Confidential to New Zealand King Salmon

Health benefits of salmon and omega 3 oil supplementation

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April 2008

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Health benefits of salmon and omega 3 fats

Several studies have demonstrated beneficial effects of n-3 LCPUFA consumption for the prevention or treatment of various diseases. However, in most cases, there are also a number of studies which have shown no beneficial effect of n-3 LCPUFA consumption on disease risk or progression. Although some of the discrepancy in results is likely to be due to differences in the experimental design and methods used in different studies, it is becoming increasingly more apparent that the genetic make-up and physiological wellbeing of individuals in the study population also has a strong influence on study outcome.

Better understanding of the mechanism of action of n-3 LCPUFAs in biological systems will likely aid in determining the type of individual most likely to benefit from n-3 LCPUFAs. In this review, several new studies have focussed on determining the mechanism of action of LCPUFAs whilst others have begun to determine the effects of LCPUFAs in particular target populations.

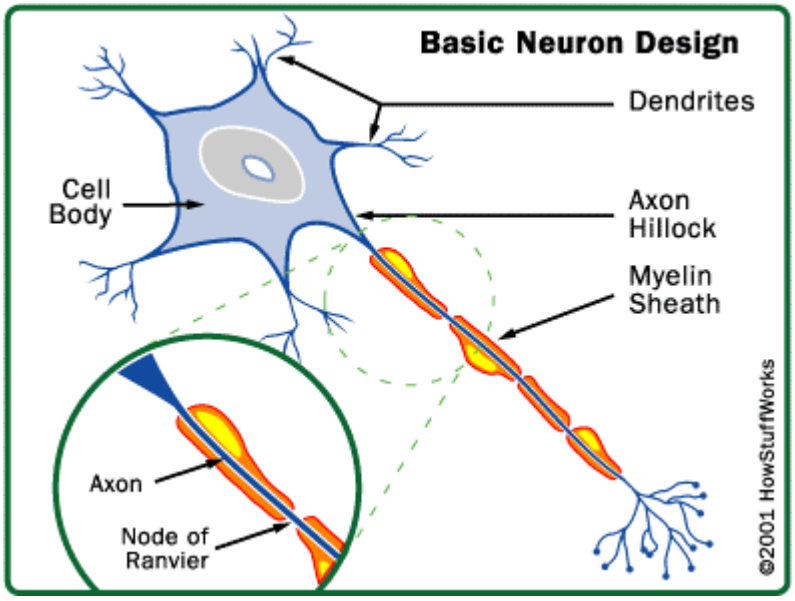
Brain Function, Brain Disease and Mental Health

n-3 LCPUFAs have an important role in brain development and function. Although it has been known for some time that the brain contains large amounts of n-3 LCPUFAs, how the LCPUFAs in the brain influence brain function is less clear. Several recent studies have examined the effect of LCPUFAs on brain function.

Multiple Sclerosis

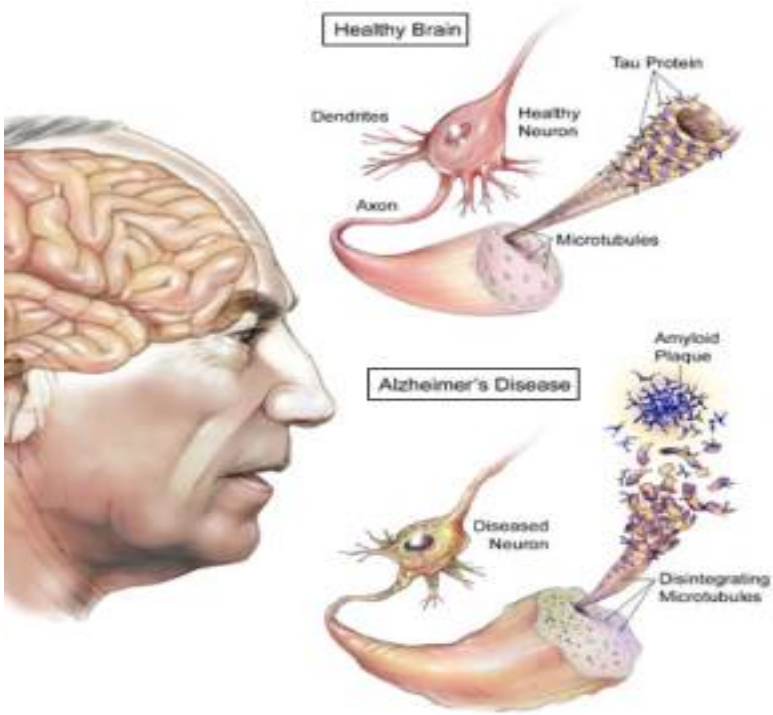
Neurons are responsible for relaying signals from the brain to other parts of the body. The axon of a neuron is surrounded by a sheath known as myelin. Myelin is composed of 80% lipids and 20% proteins and is important for signal transduction. Degradation of the myelin sheath (demyelination) is a major factor causing the symptoms of multiple sclerosis (MS). Recently, scientists in Italy investigated the effect of EPA and DHA on the expression of several myelin proteins. A single dose of EPA or DHA was injected directly into the cerebral cortex of two-day old rats. Rats were euthanised three days later and the levels of RNA for specific myelin proteins were measured in three areas of the brain, the cortex, cerebellum and medulla. Both

EPA and DHA increased expression of RNA for myelin proteins but EPA had a stronger effect than DHA (Salvati *et al.* 2008). Although much more research is required, results from this study raise the possibility that EPA or DHA treatment may aid in preventing demyelination or in the repair of a damaged myelin sheath.



Alzheimer's Disease

Alzheimer's Disease is characterised by the formation of plaques within brain tissue. These plaques are made from the protein β-amyloid which is believed to be toxic to neurons. Neuron destruction is believed to be the primary cause of the symptoms of Alzheimer's disease. LR11 is a brain protein which prevents the formation of these plaques. In late-onset Alzheimer's patients, LR11 is present in very low amounts. Investigators have found that treatment of cultured rat and human neuronal cells results in increased LR11 levels. Similarly feeding mice and rats with DHA has also been shown to increase LR11 in the animals' brain (Ma *et al.* 2007). The US NIH is currently carrying out a clinical study to investigate the effect of DHA supplementation on LR11 protein levels in patients with established Alzheimer's Disease.



Another group of researchers found feeding DHA to rats or gerbils resulted in increased levels of phosphatides and pre-synaptic and post-synaptic proteins in brain cells as well as increased numbers of dendritic spines and post-synaptic neurons (Cansev *et al.* 2008). "Synapse" is the sending of a signal from one neuron to another. Phosphatides are important components of the membranes at which synapse occurs and pre- and post-synaptic proteins are involved in the sending and receipt of the signal between neurons. Dendritic spines are formed once synapse has occurred and may be important for memory. Alzheimer's patients have smaller and fewer synapses, reduced levels of membrane phosphatides and reduced levels of synaptic proteins. It is possible that DHA treatment may aid in combating synaptic loss associated with Alzheimer's and other neurodegenerative diseases as well as neural damage which occurs as a result of stroke or trauma.

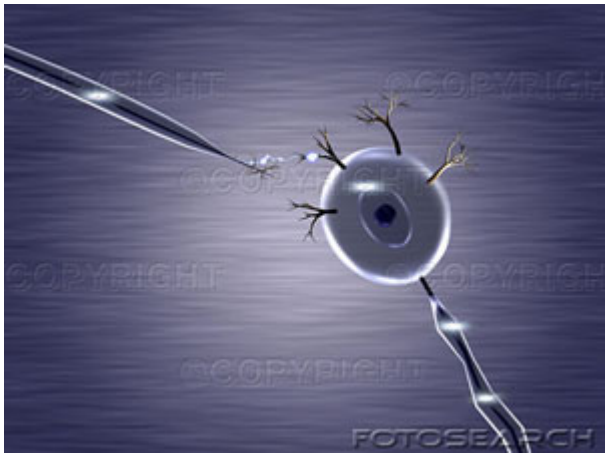


Illustration of synapse occurring between dendrites on one neuron and the axon of another neuron.

However, in a recent study in a transgenic mouse model of Alzheimer's disease, consumption of a high fish oil diet (4% menhaden oil) for 5½-7 months had no effect on cognitive function (Arendash *et al.* 2007). Although there is promising data to suggest a role for n-3 LCPUFAs in combating the neurodegeneration associated with Alzheimer's and other similar diseases, due to the complexity of the disease process as well as limitations associated with the use of model systems to study the disease, there is still much work to be done to determine which LCPUFAs are effective, in what dose and what the extent of their effects is.

Epilepsy

Detrimental changes in the integrity of the brain cell membranes as well as in the ability of brain cells to utilise energy may occur in diseases such as epilepsy. In a recent pilot study, epileptic patients were supplemented with either placebo or high doses of EPA and DHA (1g EPA and 0.8g DHA per day) for 12 weeks. Using ^{31}Phosphous neurospectroscopy, the composition of phosphorylated compounds in the patients' brains was determined. The relative amount of the different types of phosphorylated compounds in brain tissue provides information on the biochemical activity occurring within the brain. Significant effects of EPA/DHA supplementation were observed on the levels of two phosphorylated compounds: PDE (phosphodiesters), γ-NTP (gamma nucleotide triphosphate) as well as in the amount of BBC (broadband component) resonance. PDE is an index of membrane phospholipids catabolism. Levels of PDE were found to be significantly lower in

patients receiving the EPA/DHA supplement than in patients receiving the placebo suggesting the n-3 LCPUFAs may protect phospholipids membranes from degradation. BBC is an indicator of the level of phospholipids incorporated within membranes of brain cells. The level of BBC was significantly higher in LCPUFA-treated patients compared to placebo-controls suggesting LCPUFAs may increase the incorporation of phospholipids within cell membranes. Finally, levels of γ-NTP were significantly higher in EPA/DHA supplemented patients than controls indicating improved brain energy metabolism in supplemented patients (Puri *et al.* 2007). Although this was a very small study involving just seven patients (3 receiving EPA/DHA supplementation and 4 receiving placebo), that significant differences between treated and untreated patients were observed in three of the parameters measured suggests EPA/DHA treatment has a major effect on these parameters. How EPA and DHA increase brain phospholipid incorporation, decrease phospholipid degradation and improve brain energy metabolism and the consequences of this for epilepsy sufferers will no doubt be a focus for further research.

Stroke

Studies examining the effect of fish consumption on risk of stroke have yielded mixed results. In a recent study, researchers from Sweden used data collected as part of a community intervention programme on cardiovascular disease and diabetes to determine if risk of stroke in relation to fish intake differs between men and women and/or with different types of stroke. One of the factors measured in the community intervention study was the amount of lean and fatty fish consumed by study participants using a food frequency questionnaire (FFQ). Plasma phospholipid or erythrocyte membrane fatty acid composition and the total amount of mercury in erythrocytes were also measured in all study participants. Fish intake, blood fatty acid composition and erythrocyte mercury levels were obtained from 74,000 people living in North Sweden. Using this database, researchers identified 388 of the participants in the original intervention study who subsequently suffered a first stroke. For each stroke patient, two age and sex-matched controls were chosen at random from the original community intervention programme participants. The data was then analysed to determine if there were any statistically significant associations between risk of stroke and number of fish meals consumed/week, blood EPA & DHA content or levels of mercury in erythrocytes. Surprisingly the study reported a negative

association between fish intake and risk of stroke in men which may suggest that fish consumption increases the risk of stroke in men. No significant association between blood EPA and DHA levels or erythrocyte mercury levels and risk of stroke were evident. It is therefore unclear whether the apparent increased risk of stroke in men consuming more fish meals per week observed in this study was simply due to chance or whether some component of fish other than EPA and DHA or a toxin (other than mercury) which accumulates in fish increases susceptibility to stroke. Interestingly in women, there was some evidence of a decreased risk of stroke with increased fish consumption however the association failed to reach statistical significance. This may suggest that fish consumption is more beneficial in women than men for reducing stroke risk however more research is required in order to verify this finding. Due to the relatively few number of study participants who suffered a haemorrhagic stroke, whether fish consumption had a different effect on risk of ischaemic stroke compared to risk of haemorrhagic stroke could not be determined in this study (Wennberg *et al.* 2007).

Cognitive Function

For some time it has been suggested that increased n-3 LCPUFA intake may aid in reducing age-related cognitive decline. Although some studies have demonstrated a beneficial effect of n-3 LCPUFA intake on cognitive ability, others have not. This is the case with many fields of nutritional research. One reason for the apparent conflict in results obtained from studies is that certain nutrients may have certain effects in some individuals eg those with a certain genetic profile or with a certain disease but have a different effect in other individuals. There is some evidence that n-3 LCPUFAs are more beneficial in reducing cognitive decline in hypertensive patients than in normotensive individuals. In a large-scale epidemiological study in 10,000 US men and women with hypertension, a positive association between n-3 LCPUFA intake and reduced risk of 6-year cognitive decline in verbal fluency was reported (Beydoun *et al.* 2008).

Depression

Although many studies have now been reported which have examined the effect of n-3 LCPUFA intake on depression, there remains a lack of consensus as to the efficacy of LCPUFAs as potential means of reducing the severity or preventing the onset of

depression. No effect of 3 months of high dose EPA and DHA supplementation (1.5g/day) on mood or cognitive function was observed in a recent study in 190 patients with mild to moderate depression (Rogers *et al.* 2008). However in another study in 60 patients with major depressive disorder, treatment with 1g of EPA per day for 8 weeks resulted in significant improvement in the severity of depression. Combined treatment with EPA (1g/day) and fluoxetine, a pharmaceutical anti-depressant, had greater therapeutic effects in alleviating depressive symptoms than either treatment alone (Jazayeri *et al.* 2008).

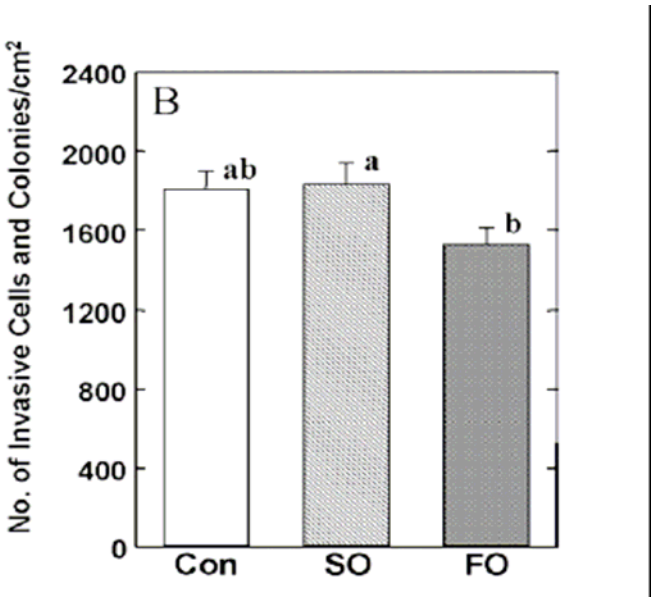
Cancer

Prostate cancer is one of the most common types of cancer particularly in Western countries. Swedish researchers recently published the results of a large-scale prospective study which aimed to determine the relationship between type and amount of dietary fat and risk of prostate cancer. Habitual fat intake was estimated based on data obtained from a 7-day food record as well as a questionnaire which assessed usual meal pattern, consumption frequency and portion size in 10,564 cancer-free men aged 45-73 years old. Study participants who subsequently developed prostate cancer were determined by monitoring Swedish National Cancer Registers. Statistical analyses were then used to determine if any correlation between dietary fat consumption and incidence of prostate cancer existed. Unexpectedly, increased consumption of EPA and DHA was associated with an increased risk of developing prostate cancer (age-adjusted relative risk for increasing quintiles of DHA intake was 1.00 ,1.26, 1.29, 1.32, 1.35 and for EPA intake 1.00, 1.09, 1.33, 1.09 and 1.33) (Wallstrom *et al.* 2007).

Nutrition at the time of surgery is known to influence surgical outcome. Takeuchi et al (2007) sought to determine whether intake of fish oil had beneficial effects on recovery from surgery in cancer patients studied. Following esophagectomy, patients are fed an enteral diet through a tube directly into the jejunum (jejunostomy). Forty patients who underwent surgery for esophageal cancer were studied. Twenty patients served as controls and received a normal enteral diet post-surgery (Group A). Six other patients received the normal enteral diet supplemented with arginine, omega-3

fatty acids and RNA, all substrates believed to have immune-enhancing effects, after surgery (Group B). Another 14 patients also received the arginine, omega-3 and RNA-supplemented enteral diet but were fed this diet both before and after surgery (Group C). The incidence of incisional wound infection and the duration of post-surgery systemic inflammation were significantly lower in patients in Group C compared to patients in Group A suggesting beneficial effects of arginine, omega-3 fats and/or RNA on surgery outcome (Takeuchi *et al.* 2007).

A second group of researchers investigated whether fish oil could reduce cancerous cell invasion. Rat ascites hepatoma cells (AH109A) were cultured with a fish oil-based or a safflower oil-based emulsion for 48 hours. The ability of the cancerous hepatoma cells to invade healthy tissues was assessed by co-culturing the hepatoma cells with a confluent culture of mesentery-derived mesothelial cells for 24 hours. Using phase-contrast microscopy, the number of hepatoma cells and colonies which had penetrated and were growing in the mesothelial cell monolayer were counted. Hepatoma cells pre-treated with fish oil emulsion were significantly less invasive than those pre-treated with safflower oil. In order to confirm the study findings under more physiologically relevant condition, the investigators then collected sera from rats fed a fish oil or safflower oil supplemented diet. Hepatoma cells were cultured in the rat sera for 48 hours before assessing cell invasiveness by co-incubating the hepatoma cells with the mesothelial cells. Results of this second experiment complemented those of the first in that hepatoma cells pre-treated with sera from rats fed a fish oil-supplemented diet were less invasive than hepatoma cells pre-treated with sera from rats fed a safflower oil-supplemented diet (Hagi *et al.* 2007).



Effects of pre-treatment of hepatoma cells with sera from rats consuming either a control diet deficient in essential fatty acids (CON) or a diet supplemented with either safflower oil (SO) or fish oil (FO) on hepatoma cell invasiveness (Hagi et al. 2007)

Dysfunctional interactions between tumour cells and normal healthy cells of the body result in a range of pathologies including fatigue, weakness, cachexia, anorexia, early satiety and chronic nausea. Collectively these pathologies are known as SIMS (systemic immune-metabolic syndrome). Reducing or eliminating SIMS is not only important for maintaining the quality of life of cancer patients but may also aid in improving the prognosis of cancer outcome. SIMS may be a side effect of the chronic systemic inflammation associated with cancer. As n-3 LCPUFAs are known to have anti-inflammatory activity, a group of researchers investigated whether inclusion of n-3 LCPUFAs in the diet of cancer patients alleviated some of the symptoms of SIMS. Twenty-two patients with advanced stage lung cancer and SIMS were supplemented with either fish oil capsules (2g per day) alone or fish oil capsules (2g per day) with the cyclooxygenase-2 (cox-2) inhibitor celecoxib (200mg) for 6 weeks. Compared to baseline, appetite was significantly improved, fatigue significantly reduced and circulating levels of the inflammatory marker C-reactive protein (CRP) were significantly reduced in all patients receiving the fish oil supplement. Body weight and muscle strength were significantly improved in patients receiving fish oil + celecoxib compared to baseline and were significantly higher than in patients receiving fish oil supplementation alone (Cerchietti *et al.* 2007). This study

demonstrates that fish oil improves symptoms of SIMS possibly by reducing inflammation. Specific inhibition of cox-2, the cyclooxygenase responsible for synthesis of pro-inflammatory eicosanoids from n-6 LCPUFAs, in conjunction with fish oil supplementation further reduces symptoms of SIMS in cancer patients.

Bone and Joint Health

Osteoarthritis is an inflammatory disease of the joints. The pain and loss of joint mobility characteristic of arthritis is largely a result of destruction of cartilage within the joint. Matrix metalloproteinases (MMPs) are enzymes responsible for the degradation of the cartilage tissue matrix. Normally MMPs remove fatigued or damaged cartilage as part of the self-repair process which occurs in all tissues. Increased expression or activity of MMPs or loss of the normal inhibitors of MMPs results in an inappropriate increase in tissue breakdown. Dogs are susceptible to cruciate ligament injury which can lead to osteoarthritis. A recent study in dogs with unilateral CCL (cruciate ligament injury) found significant reductions in expression of various MMPs in dogs receiving an n-3 LCPUFA-rich diet. Two groups of 12 dogs were fed either a control diet or a diet providing 90mg EPA and 4.5mg DHA per kg body weight per day for 8 weeks. The concentration of MMP-2 and MMP-9 in the synovial fluid of n-3 LCPUFA-fed animals was significantly lower than in control animals at certain timepoints (Hansen *et al.* 2008). Beneficial effects of n-3 LCPUFAs in reducing the symptoms associated with osteoarthritis have previously been reported in some (but not all) clinical studies. Results from the study by Hansen *et al*. (2008) may help to explain the mechanism by which n-3 LCPUFAs work.

Three recent studies have provided further insight into the role of n-3 LCPUFAs in maintaining bone mineral density. In one study, the effects of high purity ethyl esters of individual LCPUFAs on bone mass post-ovariectomy in rats were compared. Sixty-eight rats were either ovariectomised (to simulate the conditions leading to post-menopausal bone loss) (n=58) or sham-operated (n=10). Ovariectomised rats were fed a control diet (n=10) or a diet supplemented with either gamma-linolenic acid (GLA, n=12), EPA (n=12), DHA (n=12) or a mixture of all 3 LCPUFAs (n=12) for 16 weeks. Bone mineral content (BMC) and density (BMD) were measured at the lumbar

spine and femur by DEXA (dual energy x-ray absorptiometry). Over the study period, BMC decreased in ovariectomised rats fed the control diet but stayed relatively unchanged in sham-operated animals also fed the control diet. EPA had no significant effect on BMC loss however GLA appeared to exacerbate the loss of bone mineral as the decrease in femur BMC in GLA-fed animals was significantly greater than that in ovariectomised controls. DHA protected against bone loss and final BMC in the lumbar spine in DHA fed rats was significantly higher than in ovariectomised controls and not significantly different from sham-operated animals (Poulsen *et al.* 2007). This study demonstrates that different LCPUFAs have different effects in bone. Bone is continually broken down and replaced. Usually in adults bone resorption is completely balanced by bone formation and net bone mass remains unchanged. However, menopause leads to conditions which favour bone resorption over formation. Bone mass can be preserved post-menopause either by inhibiting bone resorption or by promoting bone formation. The effect of LCPUFAs on osteoclast activity (the cells responsible for bone resorption) and osteoclastogenesis have recently been investigated.

One of the signalling pathways responsible for promoting osteoclastogenesis is the RANKL pathway. This pathway involves three proteins: two receptors (RANK and OPG) and a ligand (RANKL). RANK is present on the outer membrane of osteoclast precursors. When it binds to RANKL which is mainly present on the outer membrane of mature osteoblasts (the cells responsible for bone formation), maturation of the osteoclast precursors into fully-functional osteoclasts is initiated. OPG is secreted by osteoblasts and will also bind to RANK. Binding of RANK to OPG however does not trigger osteoclast maturation and hence is known as a decoy receptor. Osteoclastogenesis can be inhibited by reducing synthesis of RANKL and/or by increasing synthesis of OPG. Inflammatory eicosanoids such as prostaglandin E2 (PGE2) synthesised from n-6 LCPUFAs promote RANKL expression and therefore promote osteoclastogenesis. The effects of DHA and EPA on the expression of RANKL and OPG in the MC3T3-E1/4 osteoblast cell line were recently studied. EPA and DHA were equally effective in inhibiting the PGE2-induced increase in RANKL expression in MC3T3-E1/4 cells. Neither had any effect on OPG secretion or on RANKL expression in cells not treated with PGE2 (Poulsen *et al.* 2008). Results of

this study suggest that EPA and DHA may have a role in inhibiting the increase in bone resorption which occurs under inflammatory conditions.

Once RANKL binds to RANK a signalling cascade is initiated which ultimately leads to osteoclastogenesis. As well as influencing RANKL expression, EPA and DHA also appear to influence factors downstream in this signalling cascade. In RAW 264.7 cells (a cell line capable of differentiating into mature osteoclasts), DHA and to a lesser extent EPA, were found to reduce the number of mature osteoclasts formed after treatment of RAW cells with RANKL (Rahman *et al.* 2008).

Results of these three studies suggest that DHA may be more effective than EPA in maintaining bone mass post-ovariectomy due at least in part to its ability to inhibit RANKL-mediated osteoclastogenesis. Whether DHA is also effective in helping to minimise bone loss in post-menopausal women remains to be investigated.

Inflammation occurs as a natural and necessary response to injury or infection. It is an important part of the process which allows infection to be repelled and damaged tissue to be repaired. However many of the pro-inflammatory eicosanoids and cytokines released in response to injury or infection also stimulate osteoclastogenesis and as a result, promote bone resorption. Substantial bone loss can occur in patients suffering from a chronic inflammatory disease. Bone loss also occurs in patients with gum disease such as the bacterial disease, periodontitis. Prostaglandin E2 (PGE2) and leukotriene B4 (LtB4) are pro-inflammatory eicosanoids produced from arachidonic acid which are known to stimulate bone resorption. In contrast, resolvin E1 (RvE1) is an anti-inflammatory/pro-resolving eicosanoid produced from EPA. Only one previous study has examined the effect of RvE1 on bone. Results from this study suggested RvE1 also inhibited osteoclast-mediated bone resorption. In a recent study published in the Journal of Immunology, Hasturk *et al.* (2007) compared the effects of topically-applied resolvin E1 (RvE1), prostaglandin E2 (PGE2) and leukotriene B4 (LtB4) on inflammation as well as on soft and bony tissue destruction in animals with periodontal disease (Hasturk *et al.* 2007). Periodontitis was induced in New Zealand white rabbits by passing silk ligatures coated with *Porphyromonas gingivalis,* the causative bacterium of periodontitis around the second premolar three times a week for 6 weeks. Following this 6 week period, periodontitis was well established in the

animals. Soft tissue destruction had occurred as well as significant bone loss (approximately 30% localised bone loss compared to baseline). Animals were then randomised into four groups and treated topically with PGE2 (n=5), LtB4 (n=5), RvE1 (n=14) or ethanol (n=10) for 6 weeks. (As RvE1, LtB4 and PGE2 were dissolved in ethanol to allow topical application, the group treated with ethanol alone served as the control group.) In the control, PGE2-treated and LtB4-treated animals, further bone loss had occurred over the 6-week treatment period and approximately 50% of bone in the infected area was destroyed. In contrast, in the animals treated with RvE1, both soft and bony tissue were completely regenerated over the 6-week treatment period (Hasturk *et al.* 2007). Although this is only the second study to explore the effects of RvE1 on bone, results suggest RvE1 has a major role in promoting tissue regeneration.

Metabolic Syndrome and Liver Disease

"Metabolic syndrome" is a term used to describe a collection of medical disorders which increase the risk of an individual developing heart disease and diabetes. The disorders which comprise the metabolic syndrome include fasting hyperglycaemia (high blood glucose level), central adiposity (excess abdominal fat), high blood pressure, high blood triglyceride levels and low blood HDL cholesterol levels. Accumulation of lipids in the liver ("fatty liver"), is associated with all aspects of the metabolic syndrome. There is some evidence that fat deposition in the liver may be the initiating factor for the metabolic syndrome. Fatty liver disease progresses from steatosis (where fat has accumulated in the liver but there is no associated inflammation) to steatohepatitis (fatty liver with associated inflammation). Steatohepatitis is a serious condition and can result in cirrhosis and permanent liver damage or failure. In a cross-sectional study, investigators from Canada reported patients with non-alcoholic steatohepatitis had lower levels of n-3 and n-6 LCPUFAs in the liver than those with first stage steatosis (Allard *et al.* 2008). The researchers suggested that the lower LCPUFA content in liver fats in patients with steatohepatitis may result in increased oxidative stress and hence inflammation. In a recent review, Mensink et al (2008) summarised results of published studies which have investigated the effect of fish oil intake on lipid accumulation in the liver. Although there is some

evidence that increased fish oil intake is beneficial in the treatment of non-alcoholic steatohepatitis, only a limited number of studies have been published in this field making it difficult to draw definitive conclusions (Mensink *et al.* 2008).

Chronic inflammation of the liver leads to death of liver cells and the development of scar tissue within the liver. This is known as liver cirrhosis and results in decreased liver function. Liver cirrhosis is a serious condition and is the seventh leading cause of death in the US. One of the consequences of cirrhotic liver disease is a reduction in plasma levels of AA and DHA. It is suspected that this relative deficiency in AA and DHA in cirrhotic patients has adverse effects. In a double-blind, placebo-control trial, 18 cirrhotic patients awaiting liver transplants were divided into 3 groups and supplemented with either placebo (n=9) or capsules providing 500mg AA and 1000mg DHA (n=5) or 250mg linolenic acid and 125mg oleic acid (n=4) for 6 weeks. Patients receiving the AA/DHA supplement had improved plasma levels of both AA and DHA (Pazirandeh *et al.* 2007). Results from this study indicate that dietary manipulation of fatty acid intake can modify plasma fatty acid profile in patients with serious cirrhotic liver disease. More research is required to determine whether rectifying the apparent plasma deficiency in AA and DHA in cirrhotic patients has a beneficial effect on clinical outcome.

Diabetes

In a collaborative study between researchers at Massey University, NZ and the University of Newcastle, Australia, the effects of consumption of a dip fortified with microencapsulated fish oil (to eliminate a fishy odour or flavour in the dip) on plasma lipid profile in patients with type II diabetes was determined. Thirteen diabetics consumed the fish oil-supplemented dip for 6 weeks. Plasma content of n-3 LCPUFAs was significantly increased in all study participants at the end of the trial period. Compared to baseline, plasma triglyceride level was significantly lower and plasma LDL and HDL cholesterol levels significantly higher following the intervention period (Garg *et al.* 2007). This study demonstrates that fish oil can significantly improve plasma lipid profile in a relatively short period of time. This may aid in reducing the risk of cardiovascular disease in diabetic patients.

Infant Growth & Development

In a recent study in mice, the LCPUFA content of the maternal diet was found to influence maternal behaviour, litter size, health of pups and the sex of pups. Female mice were fed either a control diet or a diet rich in n-3 or n-6 LCPUFAs from 4 weeks of age. At approximately 19 weeks of age, females were mated. Health of the pups was monitored in terms of body weight gain. Once pups were weaned, the behaviour of the dams was assessed using elevated-plus and open-field mazes. Animals that chose to spend more time in the closed arm of the maze were described as having greater anxiety levels than those who chose to spend more time in the open arm of the maze. Animals were mated again at approximately 27 weeks of age and the pups from the second litter were examined and the anxiety levels of the dams again assessed. Mice consuming the n-6 LCPUFA diet produced more daughters than those consuming the n-3 LCPUFA diet perhaps suggesting that, at least in mice, LCPUFAs have a role in sex determination of offspring. Dams consuming the n-3 LCPUFA diet had higher anxiety levels and produced fewer pups. Pups of n-3 LCPUFA-fed dams gained less body weight than pups of n-6 LCPUFA-fed dams which may suggest pups of n-3-fed dams were less healthy (Fountain *et al.* 2008). However, in humans n-3 LCPUFA supplementation during pregnancy may have beneficial effects on infant growth and development. In a randomised controlled double-blind study, 98 pregnant Australian women were supplemented with fish oil capsules (providing 2.2g DHA and 1.1g EPA per day) from 20 weeks' gestation to delivery. Infant growth and development was assessed at two and a half years of age. Infant growth was not affected by maternal fish oil supplementation during pregnancy. However, children of mothers who received the fish oil supplement during pregnancy had greater hand-eye coordination at two and a half years of age than children of un-supplemented mothers. The researchers concluded that fish oil supplementation during pregnancy is safe for the foetus and may have beneficial effects on infant development (Dunstan *et al.* 2008). The World Association of Perinatal Medicine, the Early Nutrition Academy and the Child Health Foundation have recently published consensus recommendations in which they endorse the consumption of n-3 LCPUFA-rich oils in pregnancy. According to the guidelines established, pregnant and lactating women should consume at least 200mg DHA per day (Koletzko *et al.* 2008).

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