Optimizing Intravenous Supply of Functional Lipid Components

Yvon A. Carpentier and Isabelle E. Dupont

L. Deloyers Laboratory for Experimental Surgery, Université Libre de Bruxelles, Brussels, Belgium

Introduction

The aims of nutritional support administered to acutely ill patients have markedly evolved with time, from supplying huge amounts of calories and nitrogen (in an attempt to abolish the catabolic response and to guarantee weight gain) to providing a limited and balanced intake of macro- and micronutrients in order to maintain or restore an adequate composition of different body compartments [1]. Currently, the focus is to improve the function of those organs, which are critical for the survival of acutely ill patients by quickly supplying conditionally essential substrates. Again, priorities have changed from protecting skeletal muscle to maintaining or improving immune defenses, intestinal integrity and barrier function, as well as cardiac work. Still, little attention has so far been paid to protecting other important functions, namely those performed by the endothelium including tissue micro-perfusion.

It has also taken some time to realize that acute phase conditions are associated with marked changes in the priorities for substrate requirements, and that the pharmacological effects of given nutrients could be used to optimize metabolic support and ultimately clinical outcome. This requires an improved knowledge of the metabolic pathways involved in the delivery of active agents, as well as of the action of such agents in key tissues and organs. In this chapter, we will consider ways of optimizing the delivery of essential fatty acids from the n-3 family to the endothelium in an attempt to maintain or optimize its functions.
Optimizing Intravenous Supply of Functional Lipid Components

**Essential or Polyunsaturated Fatty Acids**

Polyunsaturated fatty acids (PUFAs) are important components of cell membranes and the balance between n-3 and n-6 PUFAs markedly affects several key metabolic pathways. As discussed at this meeting by Grimble and Calder and reviewed elsewhere [2], the effects of n-3 PUFAs result from their direct and indirect action at several levels: as constituents of the cell membrane influencing its physical properties and the function of other components; as precursors of specific lines of eicosanoids; as second messengers in cell signaling pathways, and as modulators of various nuclear transcription factors to influence the expression of several genes.

Docosahexaenoic acid (DHA; C22:6n-3) is essential for the growth and maturation of the central nervous system in the fetus and the preterm newborn. Confirming the fact that many epidemiological observations made in populations consuming large amounts of oily fish resulted from a high n-3 PUFA intake, further studies using oral supplementation of n-3 PUFAs, namely eicosapentaenoic acid (EPA), have shown a reduction in inflammatory response and thrombotic reactions, as well as of allergic and autoimmune manifestations. n-3 PUFAs were also shown to help improve immune defenses, but only when immune depression is caused by high concentrations of prostaglandin E2, e.g., after hemorrhage or during chronic inflammation. n-3 PUFAs may also lower the level of plasma triglycerides and indirectly improve the lipoprotein profile. These effects have been recognized as particularly beneficial in several

---

**Table 1. Effects of n-3 PUFAs**

<table>
<thead>
<tr>
<th>Potential benefits in ‘chronic’ diseases</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Maturation of fetal CNS and retina (DHA)</td>
</tr>
<tr>
<td>• Reduction of inflammatory responses</td>
</tr>
<tr>
<td>• Anti-thrombotic effects</td>
</tr>
<tr>
<td>• Prevention of impaired cellular immunity when caused by ↑ PGE₂ production</td>
</tr>
<tr>
<td>• Decreased plasma triglyceride concentration</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Potential benefits in ‘acute’ conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Decreased cell reactivity to various stimuli (e.g. ventricular arrhythmia’s, etc.)</td>
</tr>
<tr>
<td>• Prevention/reversal of cancer and inflammatory cachexia</td>
</tr>
<tr>
<td>• Increased tolerance to organ transplantation and improved function of the graft</td>
</tr>
<tr>
<td>• Maintenance of tissue microperfusion</td>
</tr>
<tr>
<td>• Reduced cellular accumulation of fat</td>
</tr>
</tbody>
</table>

→ Potential interest for supplying n-3 PUFAs to ‘acute’ patients
chronic illnesses including cardiovascular diseases [3]. However, more recent observations with n-3 PUFAs suggest a number of other properties that could find clinical applications in acute conditions (Table 1). These include: a low accumulation of fat in different cells, in association with enhanced fatty acid oxidation; a substantial decrease in cell responsiveness to various stimuli, e.g. to cytokines with a reversal of cancer and inflammatory cachexia; a substantial reduction in cardiac arrhythmias including ventricular fibrillation and sudden cardiac death [4, 5], and improved tissue micro-perfusion after ischemia or organ transplantation, with an improved functioning of the grafted organ that persists over time [6]. These latter effects directly relate to endothelial function.

The Endothelium and Its Functions

The endothelium lines blood vessels and separates circulating blood and its components from the subendothelial space and vascular smooth muscle cells [7]. There are three types of endothelium, which may vary with respect to function and structure: continuous in arterioles, capillaries and venules; fenestrated in the exchange vessels of secretory and excretory organs, and discontinuous in the sinusoids of the liver and spleen. In a 70-kg individual, the endothelium covers an area of approximately 700 m² and weighs between 1.0 and 1.5 kg [8].

As a barrier between the blood and tissues, the endothelium plays an important role in regulating the exchange of water and solutes (Table 2). Specific transporters for the uptake and export of different molecules (glucose, amino acids, albumin, etc.) provide a means for endothelial cell transcytosis. Under normal conditions, lipoproteins with a size equal to or smaller than intermediate density lipoproteins (IDL), namely low density lipoproteins (LDL) and high density lipoproteins (HDL), can also cross the endothelial cell monolayer by transcytosis, so that a substantial proportion of these lipoproteins is found in the subendothelial or intimal space in normal conditions. As expected, small dense LDL penetrate the arterial wall more easily than normal LDL particles and are more atherogenic. In addition, death of endothelial cells or transendothelial migration of leukocytes, which may occur in pathological situations and in acute conditions, produces gaps between endothelial cells that increase the entry of lipoproteins into the vessel. In contrast, larger lipoproteins such as chylomicrons and very low density lipoproteins (VLDL) do not cross the endothelium and undergo intravascular hydrolysis of a substantial proportion of their triglycerides at the endothelial surface of several tissues. This process is performed by lipoprotein lipase, after activation by the cofactor apoprotein C-II.

The plasma membrane of endothelial cells is not homogenous and contains invaginations, called caveolae which represent microdomains (<5% of cell
## Table 2. Endothelium

<table>
<thead>
<tr>
<th>Anatomy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Separates blood cells and components from smooth muscle cells</td>
</tr>
<tr>
<td>700 m² (1–1.5 kg) in a 70-kg individual</td>
</tr>
<tr>
<td>3 types of endothelium (different structure and function)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Physiology (and Physiopathology)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydrolysis of circulating TG by lipoprotein lipase (LPL)</td>
</tr>
<tr>
<td>Transcytosis:</td>
</tr>
<tr>
<td>• Mechanism for tissue supply of solutes (glucose, AA, albumin, etc.)</td>
</tr>
<tr>
<td>• Also for lipoproteins (size ≤IDL)</td>
</tr>
<tr>
<td>• Substantial proportion of LDL in vessel walls (↑↑ for small dense LDL)</td>
</tr>
</tbody>
</table>

Endothelial cell lesions (or death) or leukocyte transmigration → ↑↑ lipoprotein passage.

membranes) particularly active in cholesterol efflux and cell signaling [9]. Caveolae are characterized by interactions between membrane lipids and structural proteins such as caveolin and endothelial nitric oxide synthase (eNOS), which, together with calmodulin, largely regulate nitric oxide (NO\(^•\)) production [10]. Caveolae have a specific lipid pattern with a high content of cholesterol, sphingolipids and other lipids involved in signaling pathways.

The endothelium responds not only to stimulation by neurotransmitters and hormones or substances present in the blood or produced by blood cells, but also to mediators secreted by underlying cells and to mechanical stimulation such as shear forces or high blood pressure [11]. In addition, endothelial responses are modulated by cholesterol content in caveolae, as well as by the activity of angiotensin-converting enzymes (ACE). Normal physiological, as well as pathophysiological, responses are driven by a complex molecular interplay between the endothelium, the vascular connective tissue, vascular smooth muscle cells and circulating blood cells and platelets.

The endothelium produces and releases a series of humoral and molecular factors that may often act in opposite directions. As shown in Table 3, these factors control: vascular tone, coagulation, thrombogenesis and fibrinolysis; platelet activation and inhibition; growth and migration of smooth muscle cells (SMCs) within the intimal space, as well SMC capacity to change their phenotype and to produce collagen [11].

Of particular importance, the endothelium also produces different reactive oxygen species. NO\(^•\) is a potent vasodilator produced by the constitutive eNOS in normal conditions and, in acute situations, by the inducible enzyme (iNOS),
Table 3. Endothelium: production of humoral and molecular factors

<table>
<thead>
<tr>
<th>Modulation of vascular tone</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Prostacyclin (PGI₂) (+)</td>
<td>Angiotensin II (−)</td>
</tr>
<tr>
<td>NO⁺ (+)</td>
<td>Endothelin-1 (−)</td>
</tr>
<tr>
<td>Endothelium-derived hyperpolarizing factor (+)</td>
<td>TBXA₂, PGH₂ (−)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Modulation of thromboresistance</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Antiplatelets: prostacyclin, NO⁺, ectoADPase (CD39)</td>
<td>Antifibrinolytic: PAI-1</td>
</tr>
<tr>
<td>Anticoagulant: heparin-like proteoglycans, thrombomodulin</td>
<td></td>
</tr>
<tr>
<td>Profibrinolytic: tPA, urokinase</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Modulation of SMC growth, migration and collagen production</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Heparin-like molecules (−)</td>
<td>PDGF A/B (+)</td>
</tr>
<tr>
<td>NO⁺ (−)</td>
<td>Angiotensin II (+)</td>
</tr>
<tr>
<td>TGF-β (−)</td>
<td>EGF (+)</td>
</tr>
</tbody>
</table>

All mediators and factors listed on the left side of this table improve physiological endothelial functions.

Mediators and factors listed on the right side induce endothelial dysfunction, promote coagulation, and stimulate inflammatory reactions and remodeling in the vascular wall; their production tends to be stimulated during the acute phase reaction, as well as in conditions such as hypertension, dyslipidemia, and peroxidative insult. n-3 PUFA intake generally tends to modulate the balance between these factors in favor of the physiological conditions.

which is also largely expressed in immune cells. NO⁺ acts synergistically with the other potent vasodilator prostacyclin (PGI₂) to strengthen vasorelaxation, and also to inhibit the adherence of platelets and leukocytes (by interfering with the signaling of adhesion molecules) and to suppress SMC proliferation [12, 13]. Endothelial (and SMC) membranes are rich in NADP+/NADPH and xanthine oxidases that can be activated by angiotensin II to produce superoxide anion (O⁺). The balance between O⁺, NO⁺, and superoxide dismutase (SOD) is tenuous, but particularly important: in case of imbalance (caused by excessive O⁺ production and/or insufficient SOD activity), O⁺ may react with NO⁺ to form peroxynitrite. The latter produces oxidative injury to endothelial cells as well as to LDL, markedly impairs endothelium-dependent vascular relaxation [14], stimulates endothelial cell transcriptional factors that induce the expression of pro-inflammatory cytokines and vascular cell adhesion molecule (VCAM)-1, and activates ACE to produce a vicious circle of cellular dysfunction [7].

In physiological conditions, the overall balance between endothelial factors is towards vasorelaxation, anticoagulation and platelet inactivation, as well as inhibition of SMC proliferation and migration. However, a switch towards
enhanced production of opposing factors can occur in acute conditions (or upon ACE activation), leading to impaired tissue perfusion, hypercoagulability, platelet and SMC activation, as well as adhesion molecule production and immune cell recruitment. These effects are mediated by direct action of these factors on cells to induce the expression of proto-oncogenes c-fos, c-jun, c-myc, and egr-1. In addition, with the onset of endothelial dysfunction, platelets and monocytes adhere to the vessel wall, and additional growth factors are released from these cells [8]. n-3 fatty acids may markedly counteract these effects by modulating endothelial cell response to stimuli [11].

**Endothelial Cells and Fatty Acids**

In almost all *in vitro* studies examining the effect of fatty acids on specific type tissues, fatty acids were supplied to cultured cells in the unesterified (or free) form. However, fatty acid intracellular trafficking and incorporation into phospholipids (as well as into specific phospholipid subclasses) may substantially differ when they are supplemented as triglycerides or cholesteryl esters. The type of lipoprotein carrying these lipids and the associated receptor or nonreceptor uptake pathway will also determine the intracellular fate of fatty acids [15].

A substantial proportion of triglyceride fatty acids carried by chylomicrons and VLDL is released and delivered to cells as free fatty acids after intravascular lipolysis by lipoprotein lipase. The fraction remaining in the remnant particles is delivered to cells, largely by receptor-mediated endocytosis via receptors recognizing apolipoprotein E (but also via nonreceptor particle adsorption to cell surface binding sites). In addition, for most lipoproteins, phospholipid moieties may be exchanged with cell phospholipids [16].

Triglycerides containing long-chain (≥20 carbon atoms) PUFAs are rather poor substrates for pancreatic lipase and lipoprotein lipase and PUFAs are released only in small amounts, when compared to other ‘standard’ fatty acids. Hence, digestion of fish oil triglyceride and absorption of n-3 PUFAs is relatively low and inefficient. Similarly, after intravenous infusion of fish oil-containing emulsions, n-3 PUFAs tend to concentrate into remnant particles and their delivery to cells is primarily via particle endocytosis followed by lysosomal hydrolysis.

**Intravenous Supply of n-3 PUFAs**

Lipid emulsions have been used as an important component of parenteral nutrition since the 1970s in Europe and the 1980s in the United States. They were developed on the model of the endogenous chylomicrons and are composed mainly of triglycerides (forming the major part of particle
core) emulsified by egg-derived phospholipids (forming a surface monolayer); in addition, they contain lipid-soluble vitamins and small amounts of plant sterols. The first well-tolerated preparations exclusively contained soybean oil or safflower oil, which are particularly rich in n-6 PUFAs. More recently, emulsions have been manufactured with mixtures of vegetable oils (including coconut oil rich in medium-chain triglycerides), with fish oils and/or with structured triglycerides [17].

Thus, emulsion particles and chylomicrons have a large diameter and are primarily triglyceride carriers. Following their infusion into the circulation (or during in vitro incubations in plasma), these particles rapidly acquire selected apoproteins (Apos C-I, C-II and C-III, Apo E, and probably Apo A-IV), by transfer mainly from the HDL pool (Fig. 1). This first step, which is essential for the regulation of intravascular particle metabolism, is influenced by the extent of particle surface and by the composition of both emulsion triglycerides and phospholipids [18].

**Fig. 1.** Metabolism of lipid emulsion particles. The major steps shown on this figure are: acquisition of exchangeable apoproteins (Apos) from HDL; exchanges of triglycerides (TG) and cholesteryl esters (CE) with HDL and LDL, mediated by the cholesteryl ester transfer protein (CETP); hydrolysis of a proportion of core TG by lipoprotein lipase (LPL); uptake of remodeled remnant particles by the liver and extrahepatic tissues, with intracellular TG hydrolysis and oxidation or recycling of released fatty acids (FA). PL = Phospholipids; KB = ketone bodies; EM = emulsion particles; alb-Nefa = non esterified fatty acids bound to albumin.

93
On the other hand, the plasma apoprotein content, which substantially varies between individuals, is markedly altered in acute conditions (e.g., in intensive care, post-trauma, or in patients with severe sepsis) with substantial increases in serum amyloid A and Apo J and decreases in most other apoproteins (and namely Apo A-1, Apo Cs, and Apo E). Apo C-II and C-III play an important role in modulating particle binding to the receptor site of lipoprotein lipase (LPL) and the activation of triglyceride lipolysis by the enzyme. At a later stage, Apo E and C-I have opposing effects in facilitating cellular uptake of remnant particles.

LPL hydrolyzes a substantial proportion of particle triglycerides, a process which largely reduces particle size. The extent of triglyceride hydrolysis mainly depends not only on LPL availability and activity, and other endogenous factors (hormonal balance, cytokine concentration, Apo C, etc.), but also on triglyceride composition. The released fatty acids (Nefas) are either taken up by the adjacent tissues or spilled into the circulation to increase the plasma Nefa pool. Concomitantly, emulsion particles acquire cholesteryl esters via an exchange process of neutral lipids (triglycerides and cholesteryl esters) with the cholesterol-rich HDL and LDL mediated by the cholesteryl ester transfer protein (CETP) [19].

The combination of these processes leads to the formation of smaller sized remnants which contain the nonhydrolyzed triglycerides, a major part of lipid-soluble vitamins, and cholesteryl esters. Until recently, remnant particle uptake was considered to take place essentially in the liver [20]. There is now evidence that uptake of artificial emulsion remnants occurs at an earlier stage than that of chylomicron remnants and takes place not only in the liver, but also in several extrahepatic tissues (e.g., in muscle and adipose tissues, probably in endothelial and possibly in intestinal cells, etc.). The cellular uptake of emulsion remnants can proceed via 2 pathways: one involving cell receptors—likely involving the LDL receptor (LDL-R), the LDL-R-related protein (LRP) and the VLDL receptor (VLDL-R)—and another non-receptor-mediated pathway implying particle binding to surface heparan sulfate proteoglycans and other ligands [21, 22]. Lipid components (triglycerides and phospholipids) of remnant particles will be hydrolyzed in cellular lysosomes.

Of substantial importance, differences in the composition of emulsion components may substantially affect each of these metabolic steps (e.g., Apo acquisition, CETP-mediated lipid exchanges, LPL lipolysis, remnant clearance, etc.). For instance, LPL hydrolysis of emulsions, containing a mixture of medium-chain (MCT) and long-chain triglycerides (LCT), promptly releases a major proportion of the particle medium-chain fatty acid (MCFA) as Nefas [23]. In contrast, LPL hydrolysis is not very efficient on fish oil triglycerides (and in general on triglycerides containing very long-chain fatty acids, which remain in a relatively high concentration in remnant particles) [24]. As a consequence, particle endocytosis is probably the dominant pathway for cell delivery of very
long ($\geq 20$ C atoms) PUFAs, such as EPA and DHA, but also arachidonate (C20:4n-6), together with the lipid-soluble vitamins. This pathway is fairly efficient in several cell types. Indeed, a substantial incorporation of EPA was measured in the phospholipids of white blood cell and platelet membranes after only 5 hrs of a slow infusion of a physical mixture containing 50% MCT, 40% soy LCT, and 10% fish oil (MLF 5/4/1). At that time, the EPA concentration was very low in plasma Nefas and plasma phospholipids, suggesting a direct uptake of fish oil-enriched remnants, followed by intracellular hydrolysis of fish oil triglycerides and rapid EPA reprocessing into membrane phospholipids [25].

**Optimized Delivery of n-3 PUFAs by New Lipid Particles**

More recently, we have applied these new views on the metabolism of emulsion triglyceride fatty acid to develop new preparations that would promote a prompt incorporation of n-3 PUFAs in cell membrane phospholipids, while avoiding excessive plasma accumulation of exogenous lipids. Indeed, the slow hydrolysis of n-3 PUFA containing triglycerides by lipoprotein lipase limits the rate of infusion, i.e. the total amount of conventional fish oil preparations that can be given intravenously. Previous observations on emulsions containing a mixture of different triglycerides had shown that including 50% MCT in particles with 40% soybean LCT and 10% fish oil (as in MLF 5/4/1) was associated with a fast hydrolysis of the MCT component, as well as an efficient tissue uptake of remnants.

New preparations with mixtures of 50% MCT and 50% fish oil (w:w) or with 80% MCT and 20% fish oil (together with a substantial amount of $\alpha$-tocopherol) have been tested for n-3 PUFA in vitro incorporation into endothelial cell phospholipids. Control preparations were the 100% fish oil and the 5/4/1 MLF emulsion. To better reproduce in vivo conditions, emulsion particles were allowed to acquire exchangeable apoproteins during a 45-min pre-incubation in plasma. They were then re-isolated and added (at a concentration of 50 mg triglycerides/dl) to endothelial cell (human umbilical vein endothelial cell) cultures enriched with LPL. A submaximal incorporation of n-3 PUFAs was observed after 4 hrs (Fig. 2) and, the 80% MCT/20% fish oil preparation led to a markedly higher proportion of n-3 PUFAs partitioning into cell phospholipids (Fig. 3). We speculate that the presence of MCT leads to rapid formation of small-sized remnants efficiently endocytosed by cells, while medium chain fatty acid oxidation spares n-3 PUFAs for incorporation into cell phospholipids. It remains to be determined which domains (caveolae, rafts, etc.) of endothelial cell membranes are primarily enriched and whether n-3 PUFAs supplied with remnant particles induce the same effects as those previously observed with direct supplementation of free n-3 PUFAs.

In that respect, ongoing experiments aim at determining the effect of such preparations on eNOS expression and activity in different types of cultured
Optimizing Intravenous Supply of Functional Lipid Components

Fig. 2. Incorporation of n-3 PUFAs in endothelial cell phospholipids after incubation with different fish oil (FO)-containing lipid emulsions.

Fig. 3. n-3 PUFAs increase in endothelial cell phospholipids relative to fish oil (FO) content in lipid preparations, expressed as weight percent fatty acid (FA) enrichment/FO proportion.

endothelial cells. Another set of studies is planned in a model of rat aortic rings to test the potential for preserving endothelial function after an oxidative stress with oxidized LDL. Finally, experiments will be conducted to determine the anti-arrhythmic effect of n-3 PUFA-containing emulsions in isolated hearts submitted to hypoxia.

Should these experiments lead to positive results, potential indications of early intervention with n-3 PUFA supplementation would be: patient
Optimizing Intravenous Supply of Functional Lipid Components

preparation immediately prior to revascularization procedures (on coronary or peripheral arteries); the early phase following a myocardial infarct, a stroke, or another ischemic accident; organ transplantation (with supply of n-3 PUFAs to both the donor and the recipient); severe infant prematurity and other conditions with compromised intestinal absorption of fat; excessive inflammatory reactions; etc.

Recent advances in the understanding of the metabolism of intravenous lipid vehicles have led to the development of new preparations specifically designed to promptly incorporate n-3 PUFAs into membrane phospholipids of different key organs. These preparations may represent an important adjuvant in the metabolic care of patients in acute conditions and in the preparation of patients prior to selected procedures. Indications for the use of such products largely depend on studies aiming at determining whether the rapid changes in fatty acid composition observed are adequately reflected by beneficial consequences on organ function.

Acknowledgments

The authors warmly thank Jacques Winand for fruitful discussions and advice in the writing of this chapter. The support of the FRSM (grant no. 3.4620.01) and the NIH (grant no. HL40404) for recent research in this field is gratefully acknowledged.

References

Optimizing Intravenous Supply of Functional Lipid Components


25. Siderova S, Dupont IE, Simoens C, Deckelbaum RJ, Carpentier YA. Early enrichment of WBC and platelets with ω-3 fatty acids during lipid infusion results from direct FA processing in these cells [abstract]. *Clin Nutr* 1998; 17 (suppl): A59.

**Discussion**

*Dr. Meguid:* Could you elaborate a little on your idea of targeting n-3 fatty acids into specific phospholipid groups in the membrane? Which phospholipid groups do you find most enriched by your procedures, and which do you consider ideal?

*Dr. Carpentier:* We can now separate out the caveolae and our first aim is to look at the incorporation of fatty acids in phosphatidyl inositol and in the signaling phospholipids. With respect to targeting, we think that by varying the triglyceride composition we can modify the apoproteins and other proteins that are bound by these lipid vehicles, and in this way produce preparations that are taken up by the heart and the brain about four to five times more readily than with ‘regular’ preparations. Within cells we still have to look at where exactly these fatty acids are going. We know they go to phospholipids but we don’t know which ones.

*Dr. Seidman:* Do you think the outcome of the interesting dog experiments you presented is related to alterations in the lipoprotein profiles in the endothelium, or
perhaps simply to alteration in platelet adhesiveness? We know that children with glycogen storage disease type I have marked hyperlipidemia, with particularly high triglyceride levels, and their platelets are nonadherent, so they have bleeding problems and are considered to be anticoagulated. Perhaps the rapid infusion of the lipid molecules in your animal experiments probably had an anticoagulant effect.

**Dr. Carpentier:** That work was by Alexander Leaf and his group in Boston [1]. Their view is that the probable mechanism was the binding of free fatty acids to ion channels. They have done *in vitro* experiments that showed that free fatty acids – even before they are incorporated as phospholipid esters, but while they are still within the phospholipid molecules – can have these effects. They were able to show that fibrillation of cardiomyocytes induced by the addition of calcium or ouabain could be totally prevented by preincubating the cells with n-3 fatty acids; even after fibrillation had been induced, they could restore normal rhythm by adding these fatty acids. However, when they added bovine serum albumin to remove the free fatty acids from the phospholipid membranes, the fibrillation recurred.

**Dr. Grimble:** I want to make a point in relation to the sudden death experiments in dogs, which is that large intervention studies in humans using n-3 fatty acids, such as the GISSI trial, have shown that n-3 prevent or protect against nonfatal myocardial infarction and sudden cardiac death [2]. So there is evidence for that effect in humans as well.

**Dr. Becker:** Realizing the importance of your fatty acids as structural elements, what percentage of the function of fats is still reserved for energy?

**Dr. Carpentier:** This depends on the conditions. Up to 65% or more of the fatty acids will simply be oxidized. In fact the first lipid emulsions that we used for intravenous feeding were soy bean emulsions, in which two thirds of the fatty acids were essential fatty acids, and even so there was a very high rate of oxidation. I cannot really answer your question other than by saying that you can minimize the amount of n-3 fatty acids that would be oxidized by supplying glucose at the same time, and probably by supplying medium-chain fatty acids.

**Dr. Pichard:** Putting together what Dr. Leverve told us about improved tolerance to ischemia when the ischemia is repeated and what you found in your dog model, what do you think would happen if you were to redo your experiment, exposing your dogs to ischemia or to repeated heavy exercise plus lipid? Do you think that exposure to repeated ischemia would override the effect related to lipid that you described, or might it have a synergistic effect with the lipid?

**Dr. Carpentier:** That’s a hard question to answer and I would need Dr. Leverve’s comments too. I believe that the two mechanisms are different and they could be complementary, so to some extent the effect of n-3 fatty acids in improving cellular antioxidant status could be additional to the effect of tolerance. In fact we are going to do this experiment in Dr. Leverve’s isolated heart model using our particles. We believe these vehicles are a more physiological way of providing n-3 fatty acid to tissues than free fatty acids. When you eat fish – that is, when you eat EPA & DHA in fact – EPA and DHA are esterified into triglycerides, carried into the chylomicrons, and eventually recycled into the very low-density lipoproteins; they are then more likely to enter the cells by endocytosis as free fatty acids released after intravascular hydrolysis of the triglycerides by lipoprotein lipase. So we believe that our system uses a more physiological way, while many other experiments were mainly done with free fatty acids, so we have to do the studies again.

**Dr. Leverve:** I completely agree with what Dr. Carpentier said. There are more and more data showing that energy utilization is compartmentalized in the cells. Energy that is used for contraction comes from the mitochondria, while the energy used for the membrane pump comes from glycolysis. So there is probably a connection between glycolytic ATP, which is formed at the level of pyruvate kinase, and sodium/potassium
Optimizing Intravenous Supply of Functional Lipid Components

ATPase. As sodium/potassium ATPase is also regulated by the threonic lipids, there could be a connection between lipid composition in the vicinity of sodium-potassium ATPase and glycolytic ATP, and this might be the mechanism for a preventive effect against reactive oxygen species. This is what Dr. Carpentier and I will try to study.

Dr. Rössle: With intravenous lipid emulsions, the metabolism is of course very different from enteral lipids. Can you speculate on how we could improve the absorption of n-3 fatty acids by the enteral route, given that the lipids are completely remodeled, when they are taken by this route?

Dr. Carpentier: When you eat fish, a substantial proportion of the n-3 fatty acids is re-esterified into triglycerides, and these triglycerides are only hydrolyzed to a minor extent to release free fatty acids. Our model mimics quite well what happens to triglycerides, when they are incorporated into the chylomicrons. As to how to enhance n-3 fatty acid absorption, Redgrave [3] suggested developing a chemically defined structured triglyceride with medium-chain fatty acids in positions 1 and 3 and an n-3 fatty acid in position 2. Pancreatic lipase and lipoprotein lipase would quickly hydrolyze the medium chains, releasing a monoacylglycerol with the n-3 fatty acid, which would then be able to enter cells rapidly. That seems an elegant solution, but it would be expensive and we need to see that it works. Our model is to some extent similar. We enhance hydrolysis and we also provide medium-chain fatty acids, with the idea of sparing the other fatty acids for more noble actions than oxidation.

Dr. Chioléro: Is it satisfactory to provide n-3 by the enteral route? Wouldn't it be preferable to infuse these fatty acids by the intravenous route?

Dr. Carpentier: We developed this preparation purely to obtain the effect that Alexander Leaf [4] was getting in his dogs. In routine clinical practice it is not easy to infuse free fatty acids, because you would have to bind them to albumin. This means that in addition to the costs and other problems of albumin, there is the expansion of the plasma volume to consider as well. Our model was developed to increase the availability of n-3 fatty acids within an hour or so after an injection. We had in mind that the anesthetist could inject the preparation at the start of an operation (mainly cardiac or vascular) to obtain a protective effect in patients who don’t listen to their doctors and do not eat a balanced diet. We have done experiments with similar particles, looking at the incorporation of α-tocopherol into lipoproteins in low-density lipoproteins (LDL) and blood cells. Thirty minutes after an injection, we found substantial incorporation into LDL, which are then protected against copper oxidation; within 1 hr we can almost double the α-tocopherol content of leukocytes and platelets. The enteral route will obviously take longer, and it is not really feasible in patients who have just been operated on.

Dr. Meguid: There is increased farming of fish which are fed on corn meal and so on. Is the n-3 the same, regardless of whether the fish is farmed or wild?

Dr. Carpentier: If you don’t feed your fish n-3 fatty acids, or at least α-linolenate, they will not convert those fatty acids into eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Farmed fish that are fed with n-3 fatty acids produce an oil that is adequately rich in EPA and DHA – it is not uncommon for oil from farmed salmon to contain up to 45% of both EPA and DHA. However, if the fish are fed with the wrong fatty acids they will not have these useful effects.

Dr. Waitzberg: With your strategy, are the lipids incorporated in the membrane to achieve these results, or is this more of a pharmacological effect that is not related to membrane incorporation of n-3 fatty acids?

Dr. Carpentier: What I can say at present is that we infuse these fatty acids while they are esterified in triglycerides, and within a matter of an hour or so we find them in the cell phospholipids. We still need to dissect the different cellular membranes and look at the incorporation of the fatty acids into these sites, which seem to be extremely important for cell signaling.
Dr. Waitzberg: So you challenge the idea that it requires 3 days to change the membrane composition?

Dr. Carpentier: Yes, it takes only a few hours.

Dr. Chioléro: As there are mixtures of n-3 fatty acids, and as you clearly showed us that the lipid mix is important, what is the best way to administer n-3, taking into account the various possibilities?

Dr. Carpentier: It depends on your aim. If you are feeding your patients parenterally and want to keep an unmodified fatty acid pattern, there are two emulsions that will be available in the next weeks or months. These are SMOF (Fresenius Kabi, Bad Homburg, Germany) and Lipoplus (B. Braun, Melsungen, Germany). They both contain 10% fish oil mixed with medium-chain triglycerides and either soy bean alone or soy bean and olive oil. I believe that this is a good way of supplying n-3 fatty acids, though when you look at the total of n-3 and the total of n-6 fatty acids in your patients you will find that the ratio isn’t altered. With pure fish oil preparations you will modify the n-3 to n-6 ratio, but there are limitations related to the slow elimination rate and by the fact that if you provide only that kind of fatty acid a substantial amount will be used for oxidation. I believe there is good reason to develop a preparation that will quickly modify the n-3 to n-6 ratio and optimize the incorporation of n-3 into cell phospholipids.

Dr. Grimble: Are structured lipids dead, in view of what we’ve heard from you today?

Dr. Carpentier: I don’t know if structured lipids are dead or not. It really depends on what we are talking about. What I call structured lipids are chemically defined lipids, where medium-chain fatty acids are specifically in positions 1 and 3 and where you put a fatty acid in position 2. This will be released with monoacylglycerol and will cross a cell membrane quite easily. What some companies call ‘structured lipids’ are anything but structured. They take medium-chain and long-chain triglycerides, hydrolyze them both, and let the fatty acid molecules re-esterify on the glycerol backbones at random, so you may find an MML or an LLM or an LML. What worries me is that some years ago we did studies comparing LML with MLM and found that the properties of these molecules are absolutely different. MLM is a fabulous substrate for lipoprotein lipase while LML is extremely resistant. And when you make an emulsion with these two preparations, one of them attracts cholesterol ester in an exchange mediated by CETP, while the other does not, because it is partitioned to a much greater degree in the phospholipid surface of the particle. So I believe when we discuss structured lipids, the structure should be chemically defined and not esterified at random.

Dr. Labadarios: You referred to acute phase lipoproteins. How would you define those and what would their functions be?

Dr. Carpentier: You all know that during an acute phase response there is increased production of C-reactive protein and other acute phase reactants. There is also marked production of an apoprotein called serum amyloid A. This is produced very rapidly and binds to high-density lipoproteins (HDL). It displaces the apoprotein A1 and to some extent apo A2, which are the structural apoproteins of HDL. The effect is to change the function of HDL, which is normally anti-atherogenic and antioxidiant. The altered HDL is directed towards sites of tissue damage and immune cells, probably to provide cholesterol and phospholipids for cells that are rapidly multiplying under these conditions. That single change in the composition of HDL completely changes its destination. HDL also carries large amounts of secretory phospholipase A2 and lysophospholipids, which substantially affect their anti-atherogenic effect. It is suggested that in the acute phase reaction the HDL may become pro-atherogenic and lose quite a lot of their protective effects, in particular α-tocopherol and the various enzymes that protect against LDL oxidation. I believe that transporting particles that provide cholesterol phospholipids and probably lipid-soluble vitamins to sites, where there is a need for
cell multiplication, is probably a useful mechanism, while at the same time we need to recognize that some of these modifications may lead to an increased risk of atherogenesis. As I see it, the acute phase is probably largely a survival response. We need to try to understand it better and modulate the features, which are apparently not helpful.

**Ms. Marino:** If you give an excessive amount n-3 fatty acids are they detrimental?

**Dr. Carpentier:** I believe they may be. For example, while Eskimos are protected against inflammatory reactions, cancers, and cardiovascular disease, they generally die of hemorrhagic stroke. It would be unwise to introduce any policy that provides a total imbalance of these lipids, though the American Heart Association has recommended an increased intake of n-3 fatty acid and even regular supplements in patients at risk of cardiovascular disease. It is also possible that n-3 fatty acids inhibit some functions of cellular immunity.

**References**