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Review

Membrane Lipid Replacement for chronic illnesses, aging and cancer using oral glycerolphospholipid formulations with fructooligosaccharides to restore phospholipid function in cellular membranes, organelles, cells and tissues☆

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ABSTRACT

Membrane Lipid Replacement is the use of functional, oral supplements containing mixtures of cell membrane glycerolphospholipids, plus fructooligosaccharides (for protection against oxidative, bile acid and enzymatic damage) and antioxidants, in order to safely replace damaged, oxidized, membrane phospholipids and restore membrane, organelle, cellular and organ function. Defects in cellular and intracellular membranes are characteristic of all chronic medical conditions, including cancer, and normal processes, such as aging. Once the replacement glycerolphospholipids have been ingested, dispersed, complexed and transported, while being protected by fructooligosaccharides and several natural mechanisms, they can be inserted into cell membranes, lipoproteins, lipid globules, lipid droplets, liposomes and other carriers. They are conveyed by the lymphatics and blood circulation to cellular sites where they are endocytosed or incorporated into or transported by cell membranes. Inside cells the glycerolphospholipids can be transferred to various intracellular membranes by lipid globules, liposomes, membrane-membrane contact or by lipid carrier transfer. Eventually they arrive at their membrane destinations due to 'bulk flow' principles, and there they can stimulate the natural removal and replacement of damaged membrane lipids while undergoing further enzymatic alterations. Clinical trials have shown the benefits of Membrane Lipid Replacement in restoring mitochondrial function and reducing fatigue in aged subjects and chronically ill patients. Recently Membrane Lipid Replacement has been used to reduce pain and other symptoms as well as removing hydrophobic chemical contaminants, suggesting that there are additional new uses for this safe, natural medicine supplement. This article is part of a Special Issue entitled: Membrane Lipid Therapy: Drugs Targeting Biomembranes edited by Pablo V. Escribá.

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Abbreviations: ABR, auditory brainstem responses; AD, Alzheimer's disease; AGEs, advanced glycation end products; CAPD, chronic ambulatory peritoneal dialysis; CDP-DAG, cytidinediphosphate-diacylglycerol; CFS, chronic fatigue syndrome; CL, cardiolipin; CR, caloric restriction; CVD, cardiovascular disease; DAG, diacylglycerol; DAMPs, damage associated molecular patterns; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; EPL, essential phospholipids; ETC, electron transport chain; FA, fatty acid; FDA, US Federal Drug Administration; F-MMM, Fluid–Mosaic Membrane Model; GRAS, generally recognized as safe; HDL, high density lipoproteins; HNE, 4-hydroxynonenal; IL, interleukin; LDL, low density lipoproteins; MAM, mitochondria-associated membrane; MDA, malondialdehyde; MetSyn, metabolic syndrome; MIM, mitochondrial inner membrane; MLR, Membrane Lipid Replacement; MOMP, mitochondrial outer membrane permeabilisation; MPTP, mitochondrial permeability transition pores; mtDNA, mitochondrial DNA; NCD, non-communicable diseases; NF-κB, nuclear factor kappa B; NLRP3, nucleotide-binding oligomerization domain (NOD)-like receptor protein 3; PAMPs, pathogen-associated molecular patterns; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; PS, phosphatidylserine; RNS, reactive nitrogen species; ROS, reactive oxygen species; TCA, tricarboxylic cycle; TLR, toll like receptor; TNFα, tumor necrosis factor alpha; T2D, type 2 diabetes; UCP, uncoupling protein.

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1. Introduction to Membrane Lipid Replacement

Membrane Lipid Replacement (MLR) is the oral supplementation of membrane glycerolphospholipids and antioxidants to provide replacement molecules for cellular membranes that are damaged during acute and chronic illnesses, cancer and aging [1–3]. Replacement membrane phospholipids are important for a variety of cellular and tissue functions and for general health [1–6]. For example, membrane glycerolphospholipids form the matrix for all cellular membranes and provide separation of enzymatic and chemical reactions into discrete cellular compartments and organelles. They are also essential for the function of a variety of membrane-intercalated and membrane-bound enzymes, and they afford cells with an important energy storage system [6–8]. Moreover, they provide precursors for bioactive molecules that function in signalling and recognition pathways [9–11].

Patients with chronic illnesses, and many with acute illnesses, aged individuals and cancer patients are frequently deficient in specific MLR phospholipids, because the usual dietary sources often cannot provide the amounts of MLR lipids needed for maintaining cellular membranes in undamaged states during illness [1–3,6,8]. MLR glycerolphospholipids in oral formulations [1–3], or MLR-similar bioactive lipids [9–11], have been used as supporting molecules for health maintenance and repair of therapy-damaged phospholipids, or as specific therapeutic treatments [1–3,9–12]. Essential glycerolphospholipids and their unsaturated fatty acids have been made into simple, safe, effective oral supplements that are quickly and efficiently absorbed in the upper small intestine within hours of ingestion [1–3,13].

There are multiple mechanisms for absorption of orally ingested glycerolphospholipids (for a more detailed discussion, see Section 5). The ingested phospholipids can be degraded into their constituent parts and these components absorbed; they can be taken in as intact molecules without degradation, or they can be absorbed as small lipid micelles, liposomes or phospholipid globules [3]. When present in excess in the gastrointestinal system, most phospholipids are absorbed undegraded [13]. The process appears to be driven by mass action or a 'bulk flow' process [3]. When in large excess, intact MLR phospholipids have an advantage in being able to reach their final destinations without significant degradation [14]. At their ultimate membrane sites the glycerolphospholipids can be enzymatically modified, such as substitution or modification of their fatty acid side chains or head groups, to reflect the specific compositional needs at their destinations.

It is important to replace membrane lipids frequently in most if not all acute and chronic illnesses, because cellular membranes are usually damaged in these conditions by oxidative free radicals, often produced by mitochondria [3,15,16]. During acute and chronic illnesses the concentrations of free radical reactive oxygen species (ROS), such as superoxide anion radicals, hydroxyl radicals or by hydrogen peroxide, and reactive nitrogen species (RNS), such as peroxynitrite anion, are drastically increased. Normally, natural cellular anti-oxidants neutralize these

free radical and other oxidants, but in various illnesses the concentrations of free radical and other oxidants are so high that the cellular anti-oxidants are unable to neutralize all of them. Thus excess free radical and other oxidants can damage cellular components [1–3,15,16]. Membrane phospholipids and their unsaturated fatty acids are especially sensitive to oxidative damage by ROS and RNS [3,15,16]. By oral MLR supplementation of membrane phospholipids various cellular membranes and other structures can be structurally and functionally restored [1–4,17].

Membrane lipids are vitally important to life, mainly because they fulfill four major requirements for cellular health [9,10]. They provide: (a) the matrix for all cellular membranes, permitting separation of enzymatic and chemical reactions into discrete cellular compartments; (b) energy storage reservoirs; (c) bioactive molecules that are used in certain signal transduction and molecular recognition pathways; and (d) functional molecules that interact with other membrane constituents, such as proteins and glycoproteins [3,18]. This last characteristic is an absolute requirement for the formation, structure and activities of cellular membranes [3,5,7,8,18].

2. Cellular membrane phospholipids and membrane structure

The most common membrane lipids of eukaryotic cells are glycerolphospholipids, and these are the precursors for many other membrane lipids [8,19]. As stated above, glycerolphospholipids are essential for membrane structure, but there are other important phospholipids, such as the sphingomyelins, that are also commonly found in cell membranes, in this case on their exterior surfaces [8,19]. Another common membrane constituent is cholesterol, which is the only sterol found in abundance in membranes [5,7,8,19]. MLR supplements do not contain cholesterol, but this important sterol is usually found in abundance in cells and tissues.

Membrane glycerolphospholipids have glycerol ester-linked fatty acid (FA) chains that are especially important for their properties. The chain length and saturation of the attached FAs of the phospholipids determine membrane packing and fluidity [8,18,19]. Unsaturated FAs, such as oleic acid and linoleic acid, confer a high degree of conformational flexibility of the unsaturated hydrocarbon chains within membranes due to their occupying a slightly wedge-shaped space, which results in looser packing and a more fluid membrane [5,8,20]. In contrast, saturated FA, such as stearic acid and palmitic acid, confer membrane rigidity, and this results in a less fluid or more rigid, more organized membrane [18].

Lipid compositional differences are characteristic of the different membranes of cells [8,19,21]. The concentrations of sterols (cholesterol and cholesterol esters) and sphingolipids (sphingomyelin, ceramide and gangliosides) increase from the endoplasmic reticulum to the cell surface [8,19,21]. For example, cholesterol/phospholipid ratios increase from the endoplasmic reticulum membranes to the plasma membrane

[8]. Within the same membrane there are also differences between the compositions of each side of the lipid bilayer; for example, sphingolipids, such as gangliosides, are quite asymmetrically distributed on the outer leaflets of plasma membranes [15]. Similarly, other neutral phospholipids, such as phosphatidylcholine (PC), are also found preferentially on the outer leaflet or surface of the plasma membrane, whereas anionic phospholipids, such as phosphatidylserine (PS) and phosphatidylinositol (PI), tend to reside on the inner leaflet of the plasma membrane. The asymmetric distributions of lipids between inner and outer membrane leaflets (as well as in the plane of the membrane) are important in determining membrane physical properties, such as deformation, curvature, compression, expansion, as well as functional interactions between membrane components [18,19,22–25]. For example, there are important differences in the lateral organization of lipids in the plane of the membrane in various domains [25,26]. The cooperative behavior between lipid components ensures that lipids organize laterally in a non-random, non-uniform fashion in the plane of a membrane resulting in the formation of membrane domains [25,26].

The formations of lipid domains in the matrix of cellular membranes are largely due to the interactions of glycerolphospholipids, especially PC and phosphatidylethanolamine (PE), along with sphingomyelins [20,22,25,26]. Under physiological conditions membrane phospholipids are present in various fluid, semi-solid and solid phases that are organized into domains characterized by different lipid spatial arrangements and rates of rotational and lateral movements [8,18,19,25,26]. The different lipid phases or domains in biological membranes have profound significance for their membrane activities and organizations [18,22,25,26].

The two fundamental membrane principals established over the last approximate century are that membrane lipids are present in a bilayer configuration [27] and a configuration that is non-symmetrical in its composition from one membrane side to the other [8,23,25,28]. Truly asymmetric lipid bilayers form the basic matrices of all biological membranes [7,8,23,25,26,28]. This was the basis for tri-layer models of membrane structure. The Tri-layer and Unit Membrane models were originally proposed with unfolded membrane proteins bound to the head groups of phospholipids on each side of a lipid bilayer [29–31].

For the last 40+ years the accepted basic model for all cellular membranes has been the Fluid–Mosaic Membrane Model (F-MMM) [32,33]. When it was first proposed, the F-MMM described biological membranes as a matrix of a fluid phospholipid bilayer with intercalated, mobile globular integral membrane proteins [32]. The original proposal, however, failed to take into account specialized lipid domains or regions of low lipid lateral mobility, data that was largely unavailable at the time the model was proposed. This was rectified a few years later in a more elaborate F-MMM [34]. Newer depictions of the F-MMM contain domains of fluid and structured lipids and integral membrane proteins, peripheral membrane proteins and membrane-associated protein complexes of cytoskeletal and extracellular matrix components [33,35].

Glycerolphospholipids are the major structural lipids in eukaryotic cellular membranes, and the most abundant members are: PC, PE, PS, PI, and phosphatidylglycerol (PG). The glycerolphospholipids contain hydrophobic diacylglycerol (DAG) tails of unsaturated or saturated FA of various chain lengths that constitute the main hydrophobic matrix of cellular membranes [18,25,26,28,32–35]. In mammalian cell membranes most FA molecules have at least one cis-unsaturated fatty acyl chain, which renders them fluid at room temperatures. Importantly, some membrane regions (domains) may not be in a fluid state [18–20,25,26,33,34]. Although PC usually accounts for greater than 50% of the content of phospholipids in eukaryotic cellular membranes, there are also significant percentages of PE, PI, PS and PG [8,18,19]. Another major class of membrane lipids, the sphingolipids, have hydrophobic ceramide backbones, and this class of lipids is mainly found on the exterior of cell membranes where some of these lipids display oligosaccharide chains [8,19].

Phospholipid composition can affect the curvature of a lipid bilayer. As an example, increasing PE to PC ratios in bilayers creates lateral curvature stress [36]. Lateral stress is important in conferring certain shapes that the lipid bilayer can assume, and these bilayer shapes can be seen in actual membrane structures, such as budding, blebbing, fusion and fission.

Interactions between the hydrophobic portions of membrane components must structurally match, or the membrane may be destabilized. Such hydrophobic structural matching, for example in glycerolphospholipids, is mediated mainly through protein-DAG acyl chain interactions [37]. Hydrophobic matching can be disrupted by oxidative modification of DAG acyl chains. This can be easily seen when FA acyl chains are disordered by oxidation [18], disrupting hydrophobic interactions and changing acyl chain packing. Such hydrophobic structural matching is thought to be facilitated by the conformational states of the lipid molecules or, more likely, by the selection of the appropriate glycerolphospholipids that provide the best hydrophobic match [25,38].

The hydrophobic portions of glycerolphospholipids are represented by their FA chains, and these occur in a variety of chain lengths and unsaturation states. FAs commonly found in dietary supplements are: oleic acid (9-octadecenoic acid; 18:1Δ9 or 18:1[n–9]), linoleic acid (9,12-octadecadienoic acid; 18:2Δ9,12 or 18:2[n–6]), alpha-linolenic acid (9,12,15-octadecatrienoic acid; 18:3Δ9,12,15 or 18:3[n–3]), and arachidonic acid (5,8,11,14-eicosatetraenoic acid; 20:4Δ5,8,11,14 or 20:4[n–6]) [39]. The cis-double bonds dramatically lower the melting points of phospholipids and increase their rotational properties [39,40]. This can lead to lipid lateral phase separation, lipid domain formation and differences in membrane fluidity [39]. Mammalian cells are unable to synthesize FAs with double bonds at certain specific positions, and thus some unsaturated FAs are considered essential dietary FAs [6].

Glycerolphospholipids that are synthesized are made, for the most part, in the endoplasmic reticulum, but some can be assembled in the inner mitochondrial membrane [41,42]. Their complete synthesis usually occurs in four steps: (1) synthesis of the backbone glycerol-3-phosphate molecule, (2) using FA acyl coenzyme A (CoA) attachment of FAs to this backbone to produce phosphatidic acids, (3) dephosphorylation to 1,2-DAG, and (4) addition of a hydrophilic head group, such as phosphocholine to make PC. Some glycerolphospholipids are synthesized by alterations of existing molecules, such as methylation of the ethanolamine group to form choline, or exchange of phospholipid head groups [41,42].

3. The mitochondrion and its phospholipid-containing membranes

Mitochondria have a dual membrane structure reminiscent of bacterial membranes [40,41]. The dynamic membranes of mitochondria possess discrete lipid compositions that display bilayer and lateral asymmetry. Some of their lipids are synthesized within mitochondria, while others are imported or transported into mitochondria as precursor lipids [41,42]. Between the membranes of mitochondria is an inter-membrane space, and inside the inner membrane is the mitochondrial matrix compartment. The matrix contains a complex mixture of enzymes as well as mitochondrial ribosomes, tRNAs, mRNAs and the maternally dominant mitochondrial DNA (mtDNA) [43,44].

The inner mitochondrial membrane (MIM) is the most metabolically active membrane of mitochondria. It is a highly complex structure that is freely permeable to oxygen, carbon dioxide, and water [45,46]. Embedded in the MIM are the four respiratory chain complexes, plus ATP-synthase (complex V), ubiquinone, and carnitine-palmitoyl-transferase II, most of which makes up the electron transport chain (ETC) [44–47]. Mitochondria use oxidative phosphorylation via the ETC to produce energy, using reducing equivalents from the TCA cycle. The ETC accounts for about 90% of cellular oxygen consumption and provides more than 80% of cellular energy [48].

Mitochondria provide other critical functions for cells, including the modulation of calcium signaling, regulation of cell death, the maintenance

of cellular redox balance, and innate immune signaling [49]. Mitochondria also contain important biosynthetic pathways, especially for certain lipids [47]. Because of their role in apoptosis, it is reasonable to claim that mitochondria function as gatekeepers of cell life and death [48–51].

Mitochondrial membrane phospholipids are composed of predominantly PE and PC. Mitochondria also contain the important tetra-acyl phospholipid cardiolipin (CL), which is unique to mitochondria and essential for their function. CL constitutes approximately 15–20% of the total mitochondrial phospholipid [52]. PE and CL are non-bilayer-forming phospholipids, which is best explained by their conical shapes. This allows the formation of hexagonal phases, depending on the pH and ionic strength [53]. PC and PE are abundant phospholipids that are present in all cellular and intracellular membranes. They are essential for cell survival, whereas CL is exclusively found in the MIM where it is required for oxidative phosphorylation, ATP synthesis, and mitochondrial bioenergetics. CL is functionally indispensable for MIM structure and function as well as for maintaining MIM transmembrane potential [54].

In the mitochondria CL is synthesized from PG and cytidinediphosphate-diacylglycerol by the enzyme CL synthase located on the inner surface of the MIM. Because of its location and structure, CL is highly sensitive to oxidation of its FA double bonds. For example, MIM CL possesses a high content of the unsaturated FA linoleic acid, with the exception of CL in the brain. Since CL is located adjacent to the site of ROS production in the MIM, it is at greater risk of oxidative damage than some of the other mitochondrial phospholipids. Oxidative damage to CL is of significant functional importance due to its role in maintaining MIM fluidity and osmotic stability, and its unique ability to interact with and stabilize respiratory chain proteins [54].

In terms of its most important property, providing function to the MIM, CL plays a central role in supporting the activity and organization of the mitochondrial respiratory chain. It binds to ETC complex III (cytochrome bc₁ complex) and complex IV (cytochrome c oxidase complex) that form high molecular weight super-complexes of the mitochondrial ETC. In doing this they support a system that allows for greater nutrient and precursor availability to ensure mitochondrial ETC function remains sustainable, even in periods of nutrient depletion and stress [54–56].

Mitochondria need to respond quickly to changes in MIM transmembrane potential. If this does not happen and mitochondria fail to adapt to changes in MIM potential, the result could be mitochondrial collapse, leading to mitochondria-selective autophagy, termed mitophagy, and associated cellular autophagy. Balancing mitophagy and mitochondrial biogenesis are essential for maintaining cellular homeostasis [57]. The ETC proton pump generates a noteworthy transmembrane potential of 150–200 mV across the MIM, yielding an equivalent field strength of about 30 MV/m [58]. Failure to maintain the MIM transmembrane potential results in the collapse of available cellular energy, increases free-radical ROS leakage and decreases active transport across the cell membrane.

Cellular stress caused by increases in cellular metabolic rate, hypoxia, mitochondrial membrane damage, among other events, all decidedly increase MIM ROS production [59]. Increases in intracellular ROS as well as release of ‘danger signals’ that include pathogen-associated molecular patterns (PAMPs), such as bacterial nucleic acids, peptidoglycans, lipopolysaccharides and sterile, host-derived, damage-induced molecules called damage-associated molecular patterns (DAMPs), are connected to cellular stress. Some cellular stress agents are caused by: K⁺ efflux, uric-acid crystals and extracellular ATP [60]. These stress agents induce the assembly of intracellular multi-protein inflammatory complexes called inflammasomes [61].

Inflammasomes are intracellular signaling platforms that are especially important in the detection of pathogenic microorganisms and sterile stressors as well as some environmental agents [62]. Of the known inflammasomes, the best characterized structure is formed by a pattern recognition receptor called NOD-like receptor pyrin domain

(NLRP3). The NLRP3 inflammasome is thought to sense sterile injury. These complexes, such as the nucleotide-binding oligomerization domain-like receptor (NLR) proteins, are a group of multimeric proteins consisting of an inflammasome sensor molecule, an adaptor protein ASC and caspase 1. As these multimeric protein complexes or inflammasomes form, they activate caspase 1, which in turn, proteolytically activate specific pro-inflammatory cytokines. As the pro-inflammatory cytokines are released, inflammation and a unique cell death program known as pyroptosis is initiated [63]. Innate immunity is also initiated via the NLRP3 inflammasome. This occurs through the maturation and release of pro-inflammatory cytokines. For example, the NLRP3 inflammasomes can be activated by ROS released from damaged mitochondria. This indicates that inflammatory immune responses are closely linked to mitochondria and their production of ROS [64].

4. Oxidative stress and free-radical signaling to biological membranes

Oxidative stress results from the production and eventual accumulation of surplus amounts of ROS (corresponding mainly to superoxide anion radicals, hydroxyl radicals and hydrogen peroxide) and reactive nitrogen species (RNS, corresponding mainly to peroxynitrite anion). When these ROS/RNS species are in excess of the production and amounts of natural cellular anti-oxidants, oxidative signaling and stress is the result [15,65–68]. Cellular targets of ROS/RNS include nucleic acids, proteins and lipids [16,46,65,66], although mitochondrial structures are especially sensitive to oxidative damage by ROS/RNS [46,47,66,67]. ROS/RNS can also be produced by several cellular pathways, including xanthine oxidase, NAD(P)H oxidases, monoamine oxidases, cyclooxygenases, lipoxygenases, among others [16,46,65]. There is increasing evidence that reactive oxygen species (ROS), peroxides and other reactive species formed on several proteins, lipids, and DNAs, can act as triggers for transductional signals. These signal transduction networks then act to maintain homeostasis and can prevent major changes in intracellular status, including alterations to redox potentials. Thus despite the entrenched notion that high levels of ROS can be deleterious for cells in terms of oxidative stress, low levels of mitochondrial ROS appear to act as signaling molecules of intracellular pathways important for the maintenance of physiological functions, including proper cellular differentiation, tissue regeneration, and prevention of aging (discussed in Section 8). This process has been termed redox biology, and it appears to fluctuate in response to stressors and subsequently promote adaptation to environmental changes.

The MIM, and specifically the mitochondrial ETC, is an important source of cellular free radical oxidants [46,47,67,69]. Thus ROS are found in abundance near the MIM and are produced as a consequence of direct oxygen reduction at sites outside complex IV [47,67,69]. Usually the concentrations of ROS/RNS are relatively low in cells, and any damage that is a consequence of ROS/RNS reactions is constantly being repaired [15,65,69]. Moreover, low concentrations of ROS are used in cell signaling and may be important in the aging process through the induction of mitochondrial hormesis or cellular responses to low levels of toxins [70]. But at higher concentrations ROS/RNS are toxic to cells and can damage their membranes [67,69,70]. To counteract the damaging effects of ROS/RNS, mitochondria are equipped with enzymatic and non-enzymatic systems to control the production and concentrations of ROS/RNS and prevent their build-up inside cells [65,67,70]. As discussed above, excess ROS/RNS can cause damage to mitochondria and can stimulate mitophagy and apoptosis.

In terms of specific membrane damage, ROS/RNS causes oxidative damage to unsaturated FA, CL and other lipid molecules [15,65,69]. ROS/RNS can also damage DNA and proteins [65–67]. In addition, functional changes in proteins and lipids occur with ROS/RNS reactions. For example, ROS can stimulate opening of L-type voltage-sensitive calcium channels, resulting in increased intracellular calcium concentrations, as seen in neurodegeneration and stroke patients [69–71]. Once they have

been generated, ROS/RNS can penetrate mitochondrial and other cellular membranes and diffuse outside cells to cause widespread damage to tissues [72].

ROS/RNS release results in oxidation and peroxidation of double bonds in unsaturated FA of phospholipids, and these free radicals can also react with other cellular molecules, eventually resulting in the formation and release of their metabolic end products into blood, such as malondialdehyde (MDA), 4-hydroxynonenal (HNE), 4-oxo-2-nonenal and acrolein [15,65]. These reactive end-products can covalently bind to protein thiol groups and other cellular materials, and this can negatively affect protein and enzyme activities or functions [15,65]. Oxidative stress and lipid peroxidation end-products turn out to be identifiable circulation markers of inflammation, diabetes, atherogenesis and neurodegeneration [15,68,69,72–74].

An important process during oxidative stress is the ROS/RNS free radical reaction with mitochondrial CL. CL reaction products have been implicated in mitochondrial dysfunction, and their appearance is associated with several pathological conditions, including diabetes, heart failure, hyperthyroidism, neurodegeneration and aging. These conditions are characterized by excess oxidative stress, CL deficiency, and increases in docosahexaenoic acid (DHA) [75]. Other glycerolphospholipids in mitochondria are also sensitive to ROS/RNS reactions due to their unsaturated FAs [76,77]. In fact, there is a direct relationship between the amounts of unsaturated FA in mitochondria and their abilities to maintain a productive proton gradient across the MIM [78].

In mitochondria, the end-products of oxidized-unsaturated FA are very important in inducing apoptosis via reaction with mitochondrial permeability transition pores (MPTP) [79,80]. MPTP are voltage-dependent channels that initiate calcium-dependent apoptosis. Increased mitochondrial ROS production results in oxidized-unsaturated FA reaction with MPTP that initiates Ca^{2+} release, thus modifying Ca^{2+} cell signaling and causing mitochondrial calcium loading. The mitochondrial calcium loading further increases ROS production, reiterating the process until mitochondria swell. The process eventually results in cell death [81]. However, dietary supplementation with unsaturated FA can modify mitochondrial unsaturated FA composition and alter mitochondrial Ca^{2+} homeostasis. This can delay MPTP opening and Ca^{2+} -induced apoptosis [82].

5. Lipid globules, droplets, liposomes, phospholipids and their transport

MLR glycerolphospholipids taken orally are usually absorbed in the upper small intestines as individual molecules or their constituent parts, or when present in excess, they can be transported relatively intact in small phospholipid micelles, globules or liposomes (Fig. 1) [3,8,83,84]. Some hydrolysis of glycerolphospholipids occurs in the stomach, but most phospholipid enzymatic degradation takes place in the small intestine. There FA and other parts of degraded glycerolphospholipids are transported across the epithelial cell barrier [85–87]. However, when present in the gastrointestinal system at high concentrations, most glycerolphospholipids are absorbed relatively undegraded in phospholipid micells, globules and small liposomes in an endocytotic process, not as individual molecules or their constituent parts (Fig. 1a). This could be an evolutionary adaptation to enhance phospholipid transport and thus survival when high concentrations of essential foods are only intermittently available.

Glycerolphospholipid absorption in the upper intestines has been found to be very efficient. After a large meal, over 90% of glycerolphospholipids are absorbed and transported into the blood within six hours [86,87]. In the blood circulation limited amounts of glycerolphospholipids are usually found in carrier molecules, such as lipoproteins, or in the cell membranes of erythrocytes. However, when present in excess, they can also be found in blood in lipid globules, liposomes and other forms [88]. Eventually the glycerolphospholipids are

delivered to tissues and cells where they are transferred by direct contact of lipoproteins and erythrocytes with cell membranes or by endocytosis of phospholipid micells, globules, liposomes and other forms by endothelial cells.

One problem with direct incorporation of dietary MLR polyunsaturated phospholipids into membrane structures is that they can be oxidized and degraded during their storage, ingestion, digestion and adsorption in the intestinal lumen. Therefore, to be fully available for use oral MLR phospholipids must be protected during storage and from acid degradation in the gut as well as disruption by bile salts and hydrolysis by phospholipases and other enzymes released from the pancreas and gut microflora in the small intestines [89]. This has been accomplished by complexing MLR phospholipids with specific fructooligosaccharides, called inulins, which insert between the head groups of glycerolphospholipids and protect them from excess temperatures, acidity, phospholipases and bile salts [90,91]. Inulins also protect MLR glycerolphospholipid FA from oxidation [91].

When fructooligosaccharides (inulins) are used to protect glycerolphospholipid micelles, liposomes and lipid globules, these forms can be absorbed relatively intact into gastrointestinal brush border cells (Fig. 1a) [92]. Although some hydrolysis of phospholipids will occur during this process, when in excess, most of the micellar and globule phospholipids are absorbed intact by brush border cells as unoxidized, undegraded phospholipids [89].

Using electron microscopy morphological studies have shown that undigested dietary lipids and phospholipids are present in the small intestinal brush border cells mainly as small lipid micelles, globules or larger droplets (50–1,000 Å in diameter) [14,93]. When in a protected form and in excess with respect to intestinal enzymes, the phospholipids are transported by endocytosis or pinocytosis into intestinal cells as largely intact molecules [14,94]. Although microscopic methods cannot distinguish the lipid compositions of the ingested lipid globules, droplets and chylomicrons, these forms are not present in the intestinal cells in fasting controls, indicating that they are likely derived from lipids that were previously present in the intestinal lumen [14].

As mentioned above, intestinal absorptive cells can also transport individual phospholipid molecules and their degradation products, such as FAs, using specific membrane transport systems. Thus after intestinal cell transport, glycerolphospholipids could also accumulate inside brush border cells and re-associate to form micelles, small liposomes or phospholipid globules [94]. It is thought that this form of individual molecule transport is less important when phospholipids are present at excess in the intestinal lumen [94].

In addition to the endocytotic transport of phospholipid micelles, globules, liposomes and other structures and the binding and transport of individual phospholipid molecules or their FA and other constituent parts, intestinal brush border cell membranes can directly partition phospholipids into their outer plasma membrane leaflets. For example, it was observed that intestinal microvillous plasma membranes became thicker on their outer surfaces during phospholipid absorption, and this was attributed to the direct insertion of phospholipid molecules into the outer leaflets of brush border microvilli membranes [95]. This may also serve another function. Once phospholipids like PC are enriched in the plasma membranes of the cells of the colonic mucosa, they appear to help protect this structure from pathogenic processes like ulcerative colitis and other chronic inflammatory conditions. It has been proposed that they do this by modulating the signaling state of the mucosa, a regulatory component of the inflammatory signaling pathway [96].

Individual glycerolphospholipids that are incorporated into the outer plasma membrane leaflet of colonic brush border cells can be transported into these cells by binding to transmembrane phospholipid-translocase proteins (flippases, floppases and scramblases) that can transfer the phospholipids to the opposite membrane surface [97–99]. The translocated phospholipids can then be partitioned to protein carriers that transfer the phospholipid molecules to intracellular membranes or cellular organelles [100–101], or they can be stored inside

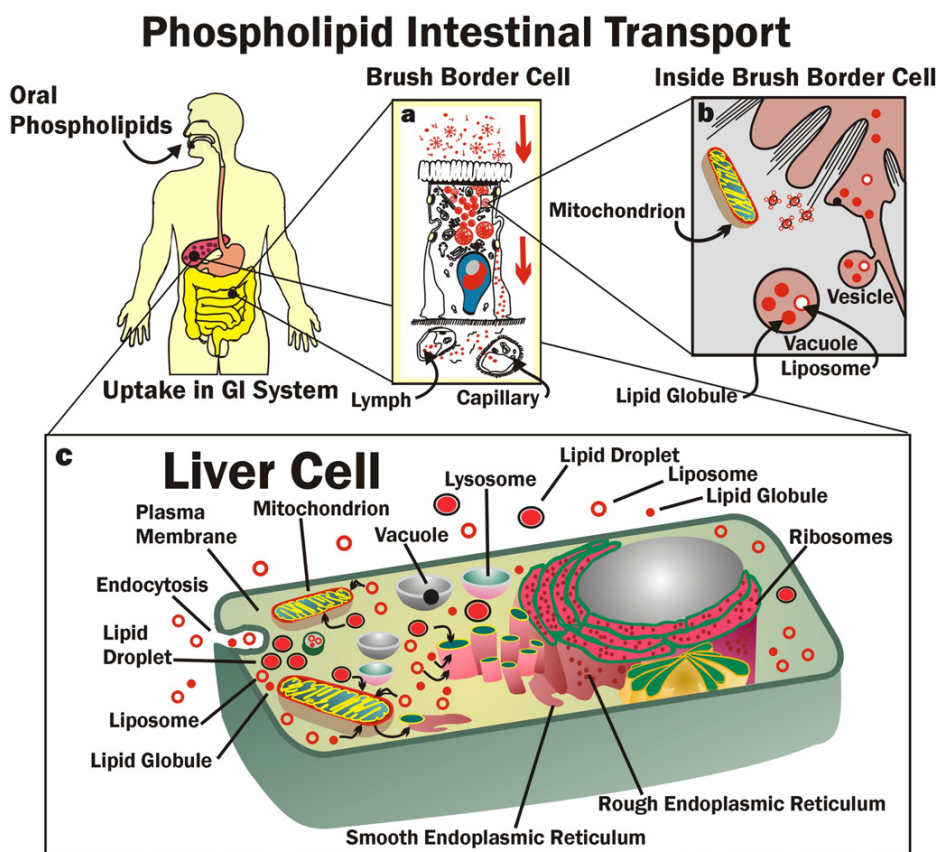


Fig. 1. Some phospholipid transport systems involved in delivery of MLR phospholipids to intracellular membranes. Most orally ingested phospholipids are initially absorbed and transported in the upper small intestines by brush border epithelial cells after their dispersion and enzymatic digestion. (a) MLR phospholipids are usually protected from complete disruption and enzymatic degradation by bound fructooligosaccharides, permitting transportation into cells as small glycerolphospholipid micelles, vesicles, globules and other forms by endocytotic processes. At the distal or basolateral regions of the brush border cells excess lipid vesicles and globules can be extruded by a reverse exocytotic/pinocytotic process and eventually transported to lymph and blood vessels. (b) The endocytotic transport of lipid droplets and vesicles into brush border epithelial cells is shown in more detail. In addition, some of the phospholipids are absorbed by the epithelial cell plasma membrane and transported as simple phospholipids or their degradation products by phospholipid carrier molecules to organelle membranes, including mitochondria. (c) A liver cells is shown with internal lipid micelles, vesicles, globules and lipid droplets. These structures can bind to various intracellular membranes and transfer glycerolphospholipids and pick up damaged lipids. Not shown in the figure are lipid transport/transfer by direct membrane-to-membrane contact and lipid droplet-, globule-, and vesicle-to-membrane contact or temporary fusion with intracellular membranes or by protein lipid carriers. (Modified from Nicolson and Ash [3]).

cells as vesicles, globules or lipid droplets and transferred to various compartments as needed [102,103]. Also, the flipping of phospholipids to the inner surface of the plasma membrane and their build-up in the inner membrane leaflet may promote formation of membrane blebs that are then released as new vesicles by inducing membrane curvature [98]. The redundancy of this entire transport process may indicate its critical role in cellular physiology.

Once inside cells, there are different ways that phospholipids can be moved and stored. Using intracellular membrane-membrane contact, contact of small vesicles and lipid globules with intracellular membranes, movements of phospholipid carriers or transport proteins and other processes can send phospholipids by fission and fusion events to various cellular and organelle membranes and compartments [102–106]. Along their transport routes, especially in the ER, and at their ultimate destinations, the glycerolphospholipids can undergo enzymatic modifications, for example, head group substitution or modification of their FA side chains. This may be done to modify glycerolphospholipids to reflect the specific compositions of the membranes at their final destinations. Importantly, the overall process appears to be driven by a 'bulk flow' or 'mass action' process [107], so when present in excess, glycerolphospholipids have an advantage in being able to reach their final destinations, even with enzymatic modifications along the way, but without their wholesale destruction. In addition, this 'bulk flow'

process may also explain the removal of damaged phospholipids from intracellular membranes by a reversal of this process.

Although less is known about the roles of small phospholipid micelles, vesicles and small globules inside cells, there is considerable information available on larger lipid structures (chylomicrons and lipid droplets) [108–110]. Intracellular lipid droplets have been defined as structures that are composed mainly of neutral lipid cores containing a coat of PC and other glycerolphospholipids (PE, PI and lesser amounts of others). Lipid droplets appear to be the principle lipid storage system for many cells, such as adipocytes, hepatocytes, and other cell types. Their identification as cellular lipid storage compartments many years ago has now been expanded to include important roles in cellular lipogenesis and homeostasis [109,110]. They also appear to be important in pathogenic processes, such as metabolic syndrome (MetSyn), fatty liver diseases, steatohepatitis, atherosclerosis, and other diseases [109,111]. Lipid droplets also have proteins on their exteriors that are used to regulate the size, structure, number and fate of these intracellular lipid storage systems [109].

Glycerolphospholipids and other lipids can be delivered to various membranes and organelles via carrier or transport proteins, mainly lipoproteins, or by lipid micelles, globules or other structures, as discussed above, and they can be stored inside cells as lipid droplets and other structures. These lipid-containing structures are found in

excess during fat absorption and storage [14,94,112]. One of these structures (chylomicrons) have been determined to be large, triglyceride-rich lipoproteins that function to transport ingested lipids to different tissues [113]. Not only can chylomicrons store lipids—tri- and monoglycerides, cholesterol, phospholipids and FAs, among others—in cells, they are also used to transfer lipids to various compartments, such as the endoplasmic reticulum, Golgi and other organelles, and even to adjacent cells [14,94,113].

Phospholipids inside cells have several fates. They can be utilized, metabolized, stored or transferred to other cells or to the surrounding extracellular fluid environment. For example, small vesicles and globules released from Golgi membranes of mucosal cells can be formed into larger chylomicrons, and these structures have been observed to be transported to the basolateral surfaces of brush border cells for release by a reverse pinocytosis or endocytotic process. Eventually they find their way to the cells lining the lymph or circulatory systems [14]. The pinocytosis/endocytosis and transport processes can be repeated until the lipid globules and other structures are eventually released into the lymph or blood for transfer to other organs and tissues.

In addition to lipid transport as micelles, vesicles, globules, membranes and other forms [14,102], lipid transfers inside cells make use of a wide variety of protein lipid carriers or transfer proteins, each specific for a given type of lipid or lipid class [100,101]. As discussed above, these lipid transport systems usually function on a bulk flow or mass action basis where sources that contain high concentrations of certain membrane lipids deliver their excess lipids to membranes that have lower concentrations of particular lipids.

Some of the membrane phospholipid-binding or transfer proteins have been isolated and examined and found to have preference for unsaturation of FA or FA with different acyl chain lengths. Thus intracellular phospholipid transfer proteins from human erythrocytes distinguish unsaturated FA acyl chains of varying lengths [114]. However, a membrane phospholipid transfer protein isolated from bovine liver preferred PC with long chain unsaturated FA (fluid phase PC) [115]. Once they arrive at their destinations, membrane phospholipids can also be modified enzymatically, and their FAs and head groups can be replaced to reflect the compositions of the membranes at their final destination [116]. As mentioned above, the bulk flow or mass action delivery of glycerolphospholipids to particular membrane sites may also be used to remove oxidized or damaged lipids from membranes and eventually degrade them or export them from cells to eventual delivery to the intestines for removal in stool [3,117].

In addition to using lipid micelles, vesicles, globules and larger lipid structures as well as protein carrier transport systems, there is an additional mechanism for transferring lipids to specific cellular compartments. Intracellular membranes and organelles can transfer lipids by direct contact and partitioning of phospholipids from one membrane to another, often via specific membrane domains. For example, the ER and mitochondria can transfer membrane lipids by direct contact-transfer through specific ER membrane domains called the mitochondria-associated membrane (MAM) [118,119]. It also appears that organelles have their own specific lipid transport systems to move phospholipids to specific regions of these structures. For example, mitochondria possess membrane lipid transport protein complexes that help shuttle membrane phospholipids between inner and outer mitochondrial membranes, probably to insure appropriate phospholipid composition of the MIM and maximal exchange of damaged membrane phospholipids with undamaged phospholipids, such as occurs during mitochondrial fusion [120–122]. There are also lipid transfer proteins in the intermembrane space between inner and outer mitochondrial membranes, and these may also be important in the transfer of glycerolphospholipids and FA between different mitochondrial compartments [122].

In the blood circulation phospholipids, steroids, FAs and other lipids can be bound to plasma carrier molecules, absorbed by lipoproteins, such as high- and low-density lipoproteins (HDL and LDL), or bound

to blood cells, such as erythrocytes. Lipoproteins are an important transport system for phospholipids in the blood circulation. Once phospholipids and other lipids are bound to lipoproteins, they are usually protected from oxidation and enzymatic digestion during transport compared to individual molecules. In man, the amounts of membrane phospholipids exchanged and preferentially transported by HDL lipoproteins are more than 20-times the amounts transported by erythrocytes [123].

An added bonus is that the excess MLR phospholipids can help remove cholesterol from the circulation by changing the phospholipid composition of erythrocyte membranes and circulating lipoproteins and in the process displacing cholesterol [123–125]. Once excess cholesterol is removed [126], it can be partitioned into circulating MLR phospholipid globules and other lipid forms and delivered back to intestinal cells for eventual export by the gastrointestinal system [3, 124].

Glycerolphospholipids transported in the circulation by blood cells, lipoproteins and lipid globules and vesicles eventually arrive at the microcirculations in organs and tissues. There the membrane phospholipids and other lipids can be transferred to the plasma membranes of endothelial cells. This process appears to be almost the reverse of the transfer of membrane phospholipids from gut endothelium to the blood and then to tissue and organ cells and eventually to intracellular membranes.

As described above, the entire sequence appears to follow a ‘bulk flow’ or ‘mass action’ process for MLR phospholipids along a concentration gradient from the gut to tissues and back again for damaged/oxidized phospholipids. This is a natural removal process that can help reduce cholesterol in membranes, cells and tissues.

6. Membrane Lipid Replacement compositions and methods

There are some limitations in the use of dietary sources, oral supplements or intravenous introduction of MLR phospholipids (a formulation called “essential” phospholipids or EPL) for the safe replacement of damaged membrane lipids [3]. For example, the average uptake of total dietary membrane lipids is considered to be in the range of 2–6 g per day [6]. Plant sources of polyunsaturated membrane glycerolphospholipids, such as legumes or cabbage, are thought to be a good source for dietary MLR supplementation [3,6, 127]. However, the amounts of plant material, such as soy beans, required to obtain a daily dose of approximately 1.8 g of membrane phospholipids is approximately 15 kg of beans [127]. Thus the exclusive use of dietary plant sources for membrane phospholipids is unappealing and impractical [3]. In addition, in most dietary sources, membrane phospholipids are unprotected from oxidation, disruption and degradation before and during digestion. In contrast, oral MLR supplements can deliver therapeutic doses of membrane phospholipids as part of a daily supplement regimen, and certain oral MLR supplements, such as NTFactor®, are protected from oxidation, bile disruption and enzymatic digestion using protective fructooligosaccharides and antioxidants [1–3,90,91].

Oral supplements for MLR utilize mixtures of glycerolphospholipids and unsaturated FA, such as n-3 and n-6 unsaturated FA, and other lipid components derived from various sources: legumes, milk, liver, fish, krill, among other sources [3,6,111,128–130]. Many of these supplement formulations have been tested in laboratory animals for their functional properties. For example, animals supplemented with n-3 unsaturated FAs showed changes in mitochondrial membrane phospholipid FA composition, improved mitochondrial function and altered Ca^{2+} -induced mitochondrial permeability transition pore function [131]. This was accomplished by replacing CL FAs with specific unsaturated FAs to improve inner membrane fluidity and ETC function. By feeding rats for 10 weeks with an unsaturated FA supplement that modified cardiac mitochondrial CL FAs, O’Shea et al. [132] found

improvements in Ca^{2+} -induced mitochondrial permeability transition pore function.

The most convenient, efficient, safe and cost effective method of membrane phospholipid administration in humans has been the use of daily oral lecithin supplements [1–3,6]. Most oral lecithin supplements are rather crude soy, egg yolk or marine preparations that lack oxidation, bile and phosphatase protection. In addition, most of these oral supplements have not been carefully analyzed for phospholipid composition, and in particular for lipid degradation products. However, there are oral MLR phospholipid supplements, such as NTFactor® and NTFactor Lipids®, that fulfill the requirements for efficacy, oxidation and degradation protection, safety and convenience [1–3]. The NTFactor® lipid supplements, and their use in clinical studies, will be discussed in more detail in Sections 8–11. NTFactor®, which also contains probiotic bacteria, growth media and other ingredients, and NTFactor Lipids®, without these additives, come in several oral forms, but almost all contain from 1–2 g of phospholipids per dose [1–3]. The recommended optimal daily oral dose of NTFactor Lipids® for most clinical conditions has been estimated at 2–4 g per day, and more recently at least 4 g per day, whereas its anti-aging use has been proposed at 2 g per day [2]. Some updates in these recommendations will be discussed in Section 12.

NTFactor®-containing supplements, such as Propax™, also contain several vitamins, minerals and other ingredients. Indeed, certain NTFactor®- and NTFactor Lipids®-containing supplements can be compositionally complex, such as a specific oral supplement for mitochondrial support (ATP Fuel®) that contains a daily dose of 4 g NTFactor® and also Coenzyme-Q10, NADH, alpha-ketoglutaric acid, L-carnitine, vitamin E and other ingredients [133]. All of these oral supplements contain some antioxidants, such as low concentrations of vitamin E and CoQ10, to protect the phospholipids and unsaturated FA from oxidation during storage and ingestion and fructooligosaccharides (inulin) to protect the phospholipids from temperature effects and acid, enzyme and bile effects in the gastrointestinal system [3]. The lipid composition of NTFactor® (and NTFactor Lipids®) is shown in Table 1.

Other MLR oral phospholipid preparations, such as PS supplements, have been used for specific purposes, such as to treat memory loss in aged subjects or in Alzheimer's disease (AD) patients. In the case of AD patients, supplementation with 300 mg per day of bovine PS for 6 months resulted in cognitive improvements compared to placebo controls [134]. Unfortunately, this result was not always seen. In another study on elderly subjects with age-associated memory impairment that received 300–600 mg PS daily for 12 weeks significant differences were not seen [135].

Although taking a single class of glycerolphospholipid alone, such as PS, has been shown to have health benefits, the use of more complex mixtures of membrane phospholipids containing PC, PS, PE, PI, etc. are considered more beneficial [1–3,133,136]. As mentioned above, the NTFactor® and NTFactor Lipids® also contain protective fructooligosaccharides and antioxidants.

MLR phospholipids, such as PC, have been introduced intravenously, such as in acute cases of toxic liver, kidney and gastrointestinal damage, hepatitis, dialysis, and other conditions [4]. Administration of membrane phospholipids ("essential" phospholipids or EPL) can deliver high phospholipid concentrations without the need for fructooligosaccharides to inhibit intestinal disruption, but they are still susceptible to enzymatic and oxidative damage. In addition, daily intravenous delivery comes with some risk for adverse events, such as infection, blood vessel damage, thrombosis, pruritus, dyspnoea and urticaria. It is also much more expensive, and administration must be professionally supervised. EPL intravenous preparations, such as Essentiale®, contain 1 g phospholipids, mainly PC (>75% PC), with some PE, PI and other phospholipids, ethanol, tocopherol, ethylvanillin, vitamins B6, B12, nicotinamide, and sodium D-pantothenate [4]. Other phospholipid products can be found in Küllenberget al. [6].

Table 1

Lipid composition of MLR product NTFactor Lipids®,^{a,b}

Abbreviation	Name	Percent [w/w]
<i>Glycerolphospholipids</i>		
PC	Phosphatidylcholine	31.62
PI	Phosphatidylinositol	24.87
PE	Phosphatidylethanolamine	18.86
PA	Phosphatidic acid	13.88
DGDG	Digalactosyldiacylglycerol	5.88
PG	Phosphatidylglycerol	2.37
LPC	Lysophosphatidylcholine	0.98
LPE	Lysophosphatidylethanolamine	0.70
PS	Phosphatidylserine	0.48
MGDG	Monogalactosyldiacylglycerol	0.31
<i>Fatty Acids</i>		
18:2Δ9,12 (n–6)	Linoleic acid	58.41
16:0	Palmitic acid	19.39
18:1Δ9 (n–7)	Oleic acid	9.68
18:3Δ9,12,15 (n–3)	Linolenic acid	5.87
18:0	Stearic acid	3.90

^a Modified from Nicolson [1]

^b NTFactor Lipids® is a patented product produced by Nutritional Therapeutics, Inc. of Hauppauge, NY

7. Safety of Membrane Lipid Replacement formulations in animal and clinical studies

One of the most important characteristics of MLR natural oral supplements is that these supplements are incredibly safe [1–3]. Preclinical and clinical studies on MLR have not shown any evidence of acute or chronic toxicity, including high dose effects, perinatal and postnatal toxicity, and mutagenic and carcinogenic potentials. Thus there has been no indication that would indicate any possible risk in ingesting these supplements. None of the preclinical studies, which were mostly conducted in laboratory animals (mice, rats and rabbits), have demonstrated any acute or chronic toxic effects of MLR phospholipids given by oral, subcutaneous or intravenous administration [3]. In addition, multiple studies on toxic or lethal doses could not establish in laboratory animals any toxic or lethal dose levels of MLR supplements.

In terms of mutagenicity and carcinogenicity, no dose levels of MLR phospholipids could be determined that caused any mutagenic or carcinogenic events. In mice, rats, rabbits and dogs daily oral doses up to 3.75 g/kg body weight produced no toxic, mutagenic or carcinogenic effects [3]. Using regimens of single or repeated dose administration no toxicity could be established in young, adult, pregnant or fetal laboratory animals. During pregnancy, no toxicity was found in pregnant rats or rabbits or in their fetal offspring at doses up to and above 1 g/kg (reviewed in [3]).

Any possible effects of MLR phospholipids on known carcinogens were also examined in laboratory animals receiving carcinogens and simultaneous administrations of MLR phospholipids. In these studies, MLR glycerolphospholipids actually inhibited the formation of tumors in animals (reviewed in [4]). As an example of this, supplementation of pure PC in rats actually reduced preneoplastic liver nodule formation [137]. Such studies along with the lack of any evidence of toxicity in clinical trials resulted in the U.S. Federal Drug Administration (FDA) classifying oral MLR phospholipids as 'Generally Recognized as Safe' (GRAS) [138].

Laboratory animals have received MLR phospholipids over long periods of time with no evidence of any toxic or pharmacologic effects [3]. For example, the pharmacological effects of MLR phospholipids on rodents was examined in life-term studies [17]. The MLR phospholipids were given in chow or water daily at doses ranging from 0.01 to 5 g/kg body weight. No effects were found in the central or peripheral nervous systems, including reflexes, analgesic, spasmolytic or spasm-influencing functions, renal function, heart and vascular function, or other measures of pharmacological toxic effects [17]. Lifetime administration of MLR phospholipids placed in the chow of laboratory rodents has shown

that MLR glycerolphospholipids were beneficial not harmful [3]. For example, Seidman et al. [153] examined the protective effect of feeding rodents MLR phospholipids on age-related hearing loss and mtDNA deletions associated with aging. Rats aged 18–20 months were fed MLR phospholipids (NTFactor®) or placebo for 6 months and their auditory brainstem responses (ABR), MIM potentials and mitochondrial DNA deletions examined every two months. ABR responses were determined by measuring hearing thresholds and sensitivities, and MIM membrane potentials were assessed with redox dyes. Using blood lymphocytes labeled with rhodamine-123 and monitoring fluorescence with a flow cytometer, any loss of MIM trans-membrane potential could be ascertained in large numbers of cells. Also, the presence of any DNA deletions in the aged rodents were established by extracting DNA from various brain regions. Using amplification of mtDNA sequences (ND1-16srRNA and other sequences) deletions of known mtDNA sequences lost during aging could be verified [139].

In the Seidman et al. study on aged rodents there were significant differences between the experimental groups of animals receiving MLR glycerolphospholipids and placebo groups in terms of ABR, MIM potential and the presence of mtDNA deletions [139]. After 4-months of administration of NTFactor®, there were significant preservations of hearing threshold at all frequencies tested in the experimental group, whereas in the placebo group the loss of hearing measured by increased threshold was apparent. In addition, NTFactor® prevented age-related decline in MIM trans-membrane potential and reduced the incidence of common mtDNA deletions found in aged rats. The anti-aging effects of the MLR phospholipids were attributed to the ability of NTFactor® to repair mitochondrial and other membranes and to the abilities of the polyunsaturated FA in NTFactor® to reduce the effects of ROS damage to mtDNA [139].

In humans no evidence of any toxicity of MLR phospholipids has also been found. For example, high doses of MLR phospholipids have been given to humans with no evidence of any toxicity or adverse events. In cases of hepatic encephalopathy due to decompensated liver cirrhosis, patients received 2 g per day of EPL intravenously for several weeks with no apparent adverse events. Patients receiving EPL showed significant improvements in their liver disease status and had significantly prolonged survivals compared to a control group that did not receive MLR phospholipids [140]. They also showed no evidence of any toxic effects of the MLR phospholipids. In terms of single glycerolphospholipids, patients with cardiovascular disease were entered into phase I/II clinical trials where the MLR phospholipid PI was given at doses over 5 g per day. Over time the PI was found to increase plasma HDL and apolipoprotein A-1 levels and reduce triglyceride levels with no evidence of any toxicity or adverse effects [141].

The use of relatively high doses of MLR phospholipids in long-term studies in humans has shown that subjects actually improved in their cardiovascular blood markers. In this study on 35 subjects (average age 60.7) who received at least 4 g per day oral NTFactor® for over 6 months beneficial results were found. During the study participants showed no evidence of any adverse events. On the other hand, their cardiovascular blood marker levels, such as homocysteine, improved [142]. Similarly, 58 patients (average age 55.0) with fatiguing illnesses received doses of 4 g per day oral NTFactor® for 2 months without incident [133]. A follow-up on these patients found that most had continued using the MLR supplement for over one or more years without any adverse events. The long-term use of MLR phospholipids in clinical studies on cardiovascular diseases has been reviewed elsewhere, and the conclusion was that there is no evidence of MLR glycerolphospholipid toxicity in man [143].

As discussed above, MLR phospholipids have been shown to be safe and effective, and they have been found to have a positive impact on human health (reviewed in [1–4,136,142–146]). Most clinical studies have used oral MLR phospholipids in the dose range of 1.5–4 g per day or intravenous administration in the dose range of 0.5–2 g per day [1–4,6,142–146]. MLR phospholipids have been obtained from soy,

egg, milk and marine sources and have been used in doses over 4 g per day orally or intravenously in doses over 2 g per day with no adverse effects. In a few cases does up to 45 g of MLR glycerolphospholipids were given orally without any adverse effects [147]. In fact, the use of MLR phospholipids actually reduced the adverse or side effects of drugs and other treatments [3,4,6,144–146].

8. Membrane Lipid Replacement in aging and energy metabolism

The signalling networks involved in the aging process are linked to a progressive accumulation over time of deleterious changes, such as reduction of physiological functions, increased fatigue, declining cognitive functionality and increased chances of diseases and death. Multiple theories have been advanced to explain the manifestation of accumulated cellular damage, but the complex mix of genetic and environmental factors involved suggests that the causes of normal aging are multi-factorial, and no single mechanism can explain all of its aspects. However, some elements of the aging process are better understood than others, and some of the molecular processes involved, including those related to membrane lipid exchanges and damage, may open up a better understanding of age-related molecular changes [148–152].

The hallmarks of aging are: (i) genomic instability, (ii) telomere attrition, (iii) mitochondrial dysfunction, (iv) cellular senescence, (v) epigenetic alterations, (vi) loss of proteostasis, (vii) deregulated nutrient sensing, (viii) stem cell exhaustion and (ix) altered intercellular communication, and (x) inflammation [148–150,152]. Of these, inflammation appears to be the most ubiquitous, in that human aging is characterised by a state of chronic, low-grade, progressive sterile inflammation (inflamm-aging), the causes of which remain unexplained [149]. Moreover, it is a primary hallmark of innate immune receptors triggered by endogenous cellular debris that are driven, in part, by increased age-related production and also by a declining ability to dispose of them via programmed autophagy and mitophagy [149,152]. Inflamm-aging is macrophage-centered, involves several tissues and organs, including the gut microbiota, and is characterised by a complex balance between pro- and anti-inflammatory responses that are increasingly linked to a wide spectrum of age-related organ disorders [150,152,153].

The inflamm-aging process impairs cellular and mitochondrial housekeeping, leading to protein aggregation and accumulation of dysfunctional mitochondria. Outcomes from this include: compromised oxidative phosphorylation, reduced MIM trans-membrane potential and increased permeabilization of the outer mitochondrial membrane [151]. Subsequent increases in ROS/RNS production and oxidative stress, can result in other lipidomic changes, such as loss in membrane fluidity due to lipid peroxidation and decreased CL content [154–156]. These are likely linked, in turn, to the reported age-related declines in mitochondrial function and other established markers of aging [154].

Importantly, MLR can beneficially impact on these events. For example, NTFactor® use in aged subjects demonstrated improved mitochondrial function, reduced fatigue and increased cognition, suggesting that the phospholipid lipid membranes of the mitochondria were functionally improved through oral MLR supplementation with NTFactor® [157]. From a research perspective, use of redox dye probes to monitor the MIM potential of the subjects' mitochondria [158] showed that the MIM potential of the aged group was restored to the same level of function as that displayed by a healthy 29 year-old control [157].

Aging has been found to be inversely related to the mitochondrial content and quality of unsaturated phospholipids [159]. Loss of membrane fluidity resulting from lipid peroxidation, as well as decreases in mitochondrial CL content, and altered activity of the respiratory chain are also features of aging [152–155]. Therefore, the regulation of phospholipid homeostasis in mitochondrial membranes through their biosynthesis, degradation and transport into and out of the mitochondrial membranes plays a crucial role in maintaining cellular viability and health and the minimization of adverse age-related changes. For

example, as discussed in Section 7, membrane translocases are generally ATP-dependent [98,99], and as ATP production declines so does the utilization of existing membrane- and serum-derived lipids for the purpose of achieving asymmetrical membrane structure and composition required to optimise age-related changes [160].

Mitochondrial production of ATP is directly linked to the regulation of synthesis, trafficking, and degradation of glycerolphospholipids and of MIM trans-membrane chemical/electrical potential [161]. Thus the provision of lipid substrates via oral MLR supplements or diet manipulation represent attractive, safe options for managing age-related membrane decline [1–3]. Dietary interventions have previously demonstrated improvements in a subset of mitochondrial FA oxidation disorders, suggesting that appropriate oral MLR supplementation and ingestion have viable roles to play in phospholipid replacement and membrane repair [162].

Numerous molecular events involving mitochondria, and in particular their membrane permeabilization, contribute to declining mitochondrial function and the release of important innate response structures called damage-associated molecular patterns (DAMPs) [163]. In addition to their impact on MIM quality and reduction in ATP production, they also bind to numerous pattern recognition receptors (PRRs). These trigger numerous defense responses, and importantly, DAMP concentrations can increase with age [164,165]. At the level of the MIM function-related cardiolipin remains vulnerable to oxidation, and susceptible to being relocated to other membrane compartments as a consequence of changes in phospholipid concentrations [166–168].

The use of MLR to inhibit CL peroxidation and relocation as well as providing precursor molecules for CL synthesis may reduce the risk for age-related alterations in apoptosis pathways. In addition, CL is also involved in the import and assembly of proteins [169]. The majority of mitochondrial proteins are nuclear-encoded and require protein translocases to be transported to the mitochondrial membranes. The importance of this system relies on its role in providing approximately 90% of the cellular ATP molecules necessary for cell survival and membrane reorganization [152,170–172].

The programmed cell death process known as autophagy and the more specific organized destruction of mitochondria known as mitophagy are highly sensitive to redox signalling and the concentrations of phospholipids in mitochondrial membranes [172–174]. Aspects of age-related changes are, in turn, related to accumulation of dysfunctional mitochondria resulting from the combination of impaired clearance of the damaged organelles by autophagy and inadequate replenishment of mitochondria by mitochondriogenesis as well as maintenance of optimal unoxidized membrane lipids to maintain MIM trans-membrane potential [174,175].

Fragments of mitochondria released during such degradation are especially prone to evoke “danger signals” as they are structurally related to their prokaryotic ancestors and many PRRs recognize molecular patterns of bacterial origin. Although nuclear molecules have important structural and genetic regulatory roles inside the cell nucleus as described below, when released into the extracellular space during cell death, they can acquire immune activity and act as DAMPs.

The release of DAMPs from these damaged membranes can be recognized by a range of PRRs, including the intracellular danger-sensing multiprotein platforms called *inflammasomes* [60–62,173–175]. When oxidized and released into the cytosol, mtDNA are recognized by Nodd-like receptor P3 (NLRP3) inflammasomes, leading to IL-1 β maturation and release. Inflammasomes are thus important intracellular molecular platforms capable of detecting perturbations in cytoplasmic homeostasis for the detonation of inflammation. Upon binding with either foreign (non-self) antigens or misplaced and/or damaged self-molecules, including cardiolipin, ATP, and urate crystals, among others, inflammasomes activate caspase 1, which leads to the maturation of certain cytokines, such as IL-1 β , a powerful pro inflammatory cytokine that contributes to age-related decline [176].

The triggering event of ROS, such as superoxide (O(2)(–)) and hydrogen peroxide (H(2)O(2)) production by damaged mitochondria, can also stimulate inflammasome formation via their role as sterile inflammation or para-inflammation promoters [177–179]. Mitochondria that remain productive, yet are avoiding appropriate mitophagy, can release up to ten-fold more hydrogen peroxide than their stable counterparts, potentially triggering yet more sterile inflammatory responses [180]. Yet nitric oxide may actually inhibit the triggering of the keystone NLRP3 inflammasome, suggesting a co-dependent oxidation relationship for the purpose of maintaining innate immune activation and subsequent adverse age-related changes in various tissue membranes [181].

Most attention has focussed on ROS and RNS, because of their damaging effects, yet evidence of their importance in regulating and maintaining normal homeostatic processes in living organisms has been accumulating [182]. Consequently a ‘redox regulation’ role seems to better describe the redox status of mitochondria and its consequences. MIMs require redox balance, and this is a significant challenge during the aging process [152]. In addition, age-related mitochondrial dysfunction is also linked to inflammaging and ‘self garbage’ production (mtDNA, ATP, cardiolipin, or formylpeptides) that can be sensed by macrophages and other immune PRRs [153].

Studies using FAs to manipulate the expression of NLRP3 inflammasomes indicate that omega-3 (n–3) unsaturated FA, such as DHA and EPA, exhibit anti-inflammatory properties via their inhibition of the inflammasome [183,184]. This is likely achieved through various mechanisms, including the manipulation of cell membrane lipids as well as inhibition of primary and secondary triggers, in particular, through the expression of NF κ B [185]. There is a distinct possibility of using of MLR dietary lipids to inhibit inflammation and compressing the rate of mitochondrial-induced DAMP production through reduction of membrane permeabilization and thus limit age-related inflammaging.

Numerous mechanisms are involved in age-related changes in mitochondria and mtDNA, including: (i) increased disorganization of mitochondrial structure, (ii) decline in mitochondrial oxidative phosphorylation (oxphos) function, (iii) accumulation of mtDNA mutations and deletions, (iv) increased mitochondrial production of ROS, (v) increased extent of oxidative damage to DNA, proteins, and lipids and (vi) insufficient antioxidative enzymes [3,153,186–196].

MLR provides protection for ROS-related damage to mtDNA, membrane proteins and lipids, and a decline in MIM trans-membrane potential. MLR also provides substrates for CL synthesis and glycerolphospholipids for membrane repair [1–3,6,8,12,120,136,146]. Thus MLR is capable of decreasing or preventing age-related mitochondrial stress-induced adverse effects and may have significant potential in the reduction of age-related disorders [139,142,157]. What is clear is that an essential aim for a healthy and long life is not simply to have low levels of provocative and proinflammatory compounds, but to be able to reach a balance between inflammatory and anti-inflammatory responses. The role of nutrition, and especially the quality and quantity of nutritional substrates, is becoming a better understood aspect of healthy aging, and is leading to safer and more effective interventions.

Mitochondria can be improved by the mechanisms of fission and fusion, and their numbers can be improved by biogenesis of mitochondria and the associated turnover of their components [197,198]. These processes are also determined by lipid and protein quality and availability. Fission is coordinated with DNA replication and is essential for mitochondrial duplication and biogenesis, which is a requirement for cell division and is strongly connected to cell cycle. Fission is also an essential phase of mitophagy, which allows recycling of sections of mitochondria that have become dysfunctional or damaged [197]. Fusion is the process by which mitochondria become interconnected. Through fusion, damaged mitochondria may acquire undamaged genetic material and maintain functionality, including the resynthesis of essential proteins [197–199].

Mitochondrial fusion can occur quickly, within a 12 hour window, and it is mediated by mitofusins utilising a three stage process [200]. These are: (i) The mitochondria align themselves, end to end, (ii) the outer membranes of the two organelles fuse with each other, (iii) the inner membranes fuse with each other, thus forming a larger intact mitochondrion [196,201]. Numerous biological signals modulate mitochondrial function, and it is only when mitochondria are fine-tuned, healthy, and efficient that all of their multiple and highly energy-demanding processes can occur normally. Fusion is a rescue mechanism for impaired mitochondria by the reorganisation of their contents (proteins, lipids and mtDNA) and the unification of the mitochondrial compartment. These events play important roles in cellular development, healthy aging and energy production [202, 203].

The use of MLR to support mitochondrial fission and fusion may be reflected in increased ATP synthesis, decreased membrane permeabilization, increased MIM potential, diminished DAMP production and eventually decreased levels of fatigue and improvements in various organ functions, such as cognition and mood [139,157].

The flexing of nutrient intake without malnutrition also offers mitochondrial repair benefits, and accordingly age-related alterations in food consumption, and may advance mitochondrial damage or facilitate its biogenesis and repair. Mitochondrial co-factors, such as Coenzyme Q10, as well as vitamin E, curcumin, EFAs, resveratrol and other components have shown improvements in aspects of age-related mitochondrial changes [204–207]. The indications are that MLR, either as a sole supplement or as part of a nutrient and lifestyle intervention, can enhance redox and associated alterations and improve membrane functionality that could contribute to meaningful improvements in age-related mitochondrial disorders [3].

9. Membrane Lipid Replacement in fatiguing illnesses: chronic fatigue syndrome, fibromyalgia, Gulf War illnesses

One of the most common complaints of patients seeking general medical care is chronic fatigue [208,209]. Fatigue is a poorly understood symptom, and it is considered to be a complex, multidimensional sensation that is perceived as loss of overall energy and feeling of exhaustion and inability to perform tasks without excessive exertion [12,209–211]. At the cellular level moderate to severe fatigue is related to loss of mitochondrial function and reduced ATP production [211–213].

Damage to mitochondria and especially mitochondrial membranes by ROS/RNS occurs during aging and in essentially all chronic and acute medical conditions [3,211,213]. Patients with excess fatigue, such as in chronic fatigue syndrome, possess evidence of oxidative damage to their DNA and lipids [214,215]. They also have oxidized blood markers and oxidized membrane lipids that are an indicator of excess oxidative stress [216,217]. Patients with chronic fatigue syndrome also have sustained, elevated levels of peroxynitrite due to excess nitric oxide [218]. Excess peroxynitrite can also result in lipid peroxidation and loss of mitochondrial function. Peroxynitrite can also stimulate changes in cytokine levels that can have a positive feedback effect on nitric oxide production [218].

In terms of incidence, fatigue is the number one symptom found in chronic medical conditions, and it is also one of the most common symptoms in cancer patients [12,145,146]. For example, it occurs in cancer patients from the earliest onset of cancer to the most progressive forms of metastatic disease [12,219,220]. Cancer-associated fatigue, pain and nausea, are the most common and disabling symptoms reported by cancer patients [219,220]. Although cancer-associated fatigue is not well understood, it is thought to be a combination of the effects of the cancer on the patient plus the effects of cancer treatments that are known to fatiguing [12,221].

Cancer-associated fatigue was until recently an uncommonly treated condition [12,219]. In general, cancer-associated fatigue was thought to be unavoidable, especially during and after cancer therapy [12,219].

Since cancer is also associated with depression and anxiety [222], historically cancer-associated fatigue was not considered an organic problem [12,219]. Fatigue or loss of energy is a core aspect in the diagnosis of depression [222,223]. Thus fatigue and depression are both often diagnosed in cancer patients, and they are considered to be part of a clinical symptom cluster or co-morbidity of cancer [223].

Cancer-associated fatigue can vary from mild to severe among different patients, and this is also true of therapy-associated fatigue, which is often seen during cancer therapy [12,145,146]. Fatigue is often mentioned as a significant reason why patients discontinue anti-cancer therapy [221]. Indeed, 80–96% of patients receiving chemotherapy and 60–93% receiving radiotherapy reported moderate to severe fatigue, which continued for months or even years after the cancer therapy was terminated [224].

MLR has been used to reduce cancer-associated fatigue and limit the fatigue caused by cancer therapy [12,144–146]. MLR supplements, such as NTFactor®, reduced cancer-associated fatigue approximately 30–40% [12,146]. In addition, MLR supplements have also been used to reduce the adverse effects of cancer therapy, such as fatigue, nausea, vomiting, malaise, diarrhea, headaches, insomnia, constipation and other adverse events [12,144–146]. For example, using a combination supplement mixture containing NTFactor® Colodny et al. [225] were able to reduce several common adverse events during and after cancer chemotherapy in colon, rectal and pancreatic cancer patients. In advanced metastatic cancers MLR supplements, such as Propax™ with NTFactor®, were used to reduce the adverse effects of treatment with multiple chemotherapy agents. MLR supplementation resulted in significantly fewer episodes of fatigue, nausea, vomiting, diarrhea, constipation, skin changes, insomnia and other effects. Eighty-one percent of the cancer patients on chemotherapy that used the NTFactor® supplement also experienced an overall improvement in quality of life parameters. In another part of the Colodny et al. [225] study a double-blind, placebo-controlled, cross-over trial was conducted on patients with advanced cancers undergoing combination chemotherapy. These patients were also given a MLR supplement containing NTFactor®. During therapy the patients had fewer and less severe adverse effects of the combination chemotherapy. For example, MLR with NTFactor® resulted in improvements in the incidence of fatigue, nausea, diarrhea, impaired taste, constipation, insomnia and other symptoms [225].

MLR has been used in several studies on fatiguing illnesses to reduce fatigue in patients with severe chronic fatigue (for quantitative data and statistical analyses on MLR fatigue reduction in various clinical conditions, see Table 2 in ref. [1], Table 1 in ref. [2], and other reviews on the subject [3,12,136,146]). For example, the effects of NTFactor® on fatigue in moderately fatigued subjects were determined to see if mitochondrial function improved in concert with reductions in fatigue using NTFactor® [157]. In this single-blinded, cross-over clinical trial there was good correspondence between reductions in fatigue and gains in mitochondrial function as assessed by increases in MIM trans-membrane potential using a redox dye. As discussed previously, mitochondrial function is directly related to MIM trans-membrane potential. After 8 weeks of MLR with NTFactor®, mitochondrial function had significantly improved, and after 12 weeks of NTFactor® supplementation, mitochondrial function was found to be similar to that found in young healthy adults [157]. Specifically, there was a 26.8% increase, ($p < 0.0001$) in mitochondrial function, measured by MIM trans-membrane potential. After 12 weeks of supplement use, subjects were switched from MLR to placebo without their knowledge for an additional 12 weeks, and their fatigue and mitochondrial function were again measured. After the 12-week placebo period, fatigue and mitochondrial function were intermediate between the initial starting values and those found after 8 or 12 weeks on the supplement, suggesting that continued MLR supplementation is likely required for further improvements in mitochondrial function and maintenances of lower fatigue scores [157]. Similar results on the effects of NTFactor® on fatigue were found in patients with chronic fatigue syndrome (CFS/ME) and/

or fibromyalgia syndrome, Gulf War Illness or chronic Lyme Disease [1–3,12,133,226,227].

Combination mitochondrial supplements have proved useful for fatiguing illnesses [3,133,146,213,226]. Supplements containing NTFactor® have also been used in combination MLR studies with other ingredients to treat long-term chronic illness patients with moderate to severe fatigue [3,133,227,228]. In these studies patients had been ill with intractable fatigue for an average of over 17 years and had been seen by an average of more than 15 physicians. In addition, they had taken an average of over 35 supplements and drugs with no effect on their fatigue [133]. Within a few weeks on the combination MLR supplement ATP Fuel®, they responded and showed significant reductions (30.7%, $p < 0.001$) in fatigue. Regression analysis of the data indicated that the reductions in fatigue were consistent, occurred with a high degree of confidence ($R^2 = 0.960$ for overall fatigue) and were gradual [133]. Thus the ATP Fuel® proved to be a safe and effective in significantly reducing fatigue in patients with intractable chronic fatigue [133,227].

Elsewhere mitochondrial cocktails have been proposed for mitochondrial cytopathies and mitochondrial dysfunction [213,228]. In his review on the subject Tarnopolsky has discussed the use of antioxidants, alpha lipoic acid, vitamins, coenzyme Q10, lycopene, creatine, riboflavin and other supplements, but not MLR supplements [229]. In a recent (2015) consensus report on the management of mitochondrial diseases from the Mitochondrial Medicine Society treatment using MLR supplementation was also not considered [230]. MLR supplements need to join vitamins and other supplements in the treatment of mitochondrial dysfunction and mitochondrial diseases [213].

10. Membrane Lipid Replacement in degenerative diseases: neurodegenerative and other diseases

Non-communicable degenerative diseases (NCD) are primarily non-infectious diseases whose prevalences increase with age or certain behaviors. The most important NCDs by prevalence and mortality rates are: cardiovascular diseases (CVDs), neoplastic diseases and neurodegenerative diseases. The CVDs include: hypertension, cardiopathies, coronary heart disease, myocardial infarction, cerebrovascular incidents, such as strokes, neurodegenerative diseases, such as Alzheimer's disease, Parkinson's disease, multiple sclerosis, among others. Other NCDs such as chronic respiratory diseases, autoimmune diseases and arthritis are also important. Obesity, metabolic syndrome (MetSyn), type 2 diabetes (T2D), hypertension, and other related diseases will be discussed in the next section.

Nutrition and environmental stress have been identified as major modifiable determinants of NCDs [231–235]. As discussed in Section 9, the proper maintenance of mitochondria is essential in order to prevent degenerative processes from occurring, leading to aging and disease. These outcomes share some basic mechanisms, such as the involvement of mitochondrial age and function [236]. Mitochondrial dynamics, such as fission and fusion to improve mitochondrial function, play critical roles in ensuring and maintaining mitochondrial health. However, if high concentrations of fats or other nutrients are consumed in excess, molecular damage may occur and spread through a population of mitochondria and affect their collective performance [237]. Computer simulations suggest that mitochondria can optimally produce ATP when they are undamaged or only marginally damaged, or alternatively if the damage is repaired, for example with MLR. When mitochondria are damaged by free radical oxidants, mitochondrial dynamics can be unfavorable and fission and fusion will often fail to recover function, suggesting that nutritional support favoring optimal mitochondrial dynamics, such as with appropriate MLR, antioxidants and other nutrients along with appropriate caloric restriction, will result in a health advantage and the availability of ample cellular energy [238].

Defects in mitochondria, systemic inflammation, and oxidative stress are the primary cause of most NCDs. Thus improved

mitochondrial bioenergetics along with positive changes in lifestyle and nutritional intervention have the potential to increase mitochondrial function and thus eventually improve health [238,239].

There are therapeutic approaches that are currently available to potentially slow down age-related functional declines that predispose a population to NCDs [240]. These include: insulin sensitizers [241], exercise to promote mitochondrial fusion and increase collective mitochondrial function [242], and targeted antioxidant treatments to reduce ROS/RNS damage to mitochondria [243]. However, the effectiveness of most existing antioxidants alone is limited, because most antioxidants are not selective for mitochondria and fail to penetrate to the MIM [243]. In addition, unlike ubiquinone or orally bioavailable mitochondria-targeted antioxidants, including MitoQ, MitoVitE, and MitoTEMPOL or tocopherols, most orally ingested nutritional antioxidants do not attach covalently to lipophilic triphenylphosphonium cations to facilitate transport across the glycerolphospholipid outer membranes into the mitochondria [244]. Other possible therapeutic approaches, such as caloric restriction or reduction in caloric intake without malnutrition or intermittent fasting, are contentious, and their benefits have not been fully determined [245].

Manipulating cellular bioenergetics represents a unique method to treat or prevent NCDs and inflammatory and immune diseases through optimal functioning of mitochondria and reprogramming of the inflammatory and metabolic responses. Using a combination of protected glycerolphospholipids and antioxidants MLR studies have shown that mitochondrial bioenergetics can be improved in animals and humans [1–3,12,136,144,145,157,226–228]. It also has the added benefit of potentially reducing inflammation [225]. Unfortunately, western diets with excessive sugar and saturated fats along with environmental insults can lead to mitochondrial dysfunction and higher susceptibilities to inflammation, apoptosis, NCDs, cancers and premature aging [246–248]. Lifestyle and behavioral changes combined with proper nutrition and MLR to provide important mitochondrial support could be an important strategy to prevent NCDs [249,250].

Recently one of the pathways involved in regulating mitochondrial function has received considerable attention. It concerns the mammalian target of rapamycin or mTOR, a well-conserved serine/threonine kinase that regulates cell growth in response to nutrient status [251]. There are two important mTOR complexes, and dysregulation of their activities is closely associated with aging and various NCDs, including diabetes, cancer and neurodegenerative diseases [252].

Signaling by TORC1 regulates mitochondrial biogenesis, oxidative stress and turnover in mammals and lower organisms. Also, defects in the clearance of damaged mitochondria by TORC1-regulated autophagy contribute to ROS/RNS accumulation [253]. By sensing the abundance of various nutrients and regulating the activity of critical processes such as autophagy and translation, the TORC1 signaling pathway lies at the intersection between nutrient, environmental and innate mechanisms of aging and NCDs. Caloric restriction without malnutrition and periodic bouts of short-term nutrient excess [254] and specific inhibitors of mTOR, such as rapamycin [255], beneficially affect mitochondrial function. This suggests that mitochondria are highly responsive nutrient sensors and effectors, some of the implications of which are discussed in the next section.

The natural sensitivity of mitochondria to energetic substrate levels and their ability to dynamically undergo morphology changes that influence the function and the integrity of the mitochondrial genome constitute a novel mechanism to explain long-term modulations of health and disease [55]. The use of MLR along with lifestyle changes may present opportunities to enhance mitochondrial fusion, reduce inflammation and oxidative stress and ultimately aid in the management, prevention and treatment of NCDs. An example of this is a small pilot trial in which MLR supplementation modified metabolism through body mass reduction and appetite restraint [256]. The study enrolled 30 subjects (mean age = 56.8; 24 females and 6 males) who used oral NTFactor® (500 mg) and alpha-amylase inhibitor (500 mg) 30 min

before each meal. Participants were told to eat and exercise normally. In the study weight, waist and hip measurements were taken weekly, and blood samples were taken prior to and at the end of the study for lipid and chemical analyses. Appetite and sweet cravings were assessed weekly by standard methods, and fatigue was determined using a fatigue instrument.

In the study described above, 63% of the participants lost an average of 6.11 ± 0.28 lb (2.77 ± 0.12 kg), along with significant average reductions of 2.51 ± 0.05 inches (6.4 ± 0.13 cm) and 1.5 ± 0.04 inches (3.8 ± 0.10 cm) from waist and hip circumferences, respectively. The entire group lost an average of 3.63 ± 0.13 lb (1.65 ± 0.11 kg) ($p < 0.001$) with average reductions of 1.59 ± 0.03 inches (4.04 ± 0.06 cm) and 1.13 ± 0.02 inches (2.87 ± 0.05 cm) from waist and hip circumferences, respectively. Weight loss and body measurement decreases were gradual, consistent and significant, along with reductions in body mass index (BMI) and basal metabolic rate (BMR). Overall hunger was significantly reduced 44.5%, with reduced cravings for sweets and fats, and there was a 23.9% reduction in fatigue. Along with fatigue reduction there was a 26.8% perceived improvement in cognition and ability to concentrate, remember and think clearly. Blood lipid profiles at the end of the trial suggested improved cardiovascular lipid profiles, and there were no adverse events reported during or after the study [256].

There is some evidence that the effects of MLR on weight loss in clinical studies may be due to the replacement of glycerophospholipids containing unsaturated FAs. Vögler et al. [257] fed rats a diet high in stearic, elaidic, oleic, linoleic and 2-hydroxyoleic acids or control for 7 days and found that in the test animals food intake was lower, and the animals lost body weight, mainly through reduction of adipose fat. Only treatment with C-18 oleic acid or 2-hydroxyoleic acid induced body weight loss (3.3 and 11.4%, respectively). They attributed the effect to enhanced energy expenditure due to changes in uncoupling protein-1 (UCP1) expression and phosphorylation state of the cyclic AMP-response element-binding protein CREB [257].

Other MLR membrane phospholipids have been used to modify NCD-related functional decline, including supplementation with oral PS to improve memory loss and cognition. For example, in one 12-week pilot study 30 male and female subjects (age 50–90 years, average 74.6 years) with memory impairments unrelated to neurological disease, stroke, intracranial hemorrhage, brain lesions, diabetes, infections or inflammatory processes were used to determine if 300 mg PS per day modified outcomes in 6 different tests of memory and cognition [258]. When the study ended, participants tested with significant improvements in memory recognition, recall, total learning, executive functions, mental flexibility, and visual spatial learning. There were no adverse events during the trial, and interestingly both mean systolic and diastolic blood pressure values were reduced in comparison to baseline [258]. Similarly, a double-blind, randomized clinical trial on 78 subjects (50–69 years) was initiated to determine if 100–300 mg oral PS per day versus placebo affected memory. In this study PS supplementation significantly improved behavioral memory functions, especially short-term memory and cognitive function in low-scoring (delayed word recall) patients without any adverse events or changes in vital signs or laboratory tests [259].

11. Membrane Lipid Replacement in metabolic diseases: metabolic syndrome, diabetes and cardiovascular diseases

The precursor to type 2 diabetes (T2D) and most cardiovascular diseases (CVD) is metabolic syndrome (MetSyn). MetSyn is made up of several interrelated disturbances of glucose and lipid homeostasis in obese individuals, including insulin resistance, changes in blood lipid profiles, abnormal glucose tolerance, hypertension and vascular inflammation, as well as a background of multiple genetic abnormalities [260, 261]. There are a number of major MetSyn risk factors, such as: (i) abdominal obesity, (ii) elevated fasting plasma glucose, (iii) arteriogenic

dyslipidemia (increased triacylglycerols, increased LDL and reduced HDL), (iv) elevated blood pressure, and (v) the presence of prothrombotic and proinflammatory molecules [260,261]. MetSyn has also been called Syndrome X [262] or insulin-resistance syndrome [263], and it is estimated that in the age group over 60 in North America, over 40% have some symptoms of MetSyn [263]. These same risk factors are also found in CVD, T2D, hypertension, and other diseases [260,264, 265].

The most important factors in the cause of MetSyn have been proposed as: obesity, genetic factors and endogenous metabolic susceptibility, such as manifested by insulin resistance and other characteristics [260–263]. From the multiple risk factors listed above and the laboratory test results listed below, a diagnosis of MetSyn can be made, although there is still some discussion as to the relative merits of using MetSyn diagnostically in clinical practice [266]. Insulin resistance, increased abdominal fat, genetic factors, physical inactivity, advancing age, inflammation and endocrine dysfunction also help establish MetSyn, which when combined with additional laboratory identifiable risk factors, such as high LDL, low HDL, high triacylglycerols, elevated blood glucose, elevated plasminogen activator inhibitor-1 and c-reactive protein, among other tests, have been found to increase the likelihood of MetSyn-associated diseases later in life [261,267].

One of the earliest signs of MetSyn is insulin resistance [268]. When the circulating concentrations of insulin are insufficient to regulate the above processes, insulin resistance occurs. This, in turn, can lead to clinically diverse syndromes, such as type A syndrome, leprechaunism, Rabson-Mendenhall syndrome and T2D [268,269]. There are several additional factors that are involved in (or characterize) insulin resistance and MetSyn: (i) multiple genes; (ii) epigenetics, such as nutrition, low birth weight, among others; (iii) visceral obesity; (iv) body-mass index; (v) caloric and carbohydrate intake; (vi) sedentary lifestyle; (vii) age; (viii) ethnicity; (ix) gender; (x) menopausal status; (xi) alcohol consumption; (xii) inflammation; and (xiii) dysbiosis [268–270].

One of the important inflammation-associated mechanisms involved in the generation of metabolic disorders is the activation of the NLRP3 inflammasome through various triggers, including mitochondrial DAMPs [271], lysosomal membrane disruption [272] and high fat diets [273]. The activation of this inflammation complex contributes to the development of visceral adiposity and insulin resistance. Because of its wide distribution in different tissues and organs, the NLRP3 inflammasome complex may represent a signaling pathway that facilitates organ metabolic damage [274]. The NLRP3 inflammasome also regulates the gastrointestinal microbiome and is activated by pathobionts and associated dysbiosis, which can affect host susceptibility to metabolic disease onset and progression, including non-alcoholic fatty liver disease, obesity and T2D [275].

Lipids are critically involved in MetSyn and associated diseases, and defects in the capacity to metabolize FAs and glucose can play important roles in insulin resistance and MetSyn [276]. Accumulations of DAG, triacylglycerol and free FAs in non-adipose tissues correlate strongly with insulin resistance [277], and increases in released, free fatty acids may block insulin signal transduction [267]. Lipids, such as DAG, have been implicated in insulin resistance by activating isoforms of protein kinase C, which in turn can directly modulate insulin signaling by phosphorylating and inhibiting the insulin receptor tyrosine kinase and activating genes responsible for FA-induced inhibition of insulin activity [277]. Differences in gene expression in adipose tissue are thought to be responsible for increasing the secretion of MetSyn-related factors, such as the pro-inflammatory cytokine TNF α and the tissue-specific protein adiponectin [278]. In muscle tissue decreased oxidative capacity and fat accumulation may also induce skeletal muscle insulin resistance and contribute to the development of T2D [276].

Conditions like MetSyn, T2D, and their associated diseases are caused by multi-factorial events, but various studies point to mitochondrial dysfunction as a major component [276,279,280]. For example, use of microarrays to monitor gene expression revealed that several

oxidative genes were found to be down-regulated, supporting the notion that mitochondrial dysfunction occurs in T2D [281]. Also, in T2D muscle there was decreased ETC activity as well as whole body anaerobic capacity, which also indicates mitochondrial dysfunction [282]. In addition, in T2D genetic polymorphisms have also been found that are involved in FA oxidation and in factors that control transcriptional activities relevant to mitochondrial function [270].

MetSyn and T2D patients both show reduced fat oxidative capacities and increases in release of FAs [279]. In obese, pre-diabetic and diabetic patients free FA levels are increased together with decreases in fat oxidative capacity, and this accumulation of FAs and acylglycerols in beta cells and other tissues correlates strongly with insulin resistance and MetSyn [261,283].

As discussed in previous sections, unsaturated FAs are particularly sensitive to ROS/RNS oxidation, resulting in the formation of lipid peroxides [284]. Lipid peroxides can be cytotoxic and lead to free-radical damage to other lipids, proteins and DNA [285]. In MetSyn, T2D, CVD and renal diseases free FAs accumulate inside cells and mitochondria, where they are prone to peroxidation. Also when mitochondrial MIM are oxidatively damaged, this can result in MIM proton leakage and ETC and mtDNA damage, and subsequent activation of the NLRP3 inflammasome [286].

Uncoupling proteins (UCPs), such as UCP2 and UCP3, which are present in the MIM, transport protons back into the mitochondrial matrix and are thus involved in regulating electron transport chain activity. UCP3 and other UCPs prevent build-up of excessive concentrations of ROS/RNS by limiting oxidative phosphorylation by pumping the protons from the intermembrane space into the mitochondrial matrix and thereby dissipating the proton gradient, reducing the ATP production and diminishing superoxide production [287]. Also, UCP3 functions to remove FAs formed by oxidative reactions that can build-up in mitochondria [285]. These FA anions can cause reactions with other lipids, proteins and DNA. Hence, UCPs appear to play important roles in redox regulation as well as mitochondrial and metabolic processes.

T2D is thought to form because of persistent hyperglycemia, which in turn causes: (i) formation of advanced glycation end-products (AGEs), their accumulation and oxidative interactions with cell receptors; (ii) activation of various isoforms of protein kinase C; (iii) induction of the polyol pathway; and (iv) increased hexosamine pathway flux [288–290]. Most of these pathways are associated with elevated oxidative stress and over-production of ROS/RNS during hyperglycemia and its development into MetSyn and eventually T2D [288]. Obesity results in hyperlipidemia, which causes an increase in FA oxidation products that stimulate insulin secretion, resulting in hyperinsulinemia. Hyperinsulinemia then down-regulates insulin receptors and increases blood glucose levels [291].

Excess oxidative stress likely contributes to progression of MetSyn to T2D by disrupting the ability of beta cells to respond to elevated blood glucose [288,289]. The excess ROS/RNS results in loss of pancreatic beta cells by apoptosis, and this further reduces production of insulin [292].

Dietary supplements can prevent some of the damage to cellular and mitochondrial membranes, and this is important in preventing loss of ETC function seen in MetSyn and T2D [292]. This can be accomplished by the dietary use of various types of antioxidants or by increasing free-radical scavenging systems [288,291,293]. In MetSyn and diseases caused or promoted by continuing excess ROS/RNS, such as T2D and CVD, dietary supplementation with low molecular weight antioxidants, plus some replacement of accessory molecules, such as the metal ion cofactors zinc, manganese, copper, vanadium, chromium and selenium necessary for antioxidant and other enzymes, plus certain vitamins with antioxidant properties and enzymes (C, E, A, CoQ₁₀) can be collectively used to maintain antioxidant levels and free-radical scavenging systems [270,284,288,291,294,295]. However, supplementation with oral antioxidants, enzymes, vitamins and other cofactors may not be sufficient to maintain cellular components free of ROS/RNS damage,

and antioxidants alone cannot replace damaged cellular components, especially the phospholipids in membranes [294,295]. Thus MLR may be necessary, in addition to the supplements listed above to optimize membrane health.

Despite the evidence for a connection between excess oxidative stress in MetSyn, T2D and associated diseases, an association between the intake of high concentrations of oral antioxidant nutrients and the prevention or delay of MetSyn progression to T2D and other diseases has not been proved [295–297]. Unfortunately, randomized, controlled clinical trials, mainly with single oral antioxidants, failed to show significant prevention benefits [298].

MLR should be useful in replacing membrane components damaged by MetSyn and restoring unoxidized phospholipids in blood lipoproteins [3,295]. Thus administration of MLR phospholipids along with changes in diet should remove oxidized phospholipids and cholesterol from HDL and LDL [123]. Treating T2D patients with oral MLR resulted in decreased serum triglyceride levels (37% reduction by 12 months) and reduced lipid peroxidation products compared to placebo [299]. There was also a significant reduction in the levels of acyl-hydroperoxides, Schiff's bases, diene/triene conjugates and MDA in patients taking oral MLR phospholipids [300]. Some studies found a significant reduction in blood sugar levels in patients with T2D given 1.2 g of oral MLR for 60 days compared to the control diet only group [301].

Another precursor to MetSyn and T2D, hypertension, is directly related to vascular dysfunction, which can be preceded by insulin resistance for decades [302]. Hypertension is also linked to insulin resistance, excess oxidative stress, mediated mainly by ROS/RNS, and changes in endothelial and smooth muscle cells, resulting eventually in vascular inflammation and initiation of apoptosis [303,304].

Chronic inflammatory damage to blood vessels due to lipid accumulation, inflammatory responses, vessel cell death and thrombosis, can cause atherosclerosis, which can eventually result in CVD. Atherosclerosis is characterized by a number of risk factors, including abnormalities in lipoproteins, increases in vascular acute phase response proteins, changes in vascular endothelial cell adhesion molecules and certain inflammatory cytokines [304,305]. ROS/RNS also play an important physiological role in maintaining vascular integrity, but when in excess, they serve a pathological role. As previously discussed, excess production of ROS/RNS is causally associated with MetSyn, T2D, hypertension, atherosclerosis, and CVD [295,304].

Endothelial and adipose dysfunction and insulin resistance are thought to be among the most basic physiologic abnormalities that link MetSyn and CVD [305,306]. Vascular damage associated with excess oxidation, inflammation and thrombosis is a primary event in the development of MetSyn, CVD and other diseases [307]. Macrophages are also recruited to adipose tissue, and changes occur in adipose cells in parallel with changes in endothelial cells, such as induction of secreted adipokines and cytokines [306].

It is doubtful that MLR alone can modify or reverse the conditions described above that result in T2D, atherosclerosis, CVD, and other diseases; however, the use of MLR phospholipids can change the composition and oxidation state of circulating lipoproteins [308]. Also, the administration of PC has resulted in removal of cholesterol from serum lipoproteins and membranes [309]. In a 10 year experiment using rhesus monkeys fed high cholesterol diets Wong et al. [310] found that seven weeks of oral lecithin in their diets significantly lowered total cholesterol, LDL cholesterol and triglyceride blood levels. Other studies have also shown that MLR supplements reduced cholesterol, LDL-cholesterol and triglycerides [261]. Mini-pigs fed a cholesterol and coconut oil diet for 24 weeks as an experimental model of atherosclerosis were then administered a MLR product (EPL) [311]. Without EPL the serum levels of triglycerides, cholesterol free FAs and beta-lipoproteins gradually increased with time, but with 8 weeks of EPL (up to 280 mg/kg body weight), there was a dose-related reduction in total lipids, cholesterol esters, free cholesterol and triglycerides. Also,

at the highest EPL dose levels used there was a reduction in atherosclerotic plaques observed in the aortas and heart valves [311].

Excessive lipoprotein lipid peroxidation is one of the first changes associated with the development of MetSyn, and it is thought to also be important in the development of hypertension, T2D, atherosclerosis and CVD. Thus agents that reduce lipoprotein lipid oxidation may inhibit or attenuate the development of these diseases [295,312]. In fact, MLR has been shown to reduce lipid peroxidation in patients with ischemic heart disease. For example, patients with angina pectoris took an oral MLR supplement containing 1.8 g phospholipids per day for 3 weeks. At the end of treatment there was a significant reduction in oxidized serum lipids, an increase in HDL cholesterol and a reduction in erythrocyte hemolysis due to peroxidation [300].

Use of MLR in a clinical setting has demonstrated that blood levels of cholesterol, LDL-cholesterol and triglycerides can be reduced. Long-term dialysis patients are at risk for ischemic cardiovascular complications, and these patients tend to have high blood lipid values. In this double-blind, randomized study two groups of ten patients who had hyperlipidemia (serum cholesterol greater than 260 mg/dl, LDL cholesterol greater than 180 mg/dl and triglycerides greater than 200 mg/dl) were given 2.7 g per day oral PC or placebo for 6 weeks [313]. The 6-week treatment was followed by a two-week wash-out period, and lipid parameters were determined at 2, 4 and 6 weeks of treatment. Two weeks after PC administration there was a significant reduction in LDL-cholesterol of 32 mg/dl compared to the stable placebo controls. By four weeks triglycerides decreased by 58.2 mg/dl ($p < 0.001$) and by six weeks there was a reduction in triglycerides of 43.3 mg/dl compared to the placebo group (4 weeks, -5.7 mg/dl and 6 weeks, -11.4 mg/dl, $p < 0.01$) [313]. In a double-blind study of type II hyperlipidemia patients participants received either three doses of oral polyenylphosphatidylcholine (0.9 g per day) or placebo, and their blood lipid levels determined [314]. Total cholesterol and LDL-cholesterol were lowered significantly, and there was a downward trend in apoprotein B, triglycerides and VLDL-cholesterol and an upward trend in apoprotein A1 compared to the placebo group [314]. As discussed above, MLR along with changes in diet and caloric restriction as well as suitable macro- and micro-nutrient concentrations can reduce and replace oxidized phospholipids and cholesterol from HDL and LDL.

MLR has the potential to reverse some of the lipid changes that are important in MetSyn development and possibly prevent the formation of MetSyn-associated diseases. It is interesting that long-term use of MLR in the form of oral NTFactor® and vitamins reduced significantly blood markers for CVD risk, such as homocysteine, erythrocyte sedimentation and fasting insulin levels [142]. In a group of patients with homocysteine levels above the threshold for risk, MLR with oral NTFactor® resulted in a reduction of test results to the normal ranges within 6 months [142]. Future studies should document whether MLR can impede or reverse the course of development of MetSyn and its associated diseases.

12. Miscellaneous uses of MLR, final comments and future directions

In addition to the many uses of MLR described in this review and shown in Table 2 for NTFactor® and NTFactor Lipids®, MLR has been used in laboratory animals and humans to treat other conditions, such as toxic liver and kidney damage caused by carbontetrachloride, alcohols, galactosamine, acetaminophen, tetracycline, solvents, detergents, thioacetamide, indomethacin, anesthetics, ionizing radiation, immune-mediated hepatitis and others. MLR was shown to reduce the toxic effects of these agents and promote organ regeneration (review [4]). In addition, there are other exposures and infections in humans where MLR has been useful, such as in the treatment of damage caused by fat embolism, non-steroidal anti-inflammatory drugs, liver-damaging anti-microbial drugs, lethal hepatic toxins, fatty liver conditions due to malnutrition and infections like hepatitis [4]. This last subject will be further considered.

The treatment of viral hepatitis using MLR phospholipids has been extensively investigated in uncontrolled settings and in controlled clinical trials [315,316]. Hepatitis patients treated with MLR using intravenous EPL reported improvements in dyspepsia, nausea, epigastric pain, fullness in the epigastrium and other symptoms as well as improvements in hepatomegaly and presence of ascites. Laboratory tests also improved, and histological analysis of liver biopsies indicated earlier regeneration of hepatocytes [315]. In a controlled clinical study patients with chronic hepatitis were treated for one year with intravenous EPL, resulting in a significant reductions in hepatomegaly, liver enzymes, hepatic excretory capacity and improvements in gamma-globulin and serum albumin levels compared to controls [316]. Elsewhere oral MLR has been used to reduce symptoms associated with complex chronic infections like Mycoplasma and Lyme disease-associated infections [133, 227].

Liver cirrhosis has also been treated with MLR phospholipids. Patients with advanced liver cirrhosis were given oral MLR phospholipids. After 3 months, nearly all blood biochemical parameters improved and were found to be within the normal range. Symptoms also improved along with reductions in hepatomegaly and ascites [317]. In other studies, patients with moderately severe to severe cirrhosis caused by hepatitis B virus were treated with IV EPL for 3 months and compared to patients who received a only a vitamin preparation. Whereas in the control group changes were not found in liver function from pre-treatment values, in the treatment group there were significant improvements in liver function and an absence of hepatitis B antigen in a majority of patients [318].

MLR has also been successfully used in chronic ambulatory peritoneal dialysis (CAPD). MLR phospholipid-treated CAPD patients showed increases in ultrafiltration with more electrolytes, creatinine, urea and phospholipids released into the ascites fluid, which were removed by dialysis. MLR phospholipids were able to restore normal physiological conditions in CAPD patients with abnormal ultrafiltration rates [319].

MLR is safe to employ during pregnancy and has been used to treat conditions that place women at risk during pregnancy. For example, during pregnancy gestosis or toxemia can occur where patients present with hypertension, edema and proteinuria. This is thought to be caused by chronic intravascular clotting and fibrin deposition in the uteroplacental bloodstream, which can affect uteroplacental perfusion and fetal development [320]. The more severe the case, the higher the levels of lipid peroxidation products have been found in the serum and erythrocyte membranes [321]. For treatment, patients were given IV or oral MLR phospholipids twice daily at a dose of 500 mg per day in the last trimester of pregnancy. When this was done, edema subsided, liver and kidney function tests normalized, and other symptoms eventually disappeared [322].

This review has documented that MLR approaches can be used to repair and replace oxidatively-damaged membrane glycerophospholipids in order to restore activities and functions of cellular membranes, and thus of cells and organs. Using mitochondria as an model for critical membrane-bound enzyme complexes and the ETC system we discussed in this review and elsewhere [1–3] how MLR can be used to restore MIM trans-membrane potential and recover mitochondrial function. Multiple clinical trials have proven the usefulness of MLR in reducing the symptoms associated with loss of mitochondrial and other functions while improving the quality of life in patients with a variety of chronic illnesses [1–3]. Although most contributions in this area have concentrated on mitochondrial function and some obvious links to cellular energy balance, such as fatigue and other issues, recent studies have focused on the effects of MLR supplements on pain, gastrointestinal and other symptoms. Also, there is evidence that MLR supplements will be useful in enhancing sperm motility and personal fertility [323].

Preliminary results in several areas suggest that new uses of MLR supplements will eventually eclipse the use of MLR for mitochondrial dysfunction. Another area of future growth for MLR is aging management. MLR provides important anti-aging effects by repairing age-

Table 2Current and potential uses of oral MLR supplements and revised dose levels.^a

Use	Subjects/patients	Age group	MLR lipid supplement	NTFL dose ^b range (g/day) (original)	NTFL dose ^c range (g/day) (revised)	Example reference
General health	Aged	Senior	NTFactor/L ^d	2	2–3	Ellithorpe et al. [228]
Fatigue	Aged	Senior	NTFactor/L	3	4	Agadjanyan et al. [157]
Fatigue	CFS/ME	Adult/teen	NTFactor/L	2–4	4	Nicolson & Ellithorpe [226]
Fatigue	CFS/ME	Adult	ATP Fuel	4	4	Nicolson et al. [133]
Fatigue	Fibromyalgia	Adult	NTFactor/L	3–4	4	Nicolson & Ellithorpe [226]
Weight loss	Obesity, fatigue ^d	Adult	NTFactor	2	3–4	Ellithorpe et al. [256]
Brain health	Neurodegen. dis.	Adult	NTFactor/L	3–4	4	Nicolson et al. [2]
CD health	CD risk/CD dis.	Adult	NTFactor/L	2–4	4	Ellithorpe et al. [142]
Metabolic health	MetSyn/diabetes	Adult	NTFactor/L	2–4	4	Nicolson [295]
Metabolic health	Diabetes	Adult	ATP Fuel	4	4	Ellithorpe et al. [256]
Neurobehavior	Autism spectrum dis.	Child	NTFactor/L	1–2	1–3	Nicolson et al. [2]
Infections	Lyme/mycoplasma	Adult	ATP Fuel	4	4	Nicolson et al. [227]
Fertility	Fertility diseases	Adult	NTFactor/L	2–3	4	Costa et al. [323]
Fatigue	Cancer	Adult	NTFactor/L	2–3	4	Nicolson and Conklin [144]
Anemia	Anemia	Adult	NTFactor/L	1–2	4	Ellithorpe et al. [228]
Injury	Spinal injury	Adult	NTFactor/L	1–2	4	Ellithorpe et al. [228]
Autoimmune	Rheumatoid arthritis	Adult	ATP Fuel	3	4	Nicolson et al. [133]
General health	Pregnancy	Adult	NTFactor/L	1–2	2	Ellithorpe et al. [228]
Chemical detox	GW illnesses	Adult	NTFactor/L	>4	>4	Nicolson et al. [2]

^a Modified from Nicolson et al. [2].^b Dose range in grams per day based on NTFactor Lipids®.^c Revised dose range in grams per day based on NTFactor Lipids®.^d NTFactor® or NTFactor Lipids®.

related damage to cells, for example, in conditions like age-related loss of fertility (Table 2). We have now increased our suggested MLR dosing over time as newer information has become available (see Table 2 for new oral MLR dosing recommendations).

As discussed in Section 5, when excess amounts of MLR phospholipids are ingested, the movement of glycerolphospholipids from the gastrointestinal system into the circulation and eventually to organs, tissues, cells and cellular membranes acts as a ‘bulk flow’ or ‘mass action’ system [107]. This simple concept explains why MLR works in the replacement of damaged glycerolphospholipids in cellular membranes. They are simply released from membranes by displacement and partitioning into intracellular lipid globules, liposomes and other structures and carried out or freed from cells and returned to the intestinal system where they are eventually excreted in stool. This also explains the usefulness of MLR in the bulk flow removal of toxic, fat-soluble chemicals from hydrophobic cellular stores.

It should be apparent that the use of MLR phospholipids for repairing and replacing membrane glycerolphospholipids requires a long-term approach. Similar to other nutritional supplements, MLR is not a quick fix for cellular damage. Cross-over clinical trials have shown that MLR supplements have to be continued as a long-term strategy for chronic diseases, because temporary changes due to MLR supplementation can be slowly reversed with time [1–3]. Even in cases of acute toxic insult, MLR administration can’t be a short-term alternative to more traditional care. In most cases, and especially in chronic conditions, the damages to cellular membranes and other structures are not temporary and continue for an unpredictable period of time. Thus dietary and behavior modifications must continue, and in order to maintain health MLR supplementation may be a lifelong requirement, especially in individuals with a typically modern sedentary lifestyle [213].

This review has focused on the use of MLR in restoring cellular membrane functions, but there are also innovative new uses of MLR in clinical care and aging [3]. The role of MLR in maintaining mitochondrial function is considered particularly important in reducing the effects of aging and helping provide for a healthy lifestyle [3]. However, MLR may also be useful as adjuncts to more traditional drug approaches, either to enhance cell and tissue absorption, modify cellular energy requirements or change drug and messenger interactions [9]. The design of new MLR formulations and especially the use of MLR phospholipids

in combination with other health supplements for specific health uses should dominate future MLR basic and clinical research.

Conflict of interest

The authors are part-time consultants to Nutritional Therapeutics, Inc. and Allergy Research Group, Inc.

Transparency document

The Transparency document associated with this article can be found, in the online version.

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