special report

The 1st Congress of the International Society for the Study of Fatty Acids and Lipids (ISSFAL): Fatty Acids and Lipids from Cell Biology to Human Disease[1]

Artemis P. Simopoulos,[2] Berthold Koletzko, Robert E. Anderson, Gerard Hornstra, Ronald P. Mensink, Babette B. Weksler, William S. Harris, Raffaele De Caterina, Reto Muggli, and Howard Sprecher

Introduction (Artemis P. Simopoulos)

The ISSFAL held its first congress on "Fatty Acids and Lipids from Cell Biology to Human Disease" in Lugano, Switzerland, June 30–July 3, 1993. Four hundred and fifty persons from 35 countries attended, and a total of 332 abstracts consisting of state-of-the-art reviews, critiques, and new data were presented at 13 plenary lectures, 10 major symposia, 10 oral communications sessions, and 14 poster sessions, covering topics such as Intracellular Communication; A New Look at Fatty Acids as Signal Transducing Molecules; Omega-3 Polyunsaturated Fatty Acids (PUFA) in the Regulation of Cytokine Synthesis; The Role of Fatty Acids During Pregnancy and Lactation; Fatty Acids and Human Physiology; Cardiovascular System; Hypertension; Diabetes; Cancer; Inflammation and Immunology; PUFA and Antioxidants; a round table discussion on The Future of Fatty Acids in Human Nutrition; Health and Policy Implications; and a final session, chaired by Dr. A. P. Simopoulos, in which summary statements were presented by the session chairmen for general discussion. During this final session it was recommended that *trans* fatty acids should be labeled as such and should not be included with other fatty acids, such as saturates or PUFA. The need for standardization of fatty acid and lipid nomenclature was recognized, and at the Board meeting of ISSFAL, a scientific committee was appointed by the President, Dr. A. Leaf, to develop a position paper. The summary statements of the symposia appear below in an abbreviated form. The full statements are included in the Congress proceedings.

Maternal and Infant Nutrition (Berthold Koletzko)

The session was cochaired by B. Koletzko and R. Uauy, and presentations were made by Drs. Koletzko, M. Hamosh, E. Lien, G. Crozier, T. A. B. Sanders, P. Budowski, and A. D. Postle.

Data from many sources indicate an essential role of docosahexaenoic acid (DHA) for early human development. In utero, the placenta appears to supply DHA and other long-chain polyunsaturated fatty acids (LCPUFA) to the fetus. After birth, breast feeding is considered the best choice of feeding to provide a healthy infant with an adequate nutrient supply. Human milk supplies significant amounts of ω-3 (n-3) and ω-6 (n-6) LCPUFA, including DHA and arachidonic acid (AA). In contrast, most of today's artificial feeding regimens for young infants are devoid of appreciable amounts of LCPUFA. Premature infants raised on formula providing linoleic (LA) and alpha-linolenic acids (LNA), but none of their products, show signs of functional deficiencies in retina and brain that are corrected by a dietary supply of preformed DHA. Also, dietary regimens that lead to a depletion of AA were associated with functional disadvantages.

In Europe, the Committee on Nutrition of the European Society for Paediatric Gastroenterology and Nutrition (ESPGAN) in 1991 recommended an enriched formula for low birthweight infants with metabolites of both LA and LNA. As the exact requirements of premature infants are not yet known, it was recommended that the average composition of human milk should be used as a model for the composition of low birthweight infant formula until better data on optimal intakes become available.

Feeding vegetable oil-based formula to healthy full-term newborns also leads to a depletion of DHA and AA. There are indications that this depletion may be associated with functional disadvantages also in full-term infants, but these data await further confirmation. However, there is general agreement that all infant diets should contain at least LNA as a source of ω-3 fatty acids, and it is considered unacceptable that there are some in-

[1]The Proceedings of the Congress will be published in the Karger series *World Review of Nutrition and Dietetics* in 1994.

[2]To whom reprint requests should be addressed at: The Center for Genetics, Nutrition and Health, 2001 S Street N.W., Suite 530, Washington, DC 20009.

fant foods still in use today in some parts of the world that provide practically no ω-3 fatty acids. It was also agreed that one should avoid extreme ratios of LA to LNA in infant formula. The range of 5 to 15 to 1 for the LA/LNA ratio in infant formula recommended by the ESPGAN Committee on Nutrition was considered reasonable.

In the past, the present level of dietary intake of *trans* isomeric fatty acids during pregnancy, lactation, and infancy was regarded safe. However, data presented at this conference demonstrate that high prenatal exposure to *trans* isomeric fatty acids is linked to poor availability of LCPUFA and lower birthweight in human infants, which is in agreement with previous observations in animal studies. These results question the safety of a high dietary supply of *trans* fatty acids in the perinatal period and add further weight to the demand for a reduction of the high *trans* fatty acid content in the current diets in many countries. As a first step in this direction, a regulation has been proposed for the European Community to restrict the maximum content of *trans* fatty acids in infant formulas to no more than 4% of total fat.

Mechanisms of Accretion of Polyunsaturates in the Nervous System (Robert E. Anderson)

The session was cochaired by N. Salem, Jr. and R. E. Anderson, and presentations were made by Drs. Salem, Anderson, N. G. Bazan, S. A. Moore, E. Yavin, and F. Cockburn.

The aim of the symposium was to explore the mechanisms by which neural tissues concentrate LCPUFA, especially those of the ω-3 family. Discussions centered around the role and function of LCPUFA in these tissues. Key points made in the presentations were:

- Humans can synthesize 20:4ω-6 and 22:6ω-3 from 18:2ω-6 and 18:3ω-3, respectively.
- The retina and the brain have mechanisms for conservation of 22:6ω-3 during ω-3 deficiency. This is achieved in the eye by recycling 22:6ω-3 between the retina and the retinal pigment epithelium.
- The liver serves as a source of 22:6ω-3 for the nervous system through synthesis from short chain precursors and incorporation into lipoproteins for delivery to target tissues. Alternatively, it was suggested that the liver may supply 22:5ω-3 to the nervous system, especially when there is a limited intake of dietary 22:6ω-3.
- Tissues in the retina and the brain can synthesize 22:6ω-3 from appropriate precursors. The relative contribution of local production and transport from the liver to the supply of LCPUFA to these tissues remains to be determined.
- The levels of 22:6ω-3 in brain and retina from infants who died of SIDS (sudden infant death syndrome) were significantly lower in those infants who had been raised on formula, compared to those raised on breast milk.

Recommendations from this symposium included the need for answers to the following questions:

- What are the specific requirements for ω-3 and ω-6 PUFA by the developing human retina and brain? How are they supplied?
- What are the long term consequences of suboptimal accretion of 22:6ω-3 in term and preterm human infants?
- Are the biochemical changes of ω-3 deficiency in human infants reversible?
- What is the best form in which to supply ω-3 and ω-6 PUFA to those infants who are not breast fed?

Essential Fatty Acids, Pregnancy and Pregnancy Complications (Gerard Hornstra)

The session was cochaired by G. Hornstra and S. W. Walsh, and presentations were made by Drs. Walsh, M. D. M. Al, G. Vilbergsson, M. M. H. P. Foreman-van Drongelen, J. D. Sorensen, and M. C. Craig-Schmidt.

It is generally accepted that essential fatty acids (EFA) play a crucial role in fetal development and pregnancy outcome, yet little information is available on the EFA status of mother and fetus in both normal and complicated pregnancy. Studies presented at the session indicate that pregnancy complications, including intrauterine growth retardation, pregnancy-induced hypertension, and preeclampsia, are associated with distinct alterations in both the maternal and the neonatal EFA status. The neonatal EFA status at birth has a rather long-lasting effect on the post-natal EFA status. Especially in preterm infants, the EFA status at birth may be of even greater importance than the dietary EFA content of formula.

It was demonstrated that the daily supplementation of pregnant women with 2.7 g ω-3 PUFA from fish oil, starting at the 30th week of pregnancy, results in significant decreases in thromboxane A_2 (measured as TxB_2) and increases in the urinary excretion of the major metabolites of prostacyclins I_2 and I_3. When related to the changes in maternal and neonatal fatty acid profiles, an inverse correlation was found between the ω-3 status and the formation of TxA_2. In addition, a positive relationship was observed between the ω-3 status and the urinary excretion of the major metabolite of prostaglandin I_3. These observations may provide an explanation for the slight (4 days) but significant prolongation in the duration of pregnancy. The studies suggest that EFA supplementation during pregnancy is likely to affect pregnancy outcome. Prospective longitudinal studies are needed to better document the possible importance of the maternal EFA status during gestation for an optimal EFA status of the neonate and its consequences in later life.

Isomeric Fatty Acids (Ronald P. Mensink)

The session was cochaired by R. P. Mensink and P. J. Nestel, and presentations were made by Drs. Mensink, Nestel, A. C. van Houwelingen, M. Sugano, K. W. J.

Wahle, and D. A. Wood. During this session various physiological effects of *trans* monounsaturated fatty acids (*trans*-C18:1) were discussed.

Trans-C18:1 may stimulate platelet aggregation. Furthermore, *trans*-C18:1 has an inhibiting effect on elongation and desaturation of the EFA, LA, and LNA. This effect can be overcome by increasing EFA availability, which can easily be achieved by dietary means. *Trans*-C18:1, however, is already present in the developing fetus, and phospholipids isolated from fetal tissue contain very low amounts of LA, whereas the LNA content is hardly measurable. Under this particular condition, the inhibiting effect of *trans*-C18:1 on EFA desaturation and elongation may have a considerable influence on the availability of the LCPUFA, which are instrumental for fetal development. As fetal *trans*-C18:1 originate from the mother, these data suggest that the maternal diet should be as low as possible in *trans* fatty acids.

Trans-C18:1 also have a negative effect on the serum lipoprotein profile. All well-controlled studies performed so far have shown an LDL-cholesterol-raising effect of *trans*-C18:1 relative to *cis*-C18:1 (oleic acid). In addition, most studies suggest an HDL-lowering effect and an Lp[a]-increasing effect of *trans*-C18:1. Finally, a case-control study suggested that *trans*-isomers of LA were associated with sudden cardiac death. It should be mentioned, however, that no such relationship was found for *trans*-C18:1.

In conclusion, several studies suggest that *trans*-C18:1 may have adverse effects on health. The question arises, however, if products high in *trans* fatty acids are indeed not beneficial, are there alternatives? Food technologists do need fats with a melting point range between about 30°C and 40°C to make products that should also be palatable for the consumer. It should be their challenge to compose fats and oils low in, or even free of, *trans* fatty acids, which meet these requirements without adversely affecting human health. In the meantime, studies should continue to obtain reliable data on the intake of *trans* fatty acids and to describe their physiological and metabolic effects in more detail.

ω-3 Fatty Acids and Thrombosis (Babette B. Weksler)

The session was cochaired by B. B. Weksler and R. Paoletti, and presentations were made by Drs. Weksler, A. Nordoy, S. Endres, E. Tremoli, G. Di Minno, and M. B. Donati.

This session examined the concept that dietary ω-3 fatty acids affect hemostatic mechanisms so as to decrease likelihood of thrombosis, especially in the setting of vascular disease.

It was pointed out that effects of ω-3 fatty acids on hemostasis and thrombosis are multifactorial. Initial studies that concentrated on antithrombotic changes in platelet function have more recently been reevaluated and show that decreases in platelet aggregation or mediator release are modest even when large amounts of ω-3 fatty acids are consumed. What must also be considered are changes in vascular endothelial and monocyte function, such as increases in vascular prostacyclin or nitric oxide production by endothelium, and decreases in tissue factor and mitogen production by monocytes, which have secondary antithrombotic effects. Prolongation of bleeding time by ω-3 fatty acids has not been shown to lead to increased surgical bleeding when tested directly. Anti-inflammatory effects of ω-3 fatty acids on neutrophils and monocytes may have important implications in decreasing vascular injury and opposing development of atherosclerotic lesions.

It was emphasized that to see antithrombotic effects of dietary ω-3 fatty acids in patients at high risk of occlusive cardiovascular disease, it is necessary to decrease dietary saturated fat content as well as to add ω-3 fatty acids. Recent data indicate that the combination of ω-3 fatty acids and lovastatin prevented exercise-induced shortening of bleeding time, indicating a favorable effect upon platelet-vessel wall interaction. This combination also enhanced decreases in plasma triglycerides and Factor VII. Elevation of the latter has been associated with high risk of cardiovascular events.

The inflammatory effects of cytokines may contribute to the development of atherosclerosis as well as to classic inflammatory diseases, particularly interleukin 1 as an inducer of prothrombotic substances in endothelial cells and in macrophages. Studies in normal subjects and patients with inflammatory disease have shown a marked suppression of IL-1 synthesis, probably taking place at the transcriptional level, during treatment with ω-3 fatty acids, that persists for many weeks after this treatment has been stopped. The long duration of treatment effect is of great interest for the design of treatment regimens. Studies on human monocyte/macrophages derived from subjects treated with ω-3 fatty acid ethyl ester concentrates, examining particularly the inhibitory effect of ω-3 treatment on monocyte procoagulant activity, indicated that long term treatment, of more than 6 weeks, was needed to show an inhibitory effect. Moreover, more prolonged treatment was required for the effect to persist. With 18 weeks of treatment, monocyte functions remained depressed for >6 months, even though the monocyte lipid concentrations had returned to normal.

ω-3 and ω-6 Fatty Acids, Lipids, and Lipoproteins (William S. Harris)

The session was cochaired by W. S. Harris and G. Crepaldi, and presentations were made by Drs. Harris, C. A. Drevon, K. C. Hayes, C. R. Sirtori, G. Crepaldi, and P. J. Nestel.

Twenty years ago the effects of dietary fats on plasma lipid levels were quite simple: animal fats raised cho-

lesterol levels, vegetable oils lowered them, monounsaturated oils had no effect, and fish oils (in the form of cod liver oil) were good for preventing rickets.

The presentations in this session made it quite clear that the situation is not so simple. The speakers discussed the impact of PUFA (especially ω-3 fatty acids) on the metabolism of chylomicrons, VLDL, LDL, and HDL; the use of PUFA in the management of hyperlipidemia; and future research directions. The speakers emphasized several relationships between dietary PUFA and lipoprotein metabolism that appear to be relatively clear. A few of these were:

- Saturated fatty acids containing 12, 14, and 16 carbon atoms are all hypercholesterolemic, but to different extents depending on the experimental conditions. Saturated fatty acids containing less than 12 or more than 16 carbon atoms are not hypercholesterolemic.
- The consumption of linoleic acid (LA) within the normal range for usual diets (5–8% en) does not lower HDL cholesterol levels.
- Practical intakes of ω-3 fatty acids (2–4 g/d) lower fasting and postprandial triglyceride levels and raise HDL_2 levels but do not significantly alter LDL cholesterol levels. The resultant impact on cardiovascular risk is unclear.

There, of course, remain many areas of continuing controversy:

- Are monounsaturated or polyunsaturated fatty acids the best substitutes for saturated fatty acids in the diet—both in terms of effects on lipoprotein levels and on lipoprotein oxidation susceptibility?
- Does a relatively high-fat diet rich in oleic acid (the Mediterranean diet) lower cardiovascular risk more than a diet low in total and saturated fats?
- What are the biochemical mechanisms by which fatty acids alter serum lipoprotein levels?

Clinical Trials with ω-3 Fatty Acids (Raffaele De Caterina)

The session was cochaired by A. Leaf and R. De Caterina, and presentations were made by Drs. De Caterina, J. Dyerberg, D. R. Robinson, R. I. Lorenz, and H. Knapp.

This symposium was aimed at covering the expanding areas of clinical use of ω-3 fatty acids in some (but by no means all) human diseases (important areas such as skin diseases, diabetes, and cancer were not covered). The bulk of the epidemiological evidence gathered so far does confirm the idea of an inverse relationship between the dietary content of ω-3 fatty acids and the incidence of coronary heart disease (CHD). Although other dietary components, especially the content of saturated fats, certainly play a major role in influencing the risk of CHD, an independent protective effect of ω-3 fatty acids has been found in several cross-cultural comparisons among and within different populations, and such evidence has been defined "sufficiently substantial" to justify the present efforts to elucidate the mechanisms for such an effect and the performance of controlled clinical studies. Although the main emphasis has hitherto been put on data describing an antithrombotic action, newer epidemiological and experimental data also point to an antiatherosclerotic effect and, possibly, to effects against the functional consequences of myocardial ischemia (i.e., the electrical myocardial instability).

A review of the clinical trials of ω-3 fatty acids in renal diseases showed that ω-3 fatty acids provide a number of biological reasons for their use in these diseases, including, among others, a possible increase in the renal vasodilatory capacity by a rearrangement of renal prostanoid production, a reduction in the production of pro-inflammatory leukotrienes, a reduction in the transcapillary escape rate of albumin, and actions limiting cyclosporine-related nephrotoxity. Animal models of renal diseases, mostly of immune-renal disease, have supported the idea of possible usefulness of these compounds in humans. The most promising areas of investigations at the moment include the reduction of proteinuria in some chronic glomerular diseases, the treatment of IgA nephropathy, and the prevention of cyclosporine-induced nephrotoxicity. In these last two areas, the results of two large, ongoing, multicenter clinical trials are eagerly awaited.

Seven well-controlled, double-blind clinical trials of the effects of dietary supplements of ω-3 fatty acids in rheumatoid arthritis have reported statistically significant beneficial effects, which were, however, of small magnitude and modest clinical impact. Such studies were conducted with doses in the range of 5–6 g/day, with minimal side effects, justifying the hypothesis that larger doses might possibly have a greater clinical impact.

Four double-blind, randomized placebo-controlled trials in ulcerative colitis with doses of ω-3 fatty acids ranging between 2.7 and 5.4 g/day have documented moderate clinical improvements, mostly in remission induction. On the other hand, in Crohn's disease, the experience gathered so far has failed to find significant clinical effects.

The experiences with ω-3 fatty acids in respiratory diseases, where the attenuation of vaso- and broncho-active leukotriene production by ω-3 fatty acids prompted studies mostly in allergic respiratory diseases, were presented. So far, one study found no change in allergic asthma with ω-3 fatty acid supplementation, and another claimed exacerbations of symptoms in aspirin-sensitive asthmatics, attributed to in vivo cyclo-oxygenase inhibition. In a nasal allergen challenge model in which inhibition of 5-lipoxygenase reduced nasal symptoms, dietary ω-3 fatty acids modified the pattern but not the overall production of leukotrienes and did not attenuate symptoms. However, since the late component of respira-

tory hypersensitivity reactions may be altered favorably by ω-3 fatty acids, further research in syndromes of pulmonary inflammation was felt to be warranted.

PUFA and Natural Antioxidants (Reto Muggli)

This session was cochaired by S. N. Meydani and R. Muggli, and presentations were made by Drs. Meydani, Muggli, M. S. Nenseter, G. Hornstra, L. B. M. Tijburg, J. P. Allard, and A. Petroni.

While there is clear evidence for a requirement of vitamin E in connection with the amount and degree of unsaturation of PUFA in the diet, the exact amount of vitamin E needed to compensate for this increased demand caused by PUFA in the diet has not been systematically investigated in humans. Thus, adequate blood levels of vitamin E are essential in clinical trials designed to prove the beneficial effects of fish and fish oil as cardioprotectors and anti-inflammatory agents. Vitamin E (d-alpha-tocopherol) in the ratio of 0.6 mg/gram of PUFA (LA) is generally regarded as the critical value of protection. The amount required to protect higher unsaturated PUFA increases approximately linearly with the number of double allylic positions. A theoretically derived formula for its calculation was proposed.

Besides vitamin E, other known (vitamin C, beta-carotene, uric acid, etc.) and unknown natural antioxidants most certainly play a role in defending against oxygen damage. In olive oil, 3,4-dihydroxy-phenyl ethanol was identified as one of several phenolic antioxidants with antithrombotic affects in vitro.

While high intakes of PUFA in animals and humans can lead to reduced plasma and tissue vitamin E levels and to symptoms of relative vitamin E deficiency, such as creatinuria, erythrocyte peroxidizability, and elevated lipid peroxide markers, lipid peroxidation, as measured by breath ethane and pentane output, was not increased in volunteers after supplementation of 5.3 g EPA and DHA in the form of methyl esters for 6 weeks.

Particularly disturbing is the nagging question as to whether PUFA, fish oil in particular, will increase the susceptibility of LDL to oxidative changes. In contrast to Hornstra, who found the oxidation resistance of LDL significantly reduced after fish oil consumption, Nenseter reported that 5 g marine ω-3 fatty acids per day for 4 months had no effect on the susceptibility of LDL to oxidation. In view of the pivotal role of oxidatively modified LDL as a possible pathogenetic factor in atherosclerosis, this discrepancy should be urgently resolved.

Fatty Acid Oxidation (Howard Sprecher)

The session was cochaired by A. A. Spector and H. Sprecher, and presentations were made by Drs. Spector, Sprecher, H. Schultz, C. R. Roe, and S. Skrede.

The initial discussion involved the role of the NADPH-dependent 2-*trans*,4-*cis*-dienoyl-CoA reductase in removing double bonds contained at even numbered carbon atoms in fatty acids. Recent evidence indicating that the epimerase reaction that inverts a fatty acyl hydroxyl group from the D- to L-configuration consists of two enzymes was also presented. A point made clear is that the role for D-hydroxyacyl-CoA derivatives in fatty acid β-oxidation is not understood because only the L-hydroxyacyl form is a substrate for β-hydroxyacyl-CoA dehydrogenase.

Additional discussion centered on a new pathway for removing fatty acid double bonds at carbon atom 5. This is a complex set of reactions called 5-reductase.

A discussion of genetic defects in mitochondrial β-oxidation followed. These defects may involve fatty acid transport into the mitochondria or with the various enzymes that carry out the β-oxidation spiral. The most frequently encountered defect is a deficiency of medium-chain acyl-CoA dehydrogenase.

Several laboratories reported that the lipoxygenase products 15-hydroxy-5,8,11,13- and 12-hydroxy-5,8,10,14-eicosatetraenoic acids are only partially β-oxidized. The chain-shortened metabolites that are released in this process may have biological activity or, alternatively, may modify cellular processes by esterification into membrane lipids.

Finally, there was a discussion of the partial β-oxidation of the 22-carbon ω-3 and ω-6 polyunsaturated fatty acids. The resulting products are esterified into membrane lipids. Such a mechanism may be involved in 22:6ω-3 and 22:5ω-6 synthesis. This process requires intracellular communication between the endoplasmic reticulum and the site of β-oxidation, which may be the peroxisomes.