature may also be valuable in defending against detection from predators or in mating. Another characteristic of krill are their large dark brown compound eyes, and like with insects, they consist of many little eyes. This gives krill the ability to see in many different directions at the same time, which helps them to locate food and detect predators. However, in comparison to human eyes that can see in only one direction, the vision quality of each little eye in krill is lower.

Krill can survive up to 200 days without food by shrinking and using their body’s bio-material for storing energy in the form of...
Antarctic krill’s biophysical make up is important for their survival. They withstand the fridge cold waters of the Antarctic and expend large amounts of energy constantly swimming up and down the depths and lengths of the Antarctic Ocean. If Antarctic krill stops swimming, their higher than water body density would make them sink [8].

Lipids. Some krill species hold their lipid reserves in wax esters and others predominantly in triglycerides. However, members of the Euphausiid family are the only known species in which phospholipids are used as their energy depot. In particular, Antarctic E. Superba krill uses phosphatidylcholine containing omega-3 fatty acids as their lipid reserve [3].

E. superba krill uses its specialized filtering apparatus in their front legs to help them feed on the tiniest of plants (phytoplankton and zooplankton) mostly in the summer, and algae from under sheets of ice in the winter, when otherwise food may be scarce. Moreover, krill can live up to seven years. Females are sexually mature by two years of age and males by three [9]. Krill shed their outgrown exoskeleton known as a moulting process. They moult in order to grow, while their new shell is still soft and in times when they shrink due to lack of food [10].

Euphausia superba (Antarctic krill)
Krill oil is a pure, natural source of eicosapentaenoic (EPA) and docosahexaenoic (DHA) omega-3 fatty acids, phospholipids (with choline), and astaxanthin. This combination of important nutrients extracted from Antarctic krill distinguishes it from fish oil and is the basis for krill oil’s uniqueness. Its naturally occurring antioxidant astaxanthin is responsible for the dark red color of krill oil. In contrast, fish oil contains no astaxanthin, and their omega-3 fatty acids are incorporated into triglycerides.

There is increasing evidence that the differences between the molecular forms of omega-3 fatty acids (triglycerides and ethyl-esters in fish oil, and phospholipids in krill oil) are important. The phospholipid form (from krill oil) has been shown to facilitate the incorporation of omega-3 fatty acids into tissues in a more effective and efficient manner compared to triglycerides and ethyl-esters (from fish oil) [14]. Therefore, it is thought that a lesser amount of omega-3 fatty acids can be taken with krill oil compared to fish oil in order to get the same amount of omega-3 health benefits. Moreover, there is evidence which points to phospholipids (and choline) itself having many health benefits.

**Astaxanthin contained in krill** is a highly potent antioxidant and accounts for krill oil’s red color [11]. It assists in keeping the omega-3 fatty acids in krill oil stable. In cells, it provides protection against free radical attack and it has been shown to normalize oxidative stress in persons such as those that are smokers or are overweight. As a result, astaxanthin has been linked to health benefits such as anti-inflammatory and pain-relieving effects, faster recovery from exercise, UV light protection in the skin [12], aging and age-related diseases, liver, heart, eye, joint, and prostate health [13].
Phospholipids have two fatty acids bound to a glycerol backbone, while triglycerides, or triacylglycerols, are a combination of three fatty acids with a glycerol backbone. Fatty acid ethyl esters are derived by a chemical process to exchange the glycerol backbone of a triglyceride with ethanol, an alcohol.

Each of krill oil’s major components (phospholipids, choline, and omega-3 fatty acids) have shown to have effects in the body’s cells and tissues. This is thought to be the basis of its health benefits in many bodily systems and health conditions.

Fatty acids are important nutritional elements of cells, and in particular phospholipids and omega-3 fatty acids are essential in cell membrane structure and function. They also play an important role in the formulation of lipoproteins, while their metabolites serve as vital molecules within pathways of the body. For example, they can modify molecules involved in inflammation (cytokines), eicosanoids, gene expression, and plasma triglyceride synthesis, to name a few. Both omega-3 fatty acids and phospholipid deficiencies can be associated with damaged cell structure and decreased fluidity, which can result in cell dysfunction.

Cellular dysfunction has been linked to health conditions of the heart, brain, liver, joints, etc. Therefore, it is thought that providing more phospholipids and omega-3 fatty acids with krill oil concentrate will result in better cell functioning and ultimately better health benefits compared to other krill and fish oil products.

Indeed, the importance of the amount of phospholipids in krill oil was shown in a comparison study of two krill oils containing either a low (600 mg; LPL) or a high (1200 mg; HPL) phospholipid amount with the same omega-3 fatty acid content of 600 mg [15]. LPL, HPL, or control oils were given to healthy male and female volunteers for 4 weeks. At study end, both plasma and red blood cell fatty acid compositions were compared for the different treatment groups. While both the LPL and HPL groups showed significantly increased plasma omega-3 levels when compared to the control group, there was no statistical difference between the LPL and HPL groups. However, when incorporation into membranes was compared, then LPL only changed EPA in red blood cells, whereas HPL changed both EPA and DHA. And the red blood cell membrane EPA and DHA incorporation was significantly higher in the HPL group when compared to the LPL participants.
Major components of krill oil and fish oil

Table 1: Typical content values (g/100g oil) for krill oil and krill oil concentrate.

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<tr>
<th>Composition</th>
<th>Krill oil</th>
<th>Krill oil concentrate</th>
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<tr>
<td>Total phospholipids</td>
<td>≥40</td>
<td>≥56</td>
</tr>
<tr>
<td>Choline</td>
<td>≥5</td>
<td>≥7</td>
</tr>
<tr>
<td>Total omega-3 fatty acids</td>
<td>24</td>
<td>≥27</td>
</tr>
<tr>
<td>EPA</td>
<td>≥12</td>
<td>≥15</td>
</tr>
<tr>
<td>DHA</td>
<td>≥5,5</td>
<td>≥7</td>
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Increasing the amount of phospholipids in krill oil increases the incorporation of EPA and DHA into membranes [15].

Since incorporation into red blood cells relates to long-term and tissue absorption in general [16], and therefore health benefits, an increased amount of omega-3 phospholipids will improve the effects of krill oil.

Hence, to profit from increased membrane incorporation rates, a new product advancement of a highly purified and concentrated version of krill oil was developed. It was made possible by a specialized ion-exchange technology, Flexitech™, that also reduces the TMA/TMAO (an osmolyte and protein stabilizer) and the salt content of the oil. This results in a product with a concentrated amount of omega-3 fatty acids, phospholipids, and astaxanthin, which is almost taste- and smell-free (see Table 1).
Sourced from the pristine waters of the Southern Ocean around Antarctica and due to its low position in the food chain, krill oil is virtually free of toxins and heavy metals. Superba™ krill oil is certified by the Marine Stewardship Council (MSC) as being sustainable and 100% traceable, from sea to shelf, with the GPS coordinates to prove it.

**Trimethylamine (TMA)** has a ‘fishy’ odor and gives the characteristic odor to seafood. Humans can convert TMA in the liver to *trimethylamine oxide* (TMAO).
Several articles on health benefits of krill oil supplementation in both animals and humans have been published over the years and positively changed health parameters are presented in more detail in the section on Krill oil in health and disease. The very latest krill oil science has emerged in the new areas of sports nutrition and skin.

**SPORT**

The immune system is a complex interplay of cells, tissues and organs to support tissue repair and prevent invasion of the body by bacteria, parasites, viruses, and fungi. Omega-3 fatty acids enhance the immune function by mixing into immune cell membranes, which reduces the amount of the pro-inflammatory fatty acid, arachidonic acid. The incorporation of omega-3 fatty acids instead promotes the formation of the less potent EPA and DHA-derived inflammatory mediators. The immune-enhancing properties of omega-3 fatty acids might particularly help in situations where the immune system function is reduced. Such a compromised immune system can occur after heavy training and sports competitions, where the risk for infection is increased, especially for upper respiratory tract infections of athletes.

*It is true* that moderate exercise in comparison to a sedentary lifestyle lowers the risk of upper respiratory tract infections (URTI). However, high intensive exercise can have immune system modulating effects that weaken the defense mechanisms of a recovering body [6].

The relationship between exercise intensity and risk of URTI was shown in a graph by Professor Nieman [17].

[Graph showing the relationship between exercise intensity and risk of upper respiratory tract infection]
The immune system changes after heavy exercise include a decrease of interleukin 2 (IL-2) and interferon gamma (IFN-γ), molecules that can regulate the activity of immune cells. Likewise, natural killer (NK) cell function was shown to be decreased after heavy exercise. NK cells are the first line of defense by reacting quickly to e.g. intruding bacteria and viruses to keep them under control until the antigen-specific immune system starts to act. Their activity can be decreased by up to 60% for several hours after extended exercise [17]. This has led to the view that after intense training or sport competitions, there is an open window of 1-9 hours with lowered host defense, which increases the likelihood for an infection.

Omega-3 supplementation has been shown to increase IL-2 and INF-γ production, as well as NK cell function and might therefore help to increase host protection after exercise.

It has been recommended by Simopoulos that athletes at a leisure level should eat up to 2 grams of fish oil per day in a 2:1 ratio of EPA to DHA [18]. Athletes at a competition level should probably consume even more omega-3 fatty acids to fully benefit from the immune-modulatory effects. In a study of 106 German winter elite endurance athletes, only one athlete was within the optimal omega-3 target range that lowers the risk for cardiovascular events and suboptimal brain function (reaction time and executive function) [19]. These surprisingly low omega-3 levels, which were even lower than in heart disease patients [20], may be explained by the high need of energy of an athletes’ body that might use these essential fatty acids as an energy source.

Omega-3 supplementation might therefore be crucial to help athletes to optimize their physical and mental performance, also in hindsight that EPA and DHA have the ability to decrease heart rate and oxygen consumption during exercise [21].

The potential of krill oil to strengthen the immune function after a simulated cycling time trial has been tested in both male and female participants [22]. The study was conducted at the University of Aberdeen, Scotland under the supervision of Dr Stuart Gray, Senior Lecturer in Exercise Physiology.

The Omega-3 index, a measure of the percentage of EPA and DHA of total fatty acids in red blood cells, was measured after 6 weeks of either 2 grams daily krill oil or placebo consumption. The results showed that those participants administered krill oil
had a statistically significant increase of their Omega-3 index.

After 6 weeks of study product supplementation, the volunteers performed an incremental maximal exercise test on a cycle ergometer. Participants cycled at 70 revolutions per minute with workload increasing by 30 Watts every minute for males, and 20 Watts every minute for females, until volitional exhaustion.

Immune function parameters, like cell signaling molecule production (IL-2, IL-4, IL-10, IL-17 and IFN-γ) and the ability to destroy target cells by NK cells were measured at baseline and in the recovery period after exercise (post-exercise, 1h and 3h).

The results demonstrated that supplementation of the diet with 2 grams per day of krill oil for 6 weeks can significantly increase the production of IL-2 and increase the toxic effect of NK cells on other cells in the recovery period after exercise. The effect was gender-independent.

Study coordinator, Dr Stuart Gray comments: “Our study is in agreement with our previous work with fish oil, where we have observed similar results. However, the krill oil EPA and DHA dose used was only a quarter from the dose given in the earlier fish oil study. It remains to be proven if the different structural form (omega-3 phospholipids from krill oil versus omega-3 triglycerides from fish oil) can explain this difference.”

Moreover, a previous double-blind 6-week study on krill oil supplementation to the Polish National Rowing Team has shown that krill oil can affect levels of pro- and anti-oxidant balance markers [23]. Study authors found that exercise significantly increased values of oxidative stress parameters in both groups, but recovery levels were significantly lower in athletes receiving 1 gram krill oil per day compared to the control group. Based on these results they concluded that supplementation with krill oil in trained rowers diminished post-exercise oxidative damage
Overall, several sport studies including omega-3 fatty acids highlight the importance of an adequate omega-3 fatty acid intake for athletes. The ability of krill oil to positively influence oxidative stress and immune function shows that regular consumption of omega-3 phospholipids from krill oil might be an effective nutritional strategy to help athletes in the post-exercise recovery phase.

**Krill oil supplementation has beneficial effects on oxidative stress and immune function and can help to improve a balanced sports nutrition for athletes.**

**Skin**

Skin is the organ that not only protects against microbes, pollution, and physical assaults, it is further important for sensory perception and in maintaining the body’s water content and temperature. It consists of several layers. The epidermis is towards the outside, with the top cell layer called the stratum corneum, which mainly consists of dead cells and oils. The body sheds the dead cells with a rate of up to 40,000 cells per hour. This can amount to as much as 3.6 kg of dead skin cells in a year. Under the epidermis lies the dermis as an inside layer including sweat glands, hair follicles, and nerve endings needed to feel temperature, pressure, and pain.

The stratum corneum is important in preventing water loss to keep the underlying living cells moisturized. If its barrier function is disturbed, the skin can become dry and flakey. Hot water, soaps, medication, low humidity, and medical conditions can all lead to dry, irritated skin. For example, eczema is characterized by dry, red skin patches that can be very itchy, which might be due to an overactive immune system, but the exact causes are unknown. Also in the case of psoriasis it is unknown why the immune system is not working properly, which leads to the formation of excess skin cells that build up in
scales and red, itchy patches.

**Healthy skin permits water loss only to a limited amount.** Damaged skin, such as in atopic eczema, is characterized by increased water loss. Krill oil was shown to alleviate water loss and dry skin in a 12-week clinical study in healthy persons.

Both omega-3 fatty acids and phospholipids are important for skin health. Deficiency leads to scaling and dryness of the skin and enhanced transepidermal water loss (TEWL) [24,25]. TEWL describes the water loss over the surface of the skin that happens by passive diffusion. The outer layer of dead cells makes the skin flexible and elastic, when it contains enough water. However, when the TEWL is high and hydration low, it becomes hard and rough. Hence, skin function is negatively affected, which can lead to discomfort or even infection.

In general, the skin holds about 30% of the body’s water and the dead cells of the stratum corneum and the lipids ‘waterproof’ the human body. Water loss and hydration of the stratum corneum is linked to its lipid content, the generation of new lipids in the skin, and ultimately the level of damage to the skin barrier function. A good balance between the skin’s water content and the amount of water passing influences skin elasticity, smoothness, and roughness and is crucial in maintaining healthy skin [26].
The skin has several functions, but one of the most critical ones is to maintain the body’s water levels and to limit water loss into the surroundings. Water makes up 75 (infants) to 55% (elderly) of the body’s weight and is vital for biological processes and life. A way to measure water loss is to assess trans-epidermal water loss (TEWL).

In contrast to e.g. saturated fatty acids and cholesterol, EPA and DHA cannot be made in the skin and must be obtained from outside sources. The skin lacks the enzymes needed to convert short-chain omega-3 fatty acids into the longer chain EPA and DHA [27]. Omega-3 fatty acid levels in human skin are rather low with under 2% of total skin fatty acids. However, significant increases can be achieved by supplementation and 3 months of 4 grams daily EPA increased skin EPA 8 times [28].

Krill oil is an attractive dietary supplement that might help in the maintenance of skin health and treatment of skin disorders by both its omega-3 and phospholipid components (see above).

To explore the effects of krill oil on skin health, 31 volunteers (middle-aged men and women with normal skin) were included in an open label, two-armed clinical trial (unpublished results). The subjects were randomized to take 3 grams daily krill oil for 13 weeks. All skin measurements were done on the upper forearm and showed that krill oil intake resulted in a significant increase in skin hydration and elasticity, as well as in a significant reduction in TEWL.

In addition, a significant correlation for the change in hydration and the changes both for elasticity and TEWL were found. Meaning that the volunteers that had a high change in skin hydration also experienced a high
change for both elasticity and TEWL. By using a digital camera, the amount of wrinkles (as a measure for roughness) and width and size of wrinkles (as a measure for smoothness) were assessed at the start and end of the trial. For both parameters, i.e. roughness and smoothness, there was a significant beneficial change observed after krill oil supplementation.

By increasing the amount of omega-3 fatty acids in the body, krill oil has the ability to not only influence skin hydration and elasticity, but also the amount and size of wrinkles. The measure of how much omega-3 accumulates in the body, given as the Omega-3 index, was increased significantly in the study and correlated with the above mentioned parameters. These results indicate that the increases in Omega-3 index by krill oil supplementation directly relate to the positive changes in skin parameters observed among study subjects.
**Phospholipids**

Carbohydrates, nucleic acids, proteins, and lipids are the four main biological compounds that determine life. Whereas, phospholipids are part of the lipid group, which are the very basic units of life.

**WHAT IS A PHOSPHOLIPID?**

A phospholipid molecule consists of two fatty acids, which are long chains of carbon and hydrogen molecules. They are attached to a glycerol backbone that is further linked to a phosphate group. The phosphate group has a head group, such as choline, resulting in phosphatidylcholine (PC).

While choline, phosphate, and glycerol make up the hydrophilic (water-friendly) side of the molecule, the fatty acid chains are the hydrophobic (water-fearing) part of the molecule.

The first phospholipid was identified in 1847 by Theodore Nicolas Gobley, a French chemist and pharmacist who analysed lecithin (a rich source of PC) in egg yolk.

**Phospholipids, as the name indicates, are made up of the mineral phosphorus and lipids (fats).**

The word lecithin originates from the Greek lekithos, egg yolk. Lecithin’s chemical name is phosphatidylcholine, while commercially it is a phospholipid mixture. Most lecithin products consist of phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, and phosphatidylinositol [29].

Phospholipids in the form of lecithin are used as emulsifiers and stabilizers in foods, in cosmetics and paints or for therapeutic reasons, and as nutraceutical supplement. In 1933, lecithin already appeared in the Italian medicine dictionary Medicamenta, for conditions such as, diabetes, tuberculosis, depression,
and recovery from infectious diseases [30].

**FUNCTIONS OF PHOSPHOLIPIDS IN THE BODY**

Phosphorus, found in the head group of phospholipids, contributes to the normal function of cell membranes, energy-yielding metabolism, and in bones and teeth [31]. The phospholipid molecule has many functions and uses in the body. For example, as a structural element in cell membranes, a source of choline for the neurotransmitter acetylcholine, an important factor in energy production and storage, assisting in blood clotting, antioxidant protection, and cholesterol solubility to name just a few. Moreover, it acts as surface-active wetting agent that lines the outside of liver, lungs, gastrointestinal tract, and kidney cells.

**CELL MEMBRANES**

The structural skin around cells and their organelles is called a membrane. Both the inside and the outside of cells is water. When phospholipids are exposed to water, they arrange themselves into a two-layered sheet (a bilayer) with all of their hydrophobic tails pointing towards the center of the sheet and the hydrophilic heads towards the surrounding water.

The amount of phospholipids in the human body is enormous! The body contains over 100 trillion cells with membranes, consisting of phospholipids, and proteins. The liver itself consists of membranes that can fill four soccer fields.

About 60% of what is made in a cell is needed for membrane structures, either for channels and receptors inside the membrane or proteins attached on the outside of membranes [33]. Membranes not only give a defined volume to a cell, they also allow for internal structures, such as organelles (e.g. nucleus) and transport vesicles between organelles.

It is important that our cells maintain a sufficient amount of phospholipids to ensure optimal cell function. Otherwise many cell functions can be compromised, such as their ability to control the exit of waste and the entry of nutrients into cells, communication
between cells, membrane-bound enzyme functions, binding of molecules (neurotransmitters, antigens, antibodies, etc.) to receptors, and more.

Two of the many factors which may affect the amount of phospholipids in cells are, i) as we age phospholipid amounts in cells decrease, and ii) cells in skin, lungs, liver, heart, and blood vessels can become vulnerable to attack from free radicals and toxins [34]. Therefore, damaged and lost phospholipids must be continuously replaced to ensure optimal cell function and health.

**Phospholipids** with their unique position in cell walls are essential for cell growth and the generation of new cells. They promote molecule transport across membranes, binding to receptors and enzyme activities and determine the fluidity of membranes. They are a source of messengers in cell signaling, choline, omega-3 fatty acids and contain phosphate for energy production. Moreover, they are needed in fat emulsification in the stomach and blood clotting.

**LIPID TRANSPORT AND CHOLESTEROL METABOLISM**

In addition to the central role of phospholipids in membrane function, they are, together with apoproteins, part of the outer layer of transport vehicles, called lipoproteins, because they are made of fat (lipid) and proteins. This combination of solubilizers allows the transport of cholesterol in the water-based bloodstream. Cholesterol is important for cell membrane function and as precursor for other compounds. However, in high amounts, it will accumulate on and in blood vessel walls and make them narrower and stiffer. The hardening of arteries via the buildup
of cholesterol and fat plaques is called atherosclerosis. If these plaques burst, they can lead to blood clots that can cause heart attacks and stroke.

By forming monolayers, phospholipids surround all lipoproteins that are classified according to their density in chylomicrons, VLDL (very low-density), LDL (low-density), IDL (intermediate-density) and HDL (high-density) particles. Whereas large lipoproteins have low density and contain more fat than protein. In short, cholesterol is taken up in the small intestine and is delivered to the liver in packages called chylomicrons. VLDL and LDL are secreted by the liver and are risk factors for atherosclerosis and heart disease by providing cholesterol to plaques. LDL is therefore often called the ‘bad’ cholesterol. In contrast, HDL, the ‘good’ cholesterol, has a protective effect by removing excess cholesterol from the arteries and bringing it back to the liver on the ‘reverse transport pathway’.

Every 1% increase in blood HDL levels has been associated with a 2-3% reduction in overall heart disease risk [35].

The levels of cholesterol in blood primarily depends on the amount of cholesterol that is ingested (from diet and bile) or made by the liver. The absorption rate of cholesterol in the gut, can vary substantially [36]. It is influenced by the amount of phospholipids available, since phospholipids are needed for the intestinal uptake of cholesterol, but high amounts of phospholipids reduce cholesterol absorption through molecular interactions. Indeed, it was found that a dose of 15mg lecithin can inhibit cholesterol absorption by 50% in rat guts [37]. There is also evidence for reduced intestinal cholesterol absorption in the gut of humans after phospholipid supplementation [38-40].

Research has shown that dietary phospholipids may help to reduce blood LDL particles, the ‘bad’ cholesterol and reduce the risk of heart disease by affecting intestinal cholesterol uptake.
Dietary phospholipids are efficiently (above 90%) taken up into the cells of the intestinal wall after they are hydrolyzed to lysoPC and free fatty acids by lipases in the small intestine. In the intestinal cells, they are reassembled and included into chylomicrons for further transport via the lymph and blood. Some studies suggest that a fraction of the dietary phospholipids is directly taken up into HDL particles already in the intestine, which later join the HDL blood pool.

Phospholipids move into cells and their membranes by selective and whole particle uptake routes. In liver cells, about two-thirds of the phospholipid uptake is by selective entry routes. Thereby, phospholipids are transferred directly from lipoproteins into the cell membrane. In addition to selective uptake pathways, cells can incorporate entire lipoproteins by endocytosis, which results in an unspecific incorporation of everything that was part of the particle, including phospholipids. Incoming phospholipids can be metabolized to triglycerides and can be used in storage or energy generation, when not used in membranes.

**PHOSPHOLIPID SOURCES**

Daily phospholipid intake is usually between 2-8 grams, which corresponds to 1-10% of the daily fat consumption [41]. Phospholipids can be found in high quantities in egg yolk, soybean, meat, fish and internal organs, while fruits, vegetables and grains have a much lower phospholipid content [42]. However, the richest sources usually also have a high content of fat and cholesterol. Also krill contains high amounts of phospholipids and *E. superba* is known to use them as energy storage form. In
the extracted krill oil concentrate, over 56% of the oil consists of phospholipids.

PHOSPHOLIPID DEFICIENCY
Not much is known about the optimal intake levels of phospholipids, since phospholipid deficiency symptoms are unknown, except the ones associated with choline deficiency that are described later on. However, due to refinement of oils and fats, cleaned raw materials, and changes towards a nutrition low in fat and cholesterol, modern diets contain only about one third of phospholipids than what they contained a century ago [43]. In particular, high-risk groups for deficiency, such as the elderly, athletes and diseased persons might therefore benefit from phospholipid supplementation.

PHOSPHOLIPIDS IN HEALTH AND DISEASE
Although dietary phospholipids are present in many foods, purified forms can help to treat various health concerns, including fatty liver, arthritis, heart disease, cachexia, hypercholesterolemia, cancer, and more [44]. Major health benefits of phospholipid supplementation are presented in Table 2 that underlie their liver-protective, anti-inflammatory, immune changing, anti-obesity, memory enhancing, anti-depressant, and anti-tumor effects [45]. Their effects on liver health and physical performance, are outlined in more detail below.

LIVER
The liver is one of the organs that get the most membrane damage because of its blood filtering function. It deals with incoming food nutrients and either stores or prepares them for further transport to other organs. It is also responsible for filtering the blood from harmful substances such as toxins, alcohol, medication, fatty food, waste products, viruses, etc. These are all stressors for the integrity of the hepatic membrane and fat accumulation or alcohol misuse can take its toll on the liver and eventually lead to fatty liver disease that can progress into cirrhosis and liver failure.

While studying 700 obese children, it was found that 15% (10% girls, 22% boys) of the children already had fatty liver disease [56]. Overall, the prevalence of suspected nonalcoholic fatty liver disease lies at 50% of obese males in the U.S. [57]. While 45% of
<table>
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<th>Health area</th>
<th>Benefit</th>
<th>Explanation</th>
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<tr>
<td>Heart disease</td>
<td>Reduced cardiovascular risks</td>
<td>PL improve parameters related to heart and cardiovascular diseases:  • Blood lipid profiles (reduction of total cholesterol, LDL and TG levels and increased HDL levels)  • Reduced hypertension  • Reduced platelet aggregation and the risk of arteriosclerosis</td>
<td>[46-48]</td>
</tr>
<tr>
<td>Immune system</td>
<td>Improvement of immunological function</td>
<td>PL-induced improvement in phagocytosis, arachidonic acid concentration and neutrophil killing activity.</td>
<td>[49]</td>
</tr>
<tr>
<td>Infant development</td>
<td>Improved brain development of fetus</td>
<td>Choline supplements during pregnancy have been shown to affect brain development of fetus and improve lifelong memory characteristics (animal studies).</td>
<td>[50]</td>
</tr>
<tr>
<td>Liver disease</td>
<td>Improved hepatic disorders</td>
<td>Reduced alcohol-induced liver damage and hepatic damage caused by toxins and virus infections (hepatitis).</td>
<td>[51]</td>
</tr>
<tr>
<td>Physical performance</td>
<td>Improved physical performance by reduction of physical stress</td>
<td>PL stimulates acetylcholine synthesis, which triggers the release of neurotransmitters in the brain, thus improving physical performance.</td>
<td>[52]</td>
</tr>
<tr>
<td>Stomach and gastrointestinal tract (GI)</td>
<td>Reduction of stomach pain induced by gastric acid</td>
<td>PL-induced protection of the GI by reduced gastric mucosal lesions. Typical GI side effects of analgetic drugs (NSAIDS) are reduced, likely due to an increased production of cytoprotective mucosal PGE2.</td>
<td>[53]</td>
</tr>
</tbody>
</table>
type-one diabetics have fatty liver [58], up to 85% of type-two diabetics are afflicted with the disease [59,60].

**Liver disorders** include fatty liver due to cellular fat accumulation in people that are e.g. overweight, have diabetes, or drink too much alcohol leading to an enlarged liver. Cirrhosis, the inflammation and scarring of liver tissue, can be due to hepatitis infection or excessive alcohol consumption, which can also lead to cancer. Autoimmune liver disorders damage liver cells by an abnormally high amount of immune cells.

<table>
<thead>
<tr>
<th><strong>Stress and depression</strong></th>
<th>Reduction in stress-related mood symptoms</th>
<th>Reduced production of stress hormones linked to strenuous exercise and eased stress-related mood symptoms.</th>
<th>[54]</th>
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<tbody>
<tr>
<td><strong>Ulcerative colitis</strong></td>
<td>Protective and healing effects in inflammatory ulcerative colitis</td>
<td>Supplementation of PC has been shown to protect and restore the intestinal lining of the gastrointestinal wall.</td>
<td>[55]</td>
</tr>
</tbody>
</table>

GI, gastrointestinal tract; HDL, high-density lipoprotein; LDL, low-density lipoprotein; NSAIDS, nonsteroidal anti-inflammatory drug; PC, phosphatidylcholine; PGE2, prostaglandin E2; PL, phospholipids

Whole phospholipid, in contrast to choline and omega-3 fatty acid supplementation alone, has been shown to influence liver fat metabolism by either affecting cholesterol and bile acid generation, degradation of fatty acids or the secretion of lipoproteins from the liver [61]. As shown in animals, a diet rich in phospholipids reduces liver fat by means of inhibiting intestinal fat uptake and influencing the activity of liver enzymes that regulate lipid metabolism. Moreover, liver damage was found to be alleviated by phospholipids. Reduced phospholipid levels in liver membranes, as for example found after alcohol consumption, can be counteracted by phos-

**STAGES OF LIVER DAMAGE**

Healthy Liver
- Deposit of fat leads to liver enlargement

Fatty Liver
- Scar tissues form

Liver Fibrosis
- Growth of connective tissues destroys liver cells

Cirrhosis
phospholipid supplementation. Accordingly, a study on chronic alcohol consumption in baboons that were given either ethanol or ethanol together with lecithin over 6.5 years reported that PC protects against liver fibrosis and cirrhosis [62]. But also other liver damages like e.g. from viral infections, can benefit from phospholipid supplementation to decrease disease activity [63].

Fat accumulation in the liver will often not lead to symptoms indicating that something is wrong. Inasmuch, fatty liver silently increases heart disease risk 3 times in men, 14 times in women and up to 10 times in type-one diabetics [64].

The majority of the studies performed with phospholipids, did not include omega-3-containing phospholipids, indicating that phospholipids in general have beneficial effects. However, other studies have demonstrated that phospholipids containing omega-3 fatty acids have more potent effects on liver and blood plasma lipid levels, compared to phospholipids without omega-3s [65,66].

In particular, Shirouchi and colleagues have concluded in their study on rats that the combination of omega-3 fatty acids with phospholipids in comparison to egg phospholipids alone can alleviate liver steatosis better through the suppression of fatty acid synthesis, enhancement of fatty acid degradation, and increase of serum adiponectin levels [66].

**BRAIN**

Phospholipids play a central role in brain function and around 60% of the brain by weight consists of phospholipids. Phospholipids are particularly enriched in dendrites and synapses and it has been shown in vitro that nerve growth increases the demand for phospholipids. Nerve growth factor, a small protein controlling nerve growth and maintenance, also stimulates phospholipid generation [67].

The omega-3 fatty acid DHA can only to a very little extent be made by the brain, and must therefore be supplied by the blood and imported over the blood-brain barrier. Phospholipids are of utmost importance in the transport of DHA, since the recently discovered DHA transporter (Mfsd2a, major facilitator super family domain containing 2a) accepts DHA only if it is bound to phospholipids; to be exact to lysoPC [68]. Mice that are genetically engineered to not have this transporter, have very low DHA amounts in the brain leading to neuronal cell loss and cognitive deficits associated with severe anxiety. In addition, humans identified with mutations in Mfsd2a present defective brain growth and intellectual disability due to
insufficient DHA-lysoPC uptake into the brain [69,70].

LysoPC in combination with DHA from the blood is therefore vital for normal brain growth and function.

The cells of the small blood vessels coming into the brain are tightly stitched together by tight junctions forming the so-called blood-brain barrier to restrict free passage of molecules. This allows for the protection of e.g. bacteria infecting the brain. Even DHA needs a special transporter that will transport DHA only when it is bound to lysoPC.

But not only bring phospholipids DHA into the brain, they also provide choline, an essential nutrient [71], which makes up about 15% of the PC molecule. Like DHA, choline is important for brain development and nerve signaling and thereby influences cognition [72]. Hence, a 47% reduced risk for getting dementia was found in the elderly that had the highest PC levels in blood [73].

Choline

WHAT IS CHOLINE?

Choline is an essential vitamin-like nutrient that is crucial for normal cellular function.

CHEMICAL STRUCTURES OF CHOLINE MOLECULES

- Choline
- Acetylcholine

Illustration adapted with permission from Dr. Christer Betsholtz
FUNCTIONS OF CHOLINE IN THE BODY

Choline as a component of phosphatidylcholine, choline plasmalogen and sphingomyelin is a major component of cell membranes.

*95% of choline in the body is found in phospholipid form as phosphatidylcholine (PC).*

The derivatives of choline are versatile and include functions such as neurotransmitters (acetylcholine), cell membrane signaling (phospholipids), lipid transport (lipoproteins), and methyl-group metabolism (homocysteine reduction) [74]. Besides, a fetus depends on choline for brain and memory development [75] and to reduce the risk for neural tube defects [76]. A lot of the maternal choline goes to the fetus, which might deplete the mother’s choline reserves.

NERVE SIGNALING

Choline is converted to acetylcholine, a neurotransmitter, in the nervous system. Acetylcholine is of importance in learning, breathing, memory, sleep, and muscles metabolism. Whereas in heart tissue, acetylcholine has an inhibitory effect and leads to reduced heart rate, in skeletal muscle it has the opposite effect.

CELL SIGNALING

Choline is a part of the phospholipids phosphatidylcholine and sphingomyelin. They are the precursors of diglycerides and ceramides, which are lipid-signaling molecules in cells. Lipid messengers can freely cross over membranes and can therefore not be stored in vesicles. They act locally on receptors and enzymes to induce a specific cellular response.

METHYL DONOR

Choline is required for the metabolism of nucleic acids and amino acids and is an important source of methyl (-CH3) groups over the generation of S-adenosylmethionine (SAMe). Up to 50 chemical reactions in mammals depend on SAMe as a methyl donor [77]. These methylation reactions are of importance in lipid biosynthesis, regulation
of metabolic pathways, and detoxification. Changes in DNA methylation, that affects gene expression, have been linked to cancer.

**WATER BALANCE**

Choline is converted to betaine, which is an osmoregulator, meaning that it can regulate the volumes and water content of cells [78]. Cells keep water levels constant by allowing ions to enter, which makes water to follow and move into cells. Betaine is an organic water attractant, in particular in cells with high osmotic pressure such as kidney or intestinal cells.

Betaine is further used to convert homocysteine, a risk factor for heart disease, to methionine.

**UPTAKE OF CHOLINE SALT VERSUS PHOSPHATIDYLCHOLINE**

Dietary choline is taken up by choline transporters in the intestine. Most of the choline is converted to PC and used in cell membranes. The liver can recycle choline and the intestine, lungs, and kidneys will send choline to the brain and liver in time of need.

Administration of choline will significantly increase blood choline con-
centrations [79,80]. It was found that choline in the form of PC, is 12 times better in raising human blood choline amounts compared to choline salt [79,80]. While choline salt show maximum levels after 30 minutes (86% increase, 4 hours until normal), PC intake raises choline by 265% and takes 12 hours until normal again [80]. It was suggested that 60% of the choline in inorganic salts, such as choline chloride, choline citrate, and choline bitartrate, is lost to conversion to TMA by intestinal bacteria [23]. In comparison, dietary PC leads to three times less TMA [24].

**CHOLINE DEFICIENCY**

Only little choline can be made by the body itself, the major part needs to be taken up over the diet. Choline is found in higher amounts in e.g. soybeans, eggs, liver, beef, and milk. Although most diets are considered to provide sufficient choline, persons at risk of choline deficiency exist. Specifically, the National Health and Nutrition Examination Survey in 2003-2004 has concluded that 90% of the American population has an inadequate intake of choline [1].

90% of the U.S. population is not consuming enough choline to ensure optimal membrane function, neurotransmission, and methyl donor availability.

The adequate intake level for choline was set at 550 mg/day for men and 425 mg/day for women in sight of preventing liver damage. Choline deficiency-induced fatty liver can occur, when not enough choline is present for the for-
formation of PC molecules that are needed for the formation of VLDL particles. If the fat becomes stuck in the liver, it accumulates and will eventually lead to liver damage. Excessive oxidative stress that damages lipids and DNA induces cell death and inflammation that can ultimately culminate in end-stage liver disease [81]. Choline deficiency is also the only nutrient deficiency that can lead to cancer.

**Genetic predisposition, gender, and age influences ones choline needs and care should be taken to ensure an adequate choline intake, since choline deficiency is linked to an increased risk for fatty liver, muscle dysfunction, atherosclerosis, and neurological disorders [82].**

**CHOLINE IN HEALTH AND DISEASE**

With its many diverse roles, it is not surprising that choline deficiency will cause disease in humans. The function of many organs such as liver, muscle, kidney, pancreas, and brain all depend on an adequate choline intake.

**BRAIN**

It was suggested that supplementation with choline, as a precursor for acetylcholine, improves brain function in persons affected by memory impairments due to aging or pathological conditions. Inasmuch, Alzheimer’s and Parkinson’s disease patients have a reduction of cholinergic neurons in the grey matter of their forebrains [83]. Also elderly persons have reduced forebrain cholinergic neurons and acetylcholine [84]. On the other hand, choline supplementation increases the amount of choline in the blood, with a concomitant increase of choline-containing compounds in the brain [85]. However, in an analysis of all available literature on studies with lecithin given to individuals with Alzheimer’s disease, Parkinsonian dementia and subjective memory problems, no clear benefit for memory performance could be found for patients with Alzheimer’s disease and Parkinsonian dementia [86]. Yet, people with subjective memory difficulties, gained a very sig-
significant benefit in one of the analysed studies [87]. Thus, while the overall analysis does not support lecithin for dementia patients, people with subjective memory complaints may profit from lecithin supplementation.

**SPORT**

Intense physical activity challenges cellular functions, which are essential to maintain for optimal sports performance. Excessive radical formation and trauma to the muscles during high-intensity exercise leads to inflammation. It has been suggested that supplementation with phospholipids, such as PC might be beneficial to endurance athletes, because of the choline head group [88]. Indeed, decreased blood choline levels were shown in endurance athletes such as cyclists and runners [89]. Supplementation with PC significantly increases circulating choline levels; in fact twelve times better than choline salts alone [79].

If, during exercise, enough choline is available in the body, then also sufficient acetylcholine is produced. Since acetylcholine is a signaling molecule important for muscle contractions, sportive performance might therefore be optimized by PC supplementation.

**Acetylcholine** is a neurotransmitter important for muscle contractions made from choline. The available free choline influences acetylcholine synthesis rate.

If no choline is supplemented, marathon runners can manifest up to a 40% decrease in blood choline levels [16,17], which is similar to the choline reductions found in cyclists [5,18]. Besides, a study with lecithin supplementation found faster recovery after a bicycle ergometer trial, when compared to the control group [26,27]

**During intense exercise**, free choline is reduced, which might impact on acetylcholine release and therefore sportive performance.
Omega-3 Fatty Acids

Omega-3 fatty acids played an essential role in human evolution and development. A turning point for the evolution of human intelligence was the diet change from red meat of animals mainly consumed by the Neanderthals to the inclusion of coastal seafood and inland freshwater sources [90]. This might have initiated the growth of the brain about two million years ago.

WHAT ARE OMEGA-3 FATTY ACIDS?
The main bioactive omega-3 fatty acids that have been described extensively are eicosapentaenoic (EPA or 20:5n-3) and docosahexaenoic acid (DHA or 22:6n-3). EPA consists of 20 carbons and 5 double bonds and can be converted enzymatically into DHA. DHA is the longest fatty acid chain, with 22 carbons and 6 double bonds. They are called omega-3 fatty acids, because they have their first double bond at three carbon atoms from the methyl end. In comparison, an omega-6 fatty acid has its first double bond at 6 carbon atoms from the methyl end.

FUNCTIONS OF OMEGA-3 FATTY ACIDS
Aside from being a source of nutritional energy, the functions of omega-3 fatty acids have a molecular basis. Inasmuch, the omega-3 fatty acids have the ability to change membrane fatty acid composition and function, regulate gene transcription, and alter metabolic and

LONG-CHAIN POLYUNSATURATED OMEGA-3 FATTY ACIDS

\[ \text{Eicosapentaenoic acid (EPA)} \]

\[ \text{Docosahexaenoic acid (DHA)} \]
signal transduction pathways. This involves a wide array of mechanisms that overlap and interplay in complicated metabolic networks that maintain the body's equilibrium.

**MEMBRANES**

Most importantly, omega-3 fatty acids, as well as the overabundant omega-6 fatty acids, are crucial as building blocks of membrane structures and a cell’s development, integrity and function. The flexible structure of omega-3 fatty acids determines the fluidity of membranes. Membrane fluidity is important for the correct functioning of membrane proteins such as receptors, ion channels, transporters, and enzymes. A change in fluidity due to altered membrane fatty acid composition will change activities and movements of these proteins. It will also affect how extracellular signals are transmitted from receptors to intracellular signaling networks, which is important in e.g. neurons, cardiac cells and hormone-secreting cells. Additionally, membrane permeability increases with the number of double bonds present in fatty acid chains of membrane phospholipids. The amount of omega-3 fatty acids that constitute membrane phospholipids can be influenced by diet.

**GENE TRANSCRIPTION AND ENZYME ACTIVITY**

When omega-3 fatty acids reach cells, they can activate transcription factors that stimulate the expression of certain genes [91-94]. This means that omega-3 fatty acids indirectly influence the production of functional gene products (often proteins) in our cells. The omega-3 fatty acids thereby influence which proteins are made in a cell and, ultimately, how the cellular metabolic functions are impacted. It is still unclear to which extent EPA and DHA bind specifically to different transcription factors, but there are at least some indications that there are preferences as to which fatty acid can regulate which gene expression [91]. However, not only can fatty acids change the expression of genes, they can also modulate enzyme activities by directly binding to them [95,96].
**EICOSANOIDs**

The ratio of omega-6 to omega-3 fatty acids is important since they compete for the same metabolic enzymes that convert them into eicosanoids (i.e. prostaglandins, prostacyclins, thromboxanes, and leukotrienes). Eicosanoids are hormone-like compounds, called cellular hormones that control key functions in the body, such as the central nervous system, immunity, and inflammation.

Whereas prostaglandins, leukotrienes, and lipoxins play a regulatory role in inflammation, thromboxanes and prostacyclins are important for controlling bleeding. Resolvins and protectins, metabolites of EPA and DHA, help reduce inflammatory responses [97].

In general, eicosanoids from omega-3 fatty acids are less inflammatory than those from omega-6 fatty acids. Hence, a disturbed ratio with high omega-6 versus omega-3 fatty acids and low omega-3 intake in general will tip the balance towards the production of pro-inflammatory eicosanoids. Therefore, a replacement of the omega-6 arachidonic acid by EPA or DHA may lead to a less inflammatory active eicosanoid profile, decreasing the risk for inflammatory diseases.

**ENDOCANNABINOIDS**

The endocannabinoid system is based on the action of endocannabinoids (ECs) that are hormones made from omega-6 fatty acids. The binding of ECs to receptors influences the expression of proteins in organs (e.g. liver, skeletal muscle, pancreas, intestine, bone, and adipose tissue) and affects the action of the central nervous system. Thereby they can influence not only enzyme activities, but also appetite, energy balance, mood, memory, pain perception, stress response, anxiety, immune functions, and reproductive processes. An overactive EC system is believed to promote increased fat mass and various markers of metabolic syndrome [98].

The quantities of ECs made, ultimately depend on the amounts of omega-6 arachidonic acid available in membranes, which depends on the amount of arachidonic acid ingested. Increased intake of fatty acids of the omega-3 family positively influences the ratio of omega-3 to omega-6 fatty acids in blood and organs. In this way, less arachidonic acid is incorporated into phospholipids, resulting in decreased conversion of arachidonic acid to ECs. Thus, dietary fatty acids constitute a means to change the body’s fatty acid composition and thereby ECs.
levels, ultimately affecting membrane signaling events and leading to changed energy metabolism (food intake and energy processing).

**OMEGA-3 DEFICIENCY**

Both omega-3 and omega-6 fatty acids are needed for optimal health. However, since there is an abundance in the Western diet of omega-6 fatty acids compared to omega-3 fatty acids, the balance between the two is highly disturbed [99,100]. The underlying reason is the vast increase in the consumption of vegetable oils rich in omega-6 fatty acids, which are present in corn, sunflower seeds, cottonseed and soybean and industrially produced meat. At the same time, the consumption of omega-3-rich fish has decreased markedly.

The human body has evolved on a delicate balance of omega-6 to omega-3 fatty acids. Cells in the body rely on a specific ratio of these fatty acids in order to function properly. If out of balance, health is affected and it is of no surprise that our modern unbalanced diet promotes health issues such as heart disease, arthritis, depression, and dementia.

Nowadays, the ratio between omega-6 to omega-3 can be as high as 10-20:1, whereas historically it was as low as 1-2:1 [101]. The recommendations today call for a ratio of 5:1 [102]. Some studies further indicate that the optimal ratio may depend on the disease state [102].

When it comes to heart disease, a ratio of 4:1 was linked to a reduction of 70% in total mortality. Rectal cell proliferation in patients with colorectal cancer were reduced at a ratio of 2.5:1, while a ratio of 2-3:1 suppressed inflammation in patients with rheumatoid arthritis. A ratio of 5:1 was needed to induce benefits for asthma patients, whereas a 10:1 ratio had negative effects.

The right amount of omega-6 and the right balance between omega-6 to omega-3 fatty acids is therefore essential for health, as otherwise too many pro-inflammatory molecules are produced from omega-6 fatty acids that can affect disease outcomes.

Most people do not consume the recommended twice a week of fatty fish and hardly reach the recommended daily intake doses of EPA and DHA. Although the recommended daily dose varies highly between different countries (1000mg in Japan and South Korea, 500mg in France, and 450mg in Norway), most countries recommend
250mg (Austria, Belgium, Czech Republic, Denmark, Finland, Germany, Greece, Iceland, Ireland, Italy, Luxembourg, Netherlands, Portugal, Slovakia, Slovenia, Spain, Sweden, Switzerland, and United Kingdom). While Australia and New Zealand recommend 160mg of EPA/DHA per day, other countries like Canada, Hong Kong, Israel, Singapore, Taiwan, and the United States do not provide an official recommendation.

**Most people** have mean intake levels below the optimal daily EPA and DHA dose of 250mg recommended by the World Health Organization (WHO) and the European Food Safety Authority (EFSA) and could therefore benefit from omega-3 supplementation.

**OMEGA-3 FATTY ACIDS IN HEALTH AND DISEASE**

More than 20,000 studies on EPA and DHA have assessed the health benefits of omega-3 fatty acids [103,104]. Through their influence on membrane integrity, gene expression, the balance with omega-6 fatty acids and the generation of eicosanoids and endocannabinoids, they have the ability to modify heart disease risk, inflammatory response, neurological and psychiatric balance, vision, and many more processes in the body [105-107].

**HEART**

Most established is the influence of EPA and DHA on cardiovascular risk, since they can modify blood triglycerides, HDL cholesterol, plaque development, heart rate, and heart muscle functions [108]. By doing so, EPA and DHA reduce the risk for cardiac death. Moreover, a meta-analysis that summarizes all available research on heart health found a connection between EPA and DHA and a reduction in blood pressure (decrease of systolic blood pressure of around 4.5mm Hg and diastolic blood pressure of 3mm Hg), especially in those with high blood pressure [109].

**Already a decrease** of 2 mm Hg blood pressure is of importance, since it is linked to a 6% reduced stroke mortality, 4% reduced coronary heart disease mortality, and 3% reduced total mortality [110].
**BRAIN**

Omega-3 fatty acids have been shown to be essential for mental health and brain development and function [111]. Research has indicated that the higher EPA and DHA intakes, the lower the risk for Alzheimer’s disease, age-related cognitive decline, depression, aggression, and more [112-114]. Omega-3 fatty acids may improve mental health by influencing nervous system activity, memory, serotonin and dopamine levels, neurotransmission, and the formation of synapses between neurons [115].

It has been shown that individuals with low blood EPA and DHA levels have smaller brains and that the lower brain volume corresponded to two years of structural brain aging in dementia-free study participants [116]. DHA was further associated with better visual memory, executive function, and abstract thinking. Generally, reduced brain volume is linked to cognitive decline and dementia. Already at the age of 30, the brain volume starts to decline due to the normal aging process, but at twice the speed, if mild dementia is present, indicating the need for optimal omega-3 intake throughout life.

Besides their positive effect on heart and brain health, EPA and DHA have been linked to a variety of health concerns of which a selection is listed in Table 3.

<table>
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<tr>
<th>Health area</th>
<th>Benefit</th>
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<tr>
<td><strong>Brain health</strong></td>
<td>DHA is highly concentrated in the brain and is important for cognitive (memory and brain performance) and behavioral function. Effects were reported for: Mood Depression Cognitive function Emotional distress ADHD symptoms Alzheimer disease Memory Age-related cognitive decline Self-harm and suicidal thinking</td>
<td>[117-121]</td>
</tr>
<tr>
<td><strong>Cancer</strong></td>
<td>Mainly in cancer with gastrointestinal origin work omega-3s as an anti-inflammatory agent.</td>
<td>[122,123]</td>
</tr>
<tr>
<td>Health Category</td>
<td>Description</td>
<td>Reference(s)</td>
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<tr>
<td>--------------------------</td>
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</tr>
<tr>
<td><strong>Eye health</strong></td>
<td>Omega-3 supplementation are reported to have a positive effect on macular degeneration (a serious age-related eye condition that can progress to blindness) and dry-eye syndrome.</td>
<td>[124,125]</td>
</tr>
<tr>
<td><strong>Heart health</strong></td>
<td>Reduced risk of heart disease by: ↓ Cholesterol ↓ Triglyceride ↓ Plaque development ↓ Arteriosclerosis ↓ Blood pressure ↑ HDL (“good cholesterol”) ↑ Omega-3 index</td>
<td>[126-129]</td>
</tr>
<tr>
<td><strong>Inflammatory diseases</strong></td>
<td>Reduced inflammatory responses in a range of diseases such as rheumatoid arthritis, inflammatory bowel diseases, systemic lupus erythematosus, and childhood asthma. The effect of omega-3s is due to a reduction in the production of many pro-inflammatory mediators such as: ↓ C-reactive protein ↓ Interleukins ↓ Prostaglandins ↓ Tumor necrosis factor alpha ↑ Omega-3 index</td>
<td>[106, 130-132]</td>
</tr>
<tr>
<td><strong>Metabolic disorders</strong></td>
<td>By reducing triglycerides and inflammatory markers and improving insulin sensitivity, omega-3s can affect non-alcoholic fatty liver disease.</td>
<td>[133]</td>
</tr>
<tr>
<td><strong>Skin health</strong></td>
<td>Maintenance of skin health by increasing hydration, reducing sunburn, aging, and skin cancer via photo-protective effects</td>
<td>[134,135]</td>
</tr>
<tr>
<td><strong>Women health</strong></td>
<td>Reduced menstrual pain</td>
<td>[136]</td>
</tr>
</tbody>
</table>

ADHD, attention deficit hyperactivity disorder; DHA, docosahexaenoic acid; HDL, high-density lipoprotein
Krill Oil: Phospholipids, Choline, And Omega-3 Fatty Acids All In One

**WHY KRILL OIL?**

To fulfill the global demand for omega-3 fatty acids with growing pressure on fish stocks, new sustainable sources are needed. Krill oil is such a source and WWF-Norway clearly says: “Management of krill is sustainable.”

Nina Jensen, CEO of WWF-Norway. “WWF-Norway and Aker BioMarine have worked together since 2008 for a sustainable management of krill resources in the Antarctica. As a result of the partnership and the MSC (Marine Stewardship Council) certification, Aker BioMarine has started an extensive mapping of fish larval by-catch, and is working with WWF-Norway to document the potential overlap/conflict between the fishery and land based predators. WWF-Norway and Aker BioMarine are also working actively towards CCAMLR (Commission for the Conservation of Antarctic Marine Living Resources) on sustainability issues. In addition, Aker BioMarine continues to be the most proactive player in the krill fishery and has implemented voluntary measures. This includes measures such as 100% observer coverage and real-time reporting procedures to ensure the continued sustainability of this fishery. By permitting scientists onboard at no cost, they are also contributing to science and research. The krill fishery has been going on for more than 30 years and the catches have been relatively stable and low. However, WWF-Norway remains concerned that while the precautionary catch limit represents a smaller catch limit than for most fisheries, it may fail to protect krill predators at the local scale. It is therefore important to have industry...
players, like Aker BioMarine, to commit to further research to understand the complex marine ecosystem in the Antarctica."

The Marine Stewardship Council (MSC) is an international nonprofit organization that focuses on the health of ocean stocks and how they are managed, in addition to assessing the effects of fisheries on the wider ecosystem. The Aker BioMarine krill fishery has been evaluated by an independent assessment team, and the evaluators have certified that the fishery is well managed, that the krill stock is healthy, and that Aker BioMarine’s krill fishery is sustainable with minimal impact on the ecosystem.

While krill oil comes from a sustainable source, it has also been found to be pure by extensive analysis for the presence of contaminants, such as dioxins, furans, dioxin-like PCBs (polychlorinated biphenyls), organochlorine pesticides, PBDEs (polybrominated diphenyl ethers), heavy metals, PAHs (polycyclic aromatic hydrocarbons), arsenic species, fluoride, trans fatty acids, and marine algal toxins. The krill’s place at the bottom of the food chain and its clean habitat impede accumulation of these contaminants, which are often found in marine life higher in the food chain.

The quality of krill oil has been further increased by reducing TMA/TMAO and salt contents giving an almost taste- and smell-free krill oil concentrate. Phospholipid, choline, and omega-3 fatty acid concentrations are increased, providing a special combination of these important nutrients in the krill oil concentrate.

**UPTAKE OF KRILL OIL IN THE BODY**

The evidence that phospholipids are a more effective delivery molecule of fatty acids to organs than triglycerides is rising [137-140]. Of particular interest is the accumulation of DHA into the brain, since it makes up about 30-40% of the fatty acids in the gray matter of the cortex and is particularly concentrated in synaptic membranes [141]. Indeed, in preclinical studies, when given single-doses of radiolabeled DHA, the DHA brain accumulation was twice as high, when DHA was supplied in phospholipid rather than triglyceride form [137,138].

**Omega-3** fatty acids from krill oil are efficiently transported and integrated into cell membranes, because they
are bound to phospholipids. As part of cell membranes, EPA and DHA have the ability to influence fluidity of the membranes, signaling processes, and metabolic parameters in the cell.

**KRILL OIL IN HEALTH AND DISEASE**

Animal studies that found benefits for krill oil supplementation include models for ulcerative colitis [142], depression [143], obesity [144-149], myocardial infarction [150], chronic inflammation [151], rheumatoid arthritis [152], and glucose tolerance [153]. Highlights from human studies with krill oil include benefits for a variety of parameters summarized in Table 4.

**OMEGA-3 INDEX**

The Omega-3 Index that has been proposed as a novel biomarker for cardiovascular risk and is defined as the percentage of EPA and DHA in red blood cell (RBC) fatty acids [161].

Steady-state, but also increased levels of the Omega-3 Index after supplementation have been shown to directly correlate with EPA and DHA levels in human cardiac tissue [132,162,163]. In contrast to plasma fatty acid measurements that reveal short-term omega-3 intake [164], the Omega-3 Index is believed to mirror overall tissue EPA and DHA levels and therefore a person's health status. An Omega-3 Index of 8% or above is considered optimal [165], while a low Omega-3 Index indicates a higher risk of sudden cardiac death.

**TARGET ZONES FOR THE OMEGA-3 INDEX**

<table>
<thead>
<tr>
<th>Bad</th>
<th>Average</th>
<th>Target Zone</th>
</tr>
</thead>
<tbody>
<tr>
<td>0%</td>
<td>4%</td>
<td>8%</td>
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Since increases in EPA and DHA levels correlate with sudden cardiac death [166], researchers have recently turned their investigations toward krill oil to see if it can increase omega-3 RBC levels. In an unpublished clinical
<table>
<thead>
<tr>
<th>Parameters</th>
<th>Explanation</th>
<th>Effects of krill oil</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ADHD symptoms</strong></td>
<td>Attention deficit hyperactivity disorder; a neurodevelopmental psychiatric disorder characterized by attention deficits, hyperactivity, and impulsiveness.</td>
<td>↑ Improvement in behavior and daily function capacity in children</td>
<td>Unpublished data</td>
</tr>
<tr>
<td><strong>Cachexia</strong></td>
<td>Loss of body mass that cannot be reversed nutritionally (cancer patients) and is characterized by anorexia, muscle wasting, and lipid profile disorder.</td>
<td>↑ Improved blood lipids in advanced cancer patients with cachexia (TG reduced, HDL increased, LDL reduced). Endocannabinoid system parameters normalized and inflammation markers reduced</td>
<td>Unpublished data</td>
</tr>
<tr>
<td><strong>CRP</strong></td>
<td>C-reactive protein; CRP blood levels rise in response to inflammation.</td>
<td>↓ Reduced CRP, inflammation, and arthritic symptoms (WOMAC scores)</td>
<td>[155]</td>
</tr>
<tr>
<td><strong>Endocannabinoids</strong></td>
<td>Lipid signaling molecules made from omega-6 fatty acids involved in a variety of physiological processes including appetite, pain-sensation, mood, and memory. Obese persons have an overactive endocannabinoid system.</td>
<td>↓ Reduced endocannabinoids in obese persons</td>
<td>[156]</td>
</tr>
<tr>
<td><strong>HDL</strong></td>
<td>High-density lipoprotein; the “good” cholesterol, since it removes excess cholesterol from the blood and brings it to the liver for other uses.</td>
<td>↑ Increased HDL (&gt; 50%) in subjects with elevated blood fat levels. HDL/TG ratio improvement in healthy subjects</td>
<td>[157,158]</td>
</tr>
<tr>
<td><strong>Immune function</strong></td>
<td>Prolonged training periods may cause a down-regulation of the immune system by decreasing natural killer (NK) cells (first line of defense to e.g. bacteria and viruses) and interleukin-2 (IL-2), a signaling molecule in the immune response.</td>
<td>↑ Increased immune function after intense exercise via higher NK cell and IL-2 levels  ↓ Reduced oxidative damage to red blood cells during recovery from rowing in national team rowers</td>
<td>[22,23]</td>
</tr>
<tr>
<td><strong>Inflammation</strong></td>
<td>Arthritis is an autoimmune disease characterized by inflammation, joint pain, and swollen joints.</td>
<td>↓ Reduced inflammation in subjects with arthritis symptoms</td>
<td>[155]</td>
</tr>
<tr>
<td><strong>LDL</strong></td>
<td>Low-density lipoprotein; LDL is often called the “bad cholesterol”, since it picks up cholesterol and fat from the liver and delivers it to artery walls causing atherosclerosis (hardening of the arteries), thereby increasing the risk for heart disease.</td>
<td>↓ Decrease or no change of LDL</td>
<td>[157,158]</td>
</tr>
<tr>
<td><strong>Omega-3 index</strong></td>
<td>Percent EPA and DHA in red blood cells. Correlates with amount of EPA and DHA in other tissues. Low Omega-3 index is linked to higher risk of heart disease.</td>
<td>↑ Significant increase of Omega-3 index</td>
<td>[127,159]</td>
</tr>
<tr>
<td><strong>PMS symptoms</strong></td>
<td>Premenstrual syndrome; PMS syndrome might have an inflammatory connection.</td>
<td>↓ Significant reduction in physical and emotional symptoms related to PMS measured as breast tenderness, stress, irritability, depression, joint pain, bloating, swelling, abdominal pain, and weight gain.</td>
<td>↓ Significant reduction in amount of painkillers used for painful cramps</td>
</tr>
<tr>
<td><strong>Triglyceride</strong></td>
<td>A lipid molecule consisting of glycerol with three fatty acids bound to it. The fatty acids can be saturated or unsaturated.</td>
<td>↓ Significant reduction of triglycerides in blood</td>
<td>[157-159]</td>
</tr>
</tbody>
</table>

ADHD, attention deficit hyperactivity disorder; CRP, C-reactive protein; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; HDL, high-density lipoprotein; IL-2, interleukin-2; LDL, low-density lipoprotein; NK, natural killer; PMS, premenstrual syndrome; TG, triglyceride; WOMAC, Western Ontario and McMaster Universities Arthritis Index
study, healthy volunteers took either 2 grams of Superba™ krill oil for 8 weeks or 2 grams of an omega-3 enriched fish oil. The goal of the study was to compare the delivery of omega-3 fatty acids, PLs versus TGs, to see if the molecular form influences the increase in Omega-3 Index. The results of the study showed that krill oil increased the Omega-3 Index significantly more than fish oil after 8 weeks of supplementation. In fact, krill oil increased the Omega-3 Index about 70% more than fish oil at the end of study after dose adjustment between the two treatment groups.

These results go hand in hand with another study, which investigated the effect of 12 weeks daily Superba™ krill oil intake in volunteers with “borderline high” or “high” blood triglyceride levels [159]. Triglycerides have an important role in lipid metabolism and are a central biomarker of heart disease risk [167].

In this study, a total of 300 volunteers were divided into five groups and supplemented with krill oil at either 0.5, 1, 2 or 4 grams per day or placebo (olive oil). The individuals included in the study had blood triglyceride values between 150 and 499 mg/dL. Blood lipids were measured at baseline, 6 weeks and 12 weeks of treatment.

Relative to subjects in the placebo group, those administered krill oil had a statistically significant 10% reduction in serum triglycerides. Moreover, LDL cholesterol levels were not increased in the krill oil groups relative to the placebo group, an important finding considering an increase in LDL cholesterol has been observed in some fish oil trials [159]. Additionally, the subjects taking 4 grams of krill oil per day raised their Omega-3 Index from 3.7%
to 6.3%. Comparable increases in the Omega-3 Index have been linked to decreased risk for sudden cardiac death in previous studies – in a prospective cohort study by about 80% [168] and in a case control study by 90% [169].

Thus, there seem to be advantages of krill oil, when it comes to raising a person’s Omega-3 Index. Most importantly, the mentioned studies show that krill oil more effectively raises the Omega-3 Index compared to fish oil, even though krill oil delivers lower amounts of EPA and DHA on a gram per gram basis compared to fish oil. Clear health benefits, in particular for heart health, have been shown by raising one’s Omega-3 Index higher than 8%.

CONCLUSION

While Superba™ krill oil has demonstrated its health benefits in numerous studies, the new krill oil concentrate holds even more promise in particular in relation to health concerns that are characterized by phospholipid and/or choline deficiency. Reduced phospholipid and choline levels are seen in e.g. liver and brain disorders, heart disease, intestinal inflammation, lung and skin diseases, or after intense exercise.

Krill oil supplementation can help to heal membranes and to optimize omega-3 fatty acid, phospholipid, and choline status in the body. The all-in-one advantage of krill oil delivering simultaneously omega-3 fatty acids, phospholipids, and choline gives it a unique standpoint in the omega-3 market. However, the full elucidation of how much each component benefits human health must await further study.

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