Emerging roles of cardiolipin remodeling in mitochondrial dysfunction associated with diabetes, obesity, and cardiovascular diseases

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Abstract

Cardiolipin (CL) is a phospholipid exclusively localized in inner mitochondrial membrane where it is required for oxidative phosphorylation, ATP synthesis, and mitochondrial bioenergetics. The biological functions of CL are thought to depend on its acyl chain composition which is dominated by linoleic acids in metabolically active tissues. This unique feature is not derived from the de novo biosynthesis of CL, rather from a remodeling process that involves in phospholipases and transacylase/acyltransferase. The remodeling process is also believed to be responsible for generation of CL species that causes oxidative stress and mitochondrial dysfunction. CL is highly sensitive to oxidative damages by reactive oxygen species (ROS) due to its high content in polyunsaturated fatty acids and location near the site of ROS production. Consequently, pathological remodeling of CL has been implicated in the etiology of mitochondrial dysfunction commonly associated with diabetes, obesity, heart failure, neurodegeneration, and aging that are characterized by oxidative stress, CL deficiency, and abnormal CL species. This review summarizes recent progresses in molecular, enzymatic, lipidomic, and metabolic studies that support a critical regulatory role of pathological CL remodeling as a missing link between oxidative stress and mitochondrial dysfunction in metabolic diseases and aging.

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INTRODUCTION

Mitochondrial dysfunction has recently been identified as a common metabolic defect associated with obesity and its metabolic complications\cite{1, 2}. A number of early studies suggest that mitochondrial oxidative function was compromised in diabetic and prediabetic humans as evidenced by reduced levels of fatty acid oxidation\cite{3}, insulin-stimulated ATP synthesis\cite{4-6}, and expression of genes involved in oxidative phosphorylation\cite{7}. However, this hypothesis has recently been challenged by findings that mitochondrial hyperactivity is associated with severe insulin resistance in Asian Indians\cite{8}. Attenuation of oxidative phosphorylation activity prevents the onset of diet-induced obesity and its related insulin resistance in mice with targeted deletion of AIF, a mitochondrial flavoprotein apoptosis inducing factor\cite{9}. Furthermore, all of the insulin sensitizers, including some of the most popular antidiabetic drugs thiazolidinediones and metformin, have been shown to suppress mitochondrial complex I activity\cite{10-13}. Therefore, the molecular mechanisms underlying a causative role of mitochondrial dysfunction in diabetes and obesity remain to be elucidated.

Cardiolipin (CL) is a mitochondrial membrane phospholipid exclusively localized in the mitochondria where it regulates mitochondrial function and oxidative stress in species from yeast to mammals\cite{14-16}. This role is mediated by the acyl composition of the side chains of CL, which is dominated by linoleic acid in insulin sensitive tissues\cite{17}. This unique acyl composition is not derived from de novo synthesis of CL, rather from a remodeling process that involves phospholipases and acyltransferases\cite{18, 19}. This remodeling process is also believed to be responsible for generating CL species that are highly sensitive to oxidative damage by reactive oxygen species (ROS), further exacerbating CL peroxidation and oxidative stress. CL is sensitive to damage of its double bonds by oxidative stress due to its rich content in linoleic acid and its location near the site of ROS production in the inner mitochondrial membrane. CL is the only phospholipid in mitochondria that undergoes early oxidation during apoptosis\cite{20}. Consequently, pathological CL remodeling has been implicated in etiology of mitochondrial dysfunction associated with a host of pathophysiological conditions including diabetes, obesity, heart failure, hyperthyroidism, neurodegeneration, and aging, all of which are characterized by increased levels of oxidative stress, CL deficiency, and enrichment of docosahexaenoic acid (DHA) content in CL\cite{22-26}. Recent progress in molecular cloning of enzymes involved in CL synthesis and remodeling, combined with the latest development in lipidomic profiling of CL, have implicated an important role of CL remodeling in regulating health and diseases.

CL SYNTHESIS, REMODELING, AND MITOCHONDRIAL FUNCTION

CL is a mitochondrial membrane phospholipid initially identified from beef heart, and is required for optimal mitochondrial respiration as a cofactor of enzymes involved in oxidative phosphorylation. CL is synthesized by three consecutive steps beginning with the biosynthesis of CDP-diacylglycerol. The committed and rate-limiting step is catalyzed by phosphatidylglycerophosphate synthase (PGS) (Fig. 1). CL is required for the reconstituted activity of a number of metabolic enzymes and carrier proteins in the mitochondria\cite{27}. CL in the inner mitochondrial membrane serves as a Ca\textsuperscript{2+}-binding site, through which Ca\textsuperscript{2+} triggers mitochondrial membrane permeabilization\cite{28}. Additionally, CL is required for cell survival, and dissociation of cytochrome c from CL triggers apoptosis\cite{29, 30}. In yeast, S. cerevisiae, mutation of the crdl gene encoding CL synthase results in impaired viability, a decrease in membrane potential, and defective oxidative phosphorylation\cite{31}. Similarly, CL deficiency in Chinese hamster ovary (CHO) cells results in stringent temperature sensitivity for cell growth in glucose-deficient medium and by reduced ATP production\cite{32}. The mutant CHO cells demonstrate an increased glycolysis, reduced oxygen consumption, and defective respiratory electron transport chain activity.

CL is the only known dimeric phospholipid, consisting of four fatty acyl chains, which is restricted to C18 chains dominated by the linoleoyl group (C18:2) in skeletal muscle and heart\cite{33}. The unique fatty acyl composition is believed to be important for its proper biological function. The hydrophobic double-unsaturated linoleic diacylglycerol species is required for high affinity binding of CL to proteins\cite{34}. Thus, alteration in molecular species composition of CL affects the activities of cytochrome c oxidase and other electron transport chain enzymes\cite{29, 34}. However, the formation of the unique fatty acyl composition of CL does not occur during de novo biosynthesis, because the enzymes of the CL biosynthetic pathway lack appropriate substrate selectivity\cite{35, 36}. This is further confirmed by the recent cloning of the human CL synthase gene from our lab and others\cite{37-40}. Thus, newly synthesized CL is believed to undergo...
a remodeling process to achieve its appropriate acyl content. In addition to CL synthesis, we have recently demonstrated that the human CLS1 is also involved in the remodeling of phosphatidylglycerol \[41\]. The recombinant hCLS1 protein expressed in COS-7 cells and Sf-9 insect cells exhibited a strong acyl-CoA dependent lysophosphatidylglycerol acyltransferase activity \[41\].

Two distinct mechanisms have been posited to carry out the CL remodeling process. The first mechanism involves transacylation of acyl groups from phosphatidylcholine or phosphatidylethanolamine to CL, which is partly catalyzed by tafazzin, an enzyme that when mutated causes defective CL remodeling and Barth syndrome \[18\] (see below). The alternative pathway involves deacylation by phospholipase A2 to lysoCL followed by reacylation to CL by acyl-CoA dependent Lyso-CL acyltransferases (Fig. 2) \[17\]. Two acyltransferases has been characterized so far, and they differ in substrate specificity and intracellular localization. In the search for a gene encoding an acyltransferase responsible for CL remodeling, we have recently identified and characterized the first CL reacylation enzyme, named acyl-CoA:lysoCL acyltransferase (ALCAT) \[19\]. The recombinant ALCAT enzyme is localized in endoplasmic reticulum and recognizes both MLCL and dilysoCL as substrates. The second acyltransferase was a monolysoCL acyltransferase initially purified from pig

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Fig. 1 CL biosynthetic and remodeling pathways. The indicated reactions of CL biosynthetic pathway are catalyzed by the following enzymes: CTP-phosphatidic acid cytidylytransferase (CPC), phosphatidylglycerolphosphate synthase (PGS), phosphatidylglycerolphosphate phosphatase (PP), and CL synthase (CLS). In addition to CL synthesis, CLS is also involved in phosphatidylglycerol remodeling by catalyzing acylation of lysophosphatidylglycerol (LPG) to phosphatidylglycerol. Phosphatidylglycerol remodeling can also by catalyzed by LPG acyltransferase (LPGAT1). The CL remodeling pathway is catalyzed by phospholipase (cPLA) and acyl-CoA:lysoCL acyltransferase (ALCAT).

Fig. 2 Proposed remodeling pathway for CL. CL is first deacylated to monolysoCL by phospholipase A2 (PLA2), and then reacylated to CL with ALCAT.
liver mitochondria, known as MLCL AT, that catalyzes the synthesis of tetratetralinoleoyl-CL (L4CL)\textsuperscript{[42]}. The gene encoding MLCL AT has recently been identified to share the same sequence homology with the mitochondrial trifunctional protein\textsuperscript{[20]}. In comparison to the MLCL AT, ALCAT1 lacks preference for linoleic acid as substrate, suggesting a possible role of ALCAT1 in pathological remodeling of CL\textsuperscript{[19, 20]}. This is corroborated by our recent reports that ALCAT1 expression is up-regulated in mammalian cells exhibiting tetratetralinoleoyl-CL deficiency and in heart and liver of mice suffering from oxidative stress induced by hyperthyroidism\textsuperscript{[43]}. However, it remains to be identified whether ALCAT1 plays a causative role in pathological remodeling of CL.

OXIDATIVE STRESS AND CL PEROXIDATION

One of the common defects associated with metabolic diseases (diabetes, obesity, cardiovascular diseases), aging, and neurodegeneration is an increased level of oxidative stress. Increased level of ROS production has been implicated in the onset of mitochondrial dysfunction and is believed to be the primary causes of diabetic complications\textsuperscript{[14]}. CL is particularly sensitive to oxidation of its double bonds by ROS due to its location near the site of ROS production in the inner mitochondria membrane\textsuperscript{[20]}. The mitochondrial electron transport chain is considered a major intracellular source of ROS including hydroxyl radicals, peroxy radicals, superoxides, and the dismutation product, H$_2$O$_2$ (Fig. 3). All of these ROS are generated both during physiologic respiration and during disrupted electron transport\textsuperscript{[20, 29]}. Oxygen free radicals are highly reactive species capable of causing oxidation of CL, a process also known as lipid peroxidation. Non-oxidized CL is required for the mitochondrial bioenergetics and the activity of key mitochondrial proteins\textsuperscript{[20]}. Consequently, CL peroxidation by ROS disrupts its binding with cytochrome c and affects the activity of complex I, III, and IV of the mitochondrial respiratory chain\textsuperscript{[45]}. A burst of ROS damages mitochondria by causing profound loss of CL\textsuperscript{[46]}. CL deficiency in ischemia and reperfusion results in mitochondrial dysfunction manifested by a decreased oxidative capacity, loss of cytochrome c, and generation of ROS. CL, but not its peroxidized form, is able to almost completely restore the ROS-induced loss of cytochrome c oxidase activity\textsuperscript{[47]}. In support of a key role of CL peroxidation in mitochondrial dysfunction, CL is the only phospholipid that undergoes oxidation during the onset of apoptosis\textsuperscript{[21]}.

![Fig. 3](image) ROS and lipid peroxidation. ROS contain free radicals from atoms that have one or more unpaired electron(s) (right panel). The imbalance in electrons results in the high reactivity of the free radicals. The process of lipid peroxidation (left panel) begins with the ROS attack on double bonds of PUFA. The carbon radical tends to be stabilized by a molecular rearrangement to form a conjugated diene. Under aerobic conditions conjugated dienes are able to combine with O$_2$ to produce a peroxyl radical, ROO$^\cdot$.

PATHOLOGICAL CL REMODELING AS A COMMON DEFECT IN MITOCHONDRIAL DYSFUNCTION IN METABOLIC AND AGING-RELATED DISEASES

Bath Syndrome (BTHS)

One of the best examples that underscores the importance of CL remodeling in metabolic diseases is BTHS, an X-linked recessive disorder manifested by cardiomyopathy, skeletal myopathy, growth retardation, and neutropenia\textsuperscript{[48]}. BTHS is caused by mutations in the tafazzin (TAZ) gene encoding an acyltransferase involved in the remodeling of phospholipids\textsuperscript{[49]}. The lipid composition of cells from patients with BTHS shows a dramatic
Diabetes and obesity

Diabetes and obesity are characterized by systemic oxidative stress which is believed to be a principal causative factor of insulin resistance and other obesity-related metabolic complications. ROS production was significantly increased in obesity and diabetes from elevated expression of NADPH oxidase and decreased expression of antioxidative enzymes. The level of superoxide dismutase, the enzyme responsible for inactivating the superoxide radical, along with the levels of antioxidants are decreased in uncontrolled diabetes. Oxidative stress impaired glucose uptake in muscle and fat, and was recently shown to be the primary cause of various forms of insulin resistance. Consistent with the notion of mitochondria as the primary source of ROS, employment of mitochondrial-targeted antioxidants ameliorated insulin resistance associated with obesity in both rodents and humans. Many of the mitochondrial defects associated with diabetes were reversed by transgenic overexpression of catalase in mice.

Pathological CL remodeling contributes to the onset of mitochondrial dysfunction and metabolic complications associated with obesity. Diabetes and obesity are associated with CL depletion in myocardium and linked with altered substrate utilization and mitochondrial dysfunction. Defective CL remodeling in the diabetic heart results in enrichment of DHA (22:6n3) in CL, which is known to cause mitochondrial dysfunction. Accumulation of DHA and other long chain polyunsaturated fatty acids (PUFA) in CL increases oxidant production and mitochondrial proton leakage in cultured mammalian cells. Treatment of diabetic mice with rosiglitazone, an insulin sensitizing drug, significantly increases CL levels and causes a substantial remodeling of CL toward an elevated linoleic acid (18:2n6) and a reduction of DHA content. Such a shift is believed to improve electron transport efficiency and decrease proton leakage. For example, when acyl composition of rat heart CL switched from 18:2n6 to 22:6n3, the activity of cytochrome c oxidase decreased by 50%, concurrent with a lower oxygen consumption rate of rat heart mitochondria.

Hyperthyroidism

Thyroid hormone is a major physiological modulator of oxidative stress and mitochondrial respiration. Thyroid hormone has been shown to increase mitochondrial mass, mitochondrial cytochrome c content, respiratory rate, and capacity of oxidative metabolism. Hyperthyroidism is associated with significant mitochondrial dysfunction. Cardiovascular tissues are particularly sensitive to ROS damage associated with hyperthyroidism, because of the high energy demand of the heart. For example, hyperthyroid hearts displayed tachycardia and low functional recovery. Their mitochondria exhibited higher level of H2O2 production and susceptibility to swelling during reperfusion. Both the levels of CL and lipid composition are profoundly altered by thyroid hormone status. Hyperthyroidism and hypothyroidism reciprocally affect the level of oxidative stress, lipid peroxidation, CL synthesis and remodeling. The hepatic and cardiac CL contents were elevated in rats treated by thyroxine, which was accompanied by an increase in CL synthase activity and level of ROS production. Moreover, cardiac mitochondrial MLCL AT activity was stimulated in hyperthyroid rats, and decreased in rats made hypothyroid. Consistent with increased level of ROS production, hyperthyroidism is associated with a marked loss of C18:2, concurrent with a significant increases in polyunsaturated fatty acids such as arachidonic acid (C20:4) and DHA (C22:6). These changes increased the double bond index by 27% and CL peroxidizibility by 266%, which is likely to contribute to the elevated level of oxidative stress associated with hyperthyroidism. In support of a possible role of ALCAT1 in pathological remodeling of CL, ALCAT1 mRNA expression was significantly up-regulated by hyperthyroidism and down-regulated by hypothyroidism.

Heart Failure

Mitochondria in the adult mammalian heart have a tremendous capacity for oxidative metabolism, and the conversion of energy by these pathways is critical for proper cardiac function. Mitochondrial ROS in the heart has been reported to increase with age. A number of endogenous mitochondrial antioxidant defenses may also diminish with age and thus reduce
the capacity for efficient management of ROS\[^{[60]}\]. CL is one of the principle phospholipids in the mammalian heart, a tissue that has perpetually high energy demands and is particularly sensitive to oxidative stress and mitochondrial dysfunction. Consequently, CL deficiency in ischemia and reperfusion results in mitochondrial dysfunction manifested by a decrease in oxidative capacity, loss of cytochrome \(c\), and generation of ROS\[^{[47,81,82]}\], CL, but not the peroxidized form, was able to almost completely restore the ROS-induced loss of cytochrome \(c\) oxidase activity\[^{[47]}\]. CL is also an immunogenic phospholipid, and development of anti-CL antibodies is associated with the onset of thrombosis\[^{[61]}\]. Loss of cardiac tetralinoleoyl CL has recently been identified to be a major defect in experimental heart failure\[^{[60]}\].

**Aging**

Oxidative injury of mitochondria impacts critical aspects of the aging process and contributes to impaired physiological function, and has been proposed as the primary cause of aging. Mitochondrial dysfunction plays a central role in a wide range of aging-related disorders and various forms of cancer, resulting in a reduced life span\[^{[65,66]}\]. In support of a causative role of oxidative stress in aging, ROS levels and phospholipid peroxidation index are inversely correlated with life span, from mice to human\[^{[67-69]}\]. Oxidative stress is also believed to contribute to an aging-associated decline in CL. Consistent with an increased level of ROS production, aging is associated with CL deficiency and profound remodeling of CL similar to that observed in metabolic diseases. Aging and physical exercise reciprocally affect mitochondrial and cardiac function by regulating CL levels in the heart. Exercise increases insulin sensitivity and the level of tetralinoleoyl CL, whereas aging decreases tetralinoleoyl CL level with concurrent increase in the DHA content in CL\[^{[25,26,84]}\]. Aging has been shown to decrease CL content in heart, liver, and kidney. Aging related loss of CL impairs mitochondrial function by decreasing the activity of mitochondrial phosphate transporter, pyruvate carrier, adenine nucleotide transporter, and cytochrome oxidase, all of which requires CL for optimum activity\[^{[99]}\]. These defects can be restored by supplementation of acyl-carnitine which is believed to restore CL levels\[^{[36]}\].

**Neurological Diseases**

Oxidative damage of mitochondrial function is implicated in the neurodegenerative process, and contributes to the onset of Parkinson’s and Alzheimer’s diseases\[^{[95]}\]. The rates of neurodegeneration are strongly correlated with rates of formation of mitochondrial reactive oxygen and nitrogen species\[^{[69]}\]. CL levels in the brain decrease with aging, which is likely to be caused by peroxidation from oxidative stress\[^{[96,97]}\]. In contrast to metabolically active tissues, such as skeletal muscle and heart, the tetralinoleoyl CL is not the predominant form of CL in the brain, representing less than 5% of the total CL\[^{[89]}\]. Hence, CL from mouse brain is dominated by long chain PUFA, including DHA (C22:6) and arachidonic acids (C20:4) which contribute to 40% of the acyl side chains of CL\[^{[90,99]}\]. Although the biological significance of the acyl composition remains elusive, the high content in PUFA renders CL in the brain highly sensitive to oxidative damage. Consequently, traumatic brain injury has been shown to selectively increase the content of DHA in CL and cause CL peroxidation\[^{[100]}\]. This selective CL peroxidation preceded peroxidation of other phospholipids and the onset of apoptosis\[^{[100]}\]. Furthermore, mice with targeted inactivation of presynaptic protein, α-synuclein, a protein implicated in Parkinson’s disease, exhibited CL deficiency and a reduction in both tetralinoleoyl CL content and mitochondrial complex I/III activity of the electron transport chain\[^{[99]}\]. The knockout mice also exhibited deficiency of PG, the precursor for CL synthesis.

**CONCLUSION**

There is now a growing body of evidence that supports a causative role of pathological remodeling of CL in mitochondrial dysfunction in metabolic and neurological disorders and aging. Collectively, the information presented in this review has implicated an important role of pathological CL remodeling as a missing link between oxidative stress and mitochondrial dysfunction associated with various pathological conditions, including diabetes, obesity, hyperthyroidism, heart failure, BTHS, neurological diseases, and aging (Fig. 4). Although much of the evidence accumulated thus far remains circumstantial and descriptive, the latest development in highly sensitive analytical methods for CL profiling combined with major progress in the identification and cloning of genes encoding CL remodeling enzymes signals a new dawn of this exciting field of research. It can be envisaged that pathological remodeling of CL might be the common denominator of mitochondrial dysfunction of all the aging-related diseases. Consequently, targeting enzymes involved in pathological remodeling of CL by chemical compounds could provide novel treatments for metabolic diseases and aging.
Fig. 4 Pathological remodeling of CL as a common denominator of mitochondrial dysfunction in metabolic and aging-related diseases. Metabolic, neurodegenerative, and aging-related disorders increase the level of ROS production which causes CL peroxidation and pathological remodeling of CL. Pathological remodeling of CL replaces linoleic acid (C18:2) with PUFA, leading to mitochondrial proton leakage, oxidative stress, and mitochondrial dysfunction which further exacerbates ROS production, metabolic complications, and the pathophysiology of the diseases.

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