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Nutritional Aspects of Single Cell Oils: Uses and Applications of Arachidonic Acid and Docosahexaenoic Acid Oils

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Introduction

One of the driving forces for the development of single cell oil (SCO) containing long-chain polyunsaturated fatty acids (LCPUFA) was the presence in human milk of two particular LCPUFA, docosahexaenoic acid (DHA) and arachidonic acid (AA). Until recently these polyunsaturated fatty acids (PUFA) have not been added to infant formulas. Once it was recognized that these two PUFA played an important role in the brain, attempts were made to provide these PUFA naturally from fish oils and egg phospholipids. It was relatively easy to obtain DHA from oils such as tuna oil (1); providing AA was more difficult. When it was found that AA-containing oils were produced by certain species of soil fungi (2), research soon established that it was possible to harvest this oil in commercial quantities. Similarly, a DHA-containing oil from a marine microalga was used to produce commercial quantities of DHA (3).

Since the brain is rich in LCPUFA, it is important to understand the role of these fatty acids (FA) in brain function. The brain has the second highest concentration of lipids in the body, after adipose tissue, with 36–60% of the nervous tissue being lipids (4). The lipids in the brain are complex lipids and include glycerophospholipids (GPL), sphingolipids (sphingomyelin and cerebrosides), gangliosides, and cholesterol with little or no triglycerides and cholesterol esters (5). Brain GPL contain a high proportions of LCPUFA, mainly DHA, AA, and docosatetraenoic acid (C22:4n-6), with very small amounts of \(\alpha\)-linolenic acid (ALA) and linoleic acid (LA). The proportion of DHA and AA in the GPL of brain gray matter is higher than the white matter (6,7), with phosphatidylethanolamine (PE) and phosphatidylserine (PS) containing the most DHA of all the GPL, while PE and PI contain the highest proportions of AA. The DHA plus AA content of the adult cerebral cortex is approximately 6% dry wt and 2% of the white matter (6). The n-6 content (20:4n-6 plus 22:4n-6) of the cerebral cortex is similar to that of the DHA level and in white matter there is a higher proportion
of n-6 than n-3 PUFA (6). The highest proportion of DHA in membrane lipids is found in the disk membranes of the rod outer segments of photoreceptor cells in the retina (8,9). Carrie et al. (10) showed that the proportion of DHA in 11 different regions of the rat brain varied from 7% GPL FA in the pituitary gland to 22% in the frontal cortex. The variation in the proportion of AA ranged from 5% in the pons medulla to 18% in the pituitary gland. This gland was the only region where the proportion of AA exceeded that of DHA.

DHA and AA are present in other tissues in the body but in lower proportions. For example, in the guinea pig the proportion of DHA of all tissues except neural tissue was <0.5% total tissue FA, while in whole brain it was 6–7% total fatty acids (TFA) (11). On a whole body basis, the brain contained approximately 22–25% of the total DHA in the body, with approximately 50% of the DHA being in the carcass (muscle and adipose tissue). The same study showed that the AA was mostly distributed in the carcass (70%), with only 2% in the brain.

The high levels of DHA and AA in the brain gray matter of over 30 different mammalian species (Table 12.1) (12), led to early speculations that these PUFA play a crucial role in the nervous system. In the 1970s, the n-6 PUFA were regarded as essential for humans, while the n-3 PUFA were only thought to be essential for fish and other marine species. The first clue for a physiological role of n-3 FA in mammals came when it was reported that dietary n-3 PUFA fed to rats led to nearly double the response of the retina to visual stimulation of rats compared with when n-6 PUFA were fed (13). Since then, intensive study of the role of DHA in the brain revealed that it plays a vital role in many different parts of the brain, the most obvious role being related to membrane function. In summary, DHA plays a crucial role in membrane-related events (membrane order that can influence the function of membrane receptors such as rhodopsin) (14,15); regulation of dopaminergic and serotonergic neurotransmission (16); regulation of membrane-bound enzymes (Na/K-dependent ATPase) (17); signal transduction via effects on inositol phos-

<table>
<thead>
<tr>
<th>FA</th>
<th>Brain</th>
<th>Liver</th>
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<tr>
<td>18:2n-6</td>
<td>12 (3–24)*</td>
<td>120 (31–470)</td>
</tr>
<tr>
<td>20:3n-6</td>
<td>7 (2–10)</td>
<td>11 (3–45)</td>
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<tr>
<td>20:4n-6</td>
<td>120 (89–150)</td>
<td>130 (41–210)</td>
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<td>22:4n-6</td>
<td>63 (42–80)</td>
<td>10 (1–56)</td>
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<td>22:5n-6</td>
<td>12 (2–29)</td>
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<td>18:3n-3</td>
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<td>20:5n-3</td>
<td>6 (1–12)</td>
<td>23 (5–78)</td>
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<td>22:5n-3</td>
<td>7 (3–19)</td>
<td>54 (3–110)</td>
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<tr>
<td>22:6n-3</td>
<td>220 (160–290)</td>
<td>98 (2–220)</td>
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*Results are shown as the mean value and range for 25 species. Source: Reference 12.
phates, diacylglycerol, and protein kinase C (18); alteration of ion flux through voltage-gated K+ and Na+ channels (19,20). In addition, DHA is involved in metabolic events (regulation of the synthesis of eicosanoids derived from AA) (21); as a precursor of docosatrienes and 17S resolvins (novel anti-inflammatory mediators) derived from DHA (Fig. 12.1) (22), and in gene expression (regulation of gene expression of many different genes in rat brain in short- and long-term studies) (23–28). Finally, DHA is involved in cellular events such as regulation of phosphatidyl serine levels (29) that appears to be involved in the protection of neural cells from apoptotic death (30); stimulation of neurite outgrowth in PC-12 brain or neuron cells (31,32); selec-

Fig. 12.1. Metabolic pathway involved in converting essential fatty acids into their longer chain metabolites and other products.
tive accumulation of DHA by synaptic growth cones during neuronal development (31,32); regulation of neuron size (33,34); regulation of nerve growth factor (35); and as a precursor of neuroprostanes (DHA oxidation products) (Fig. 12.1) (36,37).

AA is the predominant n-6 PUFA in mammalian brain and neural tissue (Table 12.1) and, like DHA, is found in sn-2 position of the glycerol backbone of membrane GPL. AA, therefore, plays a key role in membrane function. The release of AA from the membrane GPL is due to the receptor-mediated activation of phospholipase A2 or phospholipase C and diacylglycerol lipase (38). Once released, AA can become a substrate for oxidative enzymes, such as cyclooxygenases (COX-1, COX-2), lipoxygenase, or cytochrome P450 monooxygenases, that convert it to a number of bioactive eicosanoids like prostaglandins, prostacyclins, thromboxanes, and leukotrienes (Fig. 12.1) (39,40). The functional role of AA appears to be mediated either by the FA itself or through the bioactive metabolites produced by the oxidative reactions. For example, AA exerts diverse actions on acetylcholine receptors such as a short-term depression by blocking of the receptor and long-lasting potentiation by activation of protein kinase C pathway (41). Furthermore, synaptic activation of glutamate receptors has been reported to release AA. This suggests a role in synaptic transmission (42,43).

Abnormalities in AA metabolism in brain have been linked to a number of brain disorders such as bipolar disorder (44), Alzheimer's disease (45), schizophrenia (46), and ischemia (47). COX-2, an enzyme that converts AA to eicosanoids, is highly expressed in different regions of the brain such as hippocampus, cortex, and amygdala (48). Age-dependent cognitive deficits and neuronal apoptosis have been reported in transgenic mice over expressing COX-2 with a concurrent increase in prostaglandin levels in the brain (49), suggesting that neuronal COX-2 may contribute to the pathophysiology of age-related diseases. The reduced skin flushing response to niacin in schizophrenic subjects has been known for years; since the primary mechanism is conversion of AA to prostaglandin D2, this also suggests an abnormal AA metabolism in these subjects (50). Treatment of schizophrenic patients with a 2 g dose of ethyl eicosapentaenoate acid (EPA, 20:5n-3) leads to a significant improvement of the condition with an elevated level of AA in erythrocyte FA (46); it is speculated this may result from the inhibition of phospholipase A2 by EPA (46).

There is a rapid increase in the weight of the human brain postnatally, until the infant is about two years old. Associated with this, there is a rapid accretion of DHA and AA in the infant brain during the first postnatal year (51). It is thought that the DHA and AA for brain growth are largely derived from mothers' milk. Breast feeding provides at least 49 mg of DHA and 93 mg of AA to the infant each day depending on the PUFA level in the mother's milk (52). It is known that milk LC-PUFA levels can be influenced by diet; usually the LC-PUFA are in the range from 0.2 to 1.0% of total milk FA (53).

The fetal brain is believed to be able to produce a limited amount of DHA from ALA; the liver may also be able to produce some DHA (Fig. 12.1) (54), but it is believed that this is insufficient for optimal development (55). It has been argued that based on the rate of accretion of DHA into the human brain, there is a need to supply
DHA via breast milk or infant formula for at least the first six months of life (56). There has been little discussion about the capacity of newborn infants to synthesize sufficient AA for brain growth. The limited capacity of the neonatal infant to synthesize LCPUFA has been a driving force in developing infant formulas containing these FA.

Another rationale as to why it is necessary to add LCPUFA to infant formulas is based on the decline in blood LCPUFA levels after birth. Blood levels of DHA and AA in infants fed standard formulas are lower than those of breast-fed infants (57–60). Formulas containing LCPUFA can increase blood LCPUFA levels so they more closely resemble those found in breast-fed infants (61,62). Consistent with the decline in blood levels of DHA and AA in formula-fed children, two studies have examined brain PUFA levels in formula-fed and breast-fed infants. Both found that there was a significantly lower level of DHA, but not AA, in the brain tissue of children who had been mainly fed on infant formulas (lacking DHA and AA) (63,64).

These studies underpin the discussion that has been conducted throughout the world on the importance of adding DHA and AA to infant formulas. Before such action was undertaken, many studies were conducted in animals and primates.

**SCO Studies in Animals: PUFA Levels in Tissues and Functional Studies**

Many studies conducted on SCO in animals have been concerned with the efficiency of these oils in supplying tissues with PUFA, especially in relation to brain PUFA and brain function. This section will discuss several examples from the literature that illustrate that LCPUFA from SCO sources are bioavailable and can influence physiological function. Other studies have been concerned with the safety of these oils, and this is dealt with on page 188.

Ward et al. (65) studied the effect of adding LCPUFA from SCO on the brain and red blood cell FA composition. Rat pups were reared artificially from day 5 to day 18, postnaturally, using a gastromy tube. The study compared three levels of DHA and three levels of AA in a factorial design. The basal diet contained LA and ALA and no LCPUFA. The results showed that supplementing the formula with AA or DHA during the period of brain development increased deposition of these PUFA in the brain and red blood cells. Furthermore, it was found that increasing levels of each PUFA affected the levels of the other PUFA (the highest dietary AA decreased tissue DHA levels and vice versa).

Abedin et al. (66) compared the efficiency of ALA versus DHA in contributing DHA to various tissues (liver, heart, retina, and brain) in guinea pigs. In this study, LCPUFA from SCO were fed to guinea pigs from 3 weeks until 15 weeks of age. The LA content in the diets was constant (17% TFA) with the ALA content varying from 0.05% (diet S), to 1% (diet A), and to 7% (diet C). Diet A had an LA:ALA ratio of 17.5:1 and was structured to closely replicate the principal LCPUFA found in human breast milk (0.9% AA and 0.6% DHA). In the retina and brain phospholipids, the high ALA diet (diet C) or dietary DHA supplementation produced moderate increases in
DHA levels compared with diet S (low ALA diet). There was no change in retinal or brain AA following dietary AA supplementation. This was in contrast to the liver and heart in which the dietary DHA and AA supplement led to large increases (up to 10-fold) in the tissue levels of these PUFA. The data confirmed that dietary ALA was less effective than dietary DHA supplementation (on a g/g basis) in increasing tissue DHA levels, and that tissues vary greatly in their response to exogenous AA and DHA; the levels of these long-chain metabolites is most resistant to change in the retina and brain compared with the liver and heart.

A recent study in rhesus monkey neonates examined the effect of inclusion of DHA and AA in the rearing infant formula on neuromotor development (67). In this study, 28 nursery-reared rhesus macaque infants were divided into two groups, one of which was fed a formula with DHA (1% of the fat) and AA (1% of the fat) from SCO sources; the other group was fed the standard formula that was devoid of the LCPUFA. Neurobehavioral tests were conducted weekly from day 7 of life through day 30. Plasma DHA and AA concentrations in the supplemented group were significantly higher than the control group at 4 weeks of age. The monkeys fed the supplemented formula showed stronger orienting and motor skills than those fed the standard formula; the most pronounced differences were at day 7 and 14. Supplementation with LCPUFA had no influence on temperament. These data support the inclusion of LCPUFA in infant formulas for optimal development.

Some studies have compared the effects of diets with DHA alone versus those with DHA plus AA. One such study was conducted by Auestad et al. (68), in which they measured the auditory brainstem-evoked response (ABR). In previous studies, it had been found that juvenile offspring of rats fed high-DHA diets through gestation and lactation had a longer ABR that was associated with higher DHA and lower AA proportion in the brain (69). In the Auestad et al. study (68), the ABR was assessed in juvenile rats fed high-DHA diets postnatally and compared with diets containing both DHA and AA. It was found that the DHA and AA levels in the brain increased with supplementation. In contrast to the earlier study, this study found that ABR was shorter in the high-DHA group than the DHA plus AA group and not different from the unsupplemented or dam-reared suckling group. Clearly, further studies are needed to understand the relationship between dietary DHA and the development of the auditory system over a range of DHA intakes and discrete periods of development.

Blanaru et al. (70) examined the effect of an increasing dose of AA (0.3 to 0.75% fat) with a constant DHA level (0.1% fat) from SCO sources on bone mass in piglets in a study starting at day 5 and finishing at day 20 after birth. The study was initiated due to an upsurge in interest in the effect of PUFA on bone biology (71). Bone modeling was unaffected by the different treatments, but the whole body bone mineral content was elevated in piglets fed 0.6 and 0.75% AA. The effect of altering the dietary intake of DHA in this model is not known.

Some studies have compared the effects of different sources of LCPUFA on various outcomes in animals. For example, Mathews et al. (72) compared SCO sources of DHA and AA with egg phospholipids as a source of these PUFA on overall animal
health and safety. In this study, piglets consumed a skim milk formula from day 1 until day 16 after birth. The formulas with LCPUFA provided 0.3 and 0.6 g/100 g TFA as DHA and AA, respectively. The control group contained no DHA or AA. There was no difference in gross liver histology between the groups; the apparent dry matter digestibility was 10% greater in the SCO and control groups than in the group fed egg phospholipid PUFA. The plasma DHA proportion was higher in the SCO group than in the egg phospholipids group, while the plasma AA proportion was higher in the SCO group than the control group. In summary, these studies revealed that LCPUFA from SCO sources were bioavailable and that they had the capacity to alter physiological function in small animals and primates.

Safety Aspects of SCO

The main concern about SCO derived from algae and fungi has been that they are new food ingredients without a history of safe use in infant feeding anywhere in the world. This has meant that these oils have had to undergo extensive toxicological testing in various animal species. The results have been favorable (73–79), and authorities in several countries have approved their use in infant formulas. In the U.S., the Food and Drug Administration (FDA) has given generally recognized as safe status to SCO, thus permitting their use in infant formulas (80).

SCO Studies in Infants

Following the successful trials on bioavailability and safety of SCO in animals and primates, there have been a number of trials in term and preterm infants that have included LCPUFA from various sources into infant formulas. The authors have identified twelve randomized clinical trials (RCT) designed to test the efficacy and safety of adding either n-3 LCPUFA (DHA and EPA) or a combination of DHA and AA to formulas for term infants, that have been published in full. Four of these studies used SCO (62,81–84), while the remainder used other lipid sources (61,85–94). It is doubtful whether the source of LCPUFA has much effect on LCPUFA status of the infant (95).

Trial Design and Treatments

The four trials involved healthy term infants fed formulas from near birth and all but one had a breast-fed reference group. Most trials appeared to have adequate randomization and masking procedures, and most presented power calculations for their primary outcome measurements. Therefore, the trials involving term infants are generally of good methodological quality.

The levels of n-3 LCPUFA used in the trials ranged from 0.1 to 1% total fat while the AA ranged from 0.4 to 0.7% TFA. Four trials assessed the effect of supplementation with n-3 LCPUFA with no AA (61,85,89,92).
Outcomes

Benefits of adding LCPUFA to formulas on visual acuity assessed by both visual evoked potential or Teller cards have been reported in some studies (81), while other studies have shown no difference between LCPUFA supplemented and unsupplemented infants (85). A systematic review of three trials on visual acuity in term infants indicated an improvement in card acuity with LCPUFA treatment at two months of age only (96). There is also mixed evidence for the support of an effect of dietary LCPUFA on more global measures of development (Bayley Scales of Infant Development). Birch et al. (82) have reported benefits of dietary LCPUFA; however, the larger studies conducted by Scott et al. (86) and Auestad et al. (62) showed no effect of LCPUFA supplementation on the Bayley Scales of Infant Development. Possible interpretations of these data include a small individual effect, or that only a proportion of infants will benefit, or the presence of confounding variables. Further studies are needed to elucidate this issue.

There have been no negative findings in relation to growth in term infants regarding LCPUFA supplementation of infant formulas. This is despite the fact that four trials have supplemented formulas with DHA alone without added AA for periods of up to one year, resulting in the AA status of infants being depleted. Therefore, there is no evidence that n-3 LCPUFA supplementation of term infant formulas causes perturbations of growth.

Trials Involving LCPUFA in Preterm Infants

The last trimester of pregnancy is the time when DHA accretion in the brain and nervous system is at its greatest velocity. Therefore, many preterm infants, especially those born before 30 weeks, are born with negligible body stores of DHA; subsequently they are fed with milks that contain no DHA or levels that are much lower than what these infants would have received if they were still in utero. Preterm infants are more at risk than term infants of disturbed DHA accumulation and thus may have the most to gain from DHA supplementation.

The authors are aware of 13 RCT reported in at least 20 separate papers. Four of these studies used SCO (97–102), while the remainder used lipids from other sources (103–116). These trials were designed to test the efficacy and safety of varying levels of DHA, EPA, and AA in the diets of preterm infants.

Trial Design and Treatments

All the trials reported adequate concealment of allocation and in general their methodological quality is more robust than earlier trials.

Outcomes

The original trials that showed a benefit on electroretinographic responses and visual acuity all supplemented with fish oil. Of the SCO trials, only that of O'Connor et al.
assessed visual function and showed benefit to improving visual evoked potential (VEP) acuity but not Teller card acuity. Of the three trials that have assessed global development, one reported an advantage of LCPUFA supplementation on psychomotor development in infants born at less than 1250 g (100). The data from O'Conner et al. (100) suggested that the more immature and sick infants may have the most to gain from LCPUFA supplementation; this highlights the notion that some subgroups may be more sensitive to the effects of LCPUFA. Further work is needed to best maximize the potential benefits on early childhood development.

Although most trials show that there is no effect of LCPUFA supplementation on growth, a recent trial suggested an enhancement of growth (99). Two separate systematic reviews and meta-analyses combining growth data from all published RCT showed no difference in any growth parameter between supplemented and unsupplemented infants (117,118). Although the outcome data from the individual LCPUFA intervention trials with visual outcomes consistently indicated beneficial effects of LCPUFA supplementation, the two available systematic reviews/meta-analyses of these data have not been complete (118,119). One review could not combine the visual outcome data because of different assessments and methodologies and differing assessment times (118). The other review included data from three randomized trials and one non-randomized study and concluded that there was a beneficial effect of LCPUFA treatment on visual acuity at two and four months corrected age (119). Thus, despite the promising beneficial effect of DHA supplementation on the neural outcomes of preterm infants, trials with standard methodologies and follow-up of infants beyond 12 months corrected age are necessary to more precisely assess the extent of benefit offered by LCPUFA supplementation.

**SCO Studies in Adults**

There have been relatively few studies on SCO in adults. Nelson and colleagues conducted two separate studies that involved feeding a small group of volunteers with either DHA or AA derived from SCO. These studies led to a number of papers by the group, published in the period 1997–1999 (120–128).

The aim of the DHA study was to examine the effects of feeding DHA-rich triacylglycerol (TAG) on the FA composition, eicosanoid production, select activities of human peripheral blood mononuclear cells, plasma lipoprotein concentration, and the FA composition of plasma lipids and adipose tissue. The 120-d study with 11 healthy men was conducted at the Metabolic Research Unit of Western Human Nutrition Research Center. Four subjects (control group) were fed the stabilization diet or basal diet (15, 30, and 55% energy from protein, fat, and carbohydrate, respectively) throughout the study; the remaining seven subjects were fed the basal diet for the first 30 d, followed by 6 g DHA/d for the next 90 d. DHA replaced an equivalent amount of LA; the two diets were comparable in their total fat and all other nutrients. The ratio of saturated plus trans FA to monounsaturated FA to PUFA in the diets was 10:10:10. Both diets were supplemented with 20 mg D α-tocopherol acetate/d.
The white blood cell FA composition, eicosanoid production, immune cell functions, plasma lipoprotein concentrations, and the plasma and adipose tissue FA composition were examined on day 30 and 120. There was an increase in white blood cell DHA from 2.3 to 7.4% and a decrease in the proportion of AA from 19.8 to 10.7%. There was also a lowered prostaglandin E₂ (PGE₂) and leukotriene B₄ (LTE₄) production by 60–75%, in response to lipopolysaccharide. Natural killer cell activity and in vitro secretion of interleukin-1β and tumor necrosis factor α were significantly reduced by DHA feeding (120,121). The concentration of plasma cholesterol, low-density lipoprotein and apolipoproteins [A₄, B, and lipoprotein (a)] were unchanged after 90 d, but the TAG levels were significantly reduced and the high-density lipoprotein-C and apolipoprotein-E levels were increased significantly. The proportion of plasma DHA rose from 1.8 to 8.1% after 90 d on the high-DHA diet. Interestingly, the plasma EPA levels rose from 0.4 to 3.4% in subjects on the high-DHA diet, despite the diet being devoid of EPA. The DHA proportion in adipose tissue rose significantly from 0.1 to 0.3%, but the amount of EPA did not change (122). The effects of the high-DHA diet on platelet aggregation were also studied in this experiment; no significant effects were found in blood coagulation parameters, platelet function or thrombotic tendency (122,123).

These authors also studied the effect of the DHA-rich diet on the conversion of deuterium-labelled 18:2n-6 and 18:3n-3 to LCPUFA. The labeled compounds were administered as TAG at the end of the DHA-feeding period and blood samples were taken over the following 72 h. The DHA supplementation significantly reduced the concentrations of most deuterium-labeled n-6 and n-3 LCPUFA metabolites in plasma lipids. For example, the accumulation of deuterium-labeled 20:5n-3 and 22:6n-3 was depressed by 76 and 88%, respectively. The accumulation of deuterium-labeled 20:3n-6 and 20:4n-6 also decreased by 72% for both PUFA (124). These authors calculated that the accumulation of n-3 LCPUFA metabolites synthesized from 18:3n-3 would be reduced from about 120 mg/d to 30 mg/d by supplementation with 6.5 g DHA/d. It was calculated that the accumulation of n-6 LCPUFA metabolites, synthesized from 18:2n-6, would be reduced from about 800 mg/d to 180 mg/d. The authors suggested that health benefits associated with this level of DHA supplementation would be the result of reduced accretion of n-6 LCPUFA and an increase in n-3 LCPUFA levels in tissue lipids.

A study on AA was conducted by Nelson et al. (125). In this study, 10 healthy men lived at the Metabolic Research Unit for 130 d. All subjects were fed a basal diet containing 27 energy percentage (en%) fat, 57 en% carbohydrate, 16 en% protein, and 200 mg AA/d for the first and last 15 d of the study. Additional AA (1.5 g/d) was incorporated into the diet of six men from day 16 to 65 while the remaining four subjects continued to eat the basal diet. The ratio of saturated plus trans FA to monounsaturated FA to PUFA in the diets was 7:10:7. The diets of the two groups were crossed over from day 66 to 115. Dietary AA had no significant effect on the blood cholesterol levels, lipoprotein distribution, or apoprotein levels.

The plasma TFA composition was markedly enriched in AA after 50 d (P < 0.005). The AA proportion in plasma PL increased from 10.3 on the basal diet to
19.0% after the AA-enriched diet. There was a significant rise in the proportion of AA in the red blood cells that mainly replaced LA. The adipose tissue FA composition was not influenced by the AA-enriched diet (125). Platelet aggregation in the platelet-rich plasma was determined using ADP, collagen, and AA. There were no significant differences in platelet aggregation before and after consuming the AA-enriched diet (126). There were no significant changes in any of the indices of blood clotting (prothrombin time, partial thromboplastin time, antithrombin III levels, and in vivo bleeding times) between diet groups. Surprisingly, there was only a small change in platelet AA proportion during the AA feeding period. The in vitro secretion of LTB$_4$ and PGE$_2$, from in vitro stimulated white blood cells, was significantly increased after the AA-enriched diet, but it did not alter the secretion of tumor necrosis factor $\alpha$, interleukins-1 $\beta$, -2, -6; and the receptor for interleukin-2 (127). At the end of each diet phase, each subject was dosed with about 3.5 g of deuterium-labeled 18:2n-6 as TAG. The concentration of deuterium-labeled 20:3n-6 and 20:4n-6 were both 48% lower ($P < 0.05$) in plasma lipids from the AA-enriched group compared with the low AA diet group (128).

**Conclusion**

The commercial development of SCO and their FDA approval has allowed the addition of LCPUFA from SCO sources to infant formulas. This advance has enabled the composition of infant formulas to approach that of human milk, a goal that is sought by both formula companies and parents. Future research on SCO should examine the nutritional benefits of SCO in adults.

**Acknowledgments**

Robert Gibson and Maria Makrides are both funded through NHMRC Senior Research Fellowships. Anura Jayasooriya was a postgraduate student funded through RMIT University at the time of preparation of the manuscript. The assistance of Anupama Pasam in the early stages of the manuscript preparation is gratefully acknowledged.

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