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Tetra-linoleoyl cardiolipin depletion plays a major role in the pathogenesis of sarcopenia

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Abstract

Sarcopenia, the progressive loss of muscle mass, strength, and physical performance that occurs during aging, is highly prevalent among the elderly. Sarcopenia increases the risk of falls, disability, and death. The biological basis for sarcopenia is not well understood. There are no specific preventive or therapeutic strategies for sarcopenia except exercise. The elucidation of biological pathways and identification of therapeutic targets for treating or preventing sarcopenia remain a high priority in aging research. Mitochondria play a critical role in skeletal muscle by providing energy in the form of ATP, regulation of signaling, calcium homeostasis, autophagy, and other functions. Cardiolipin, a unique dimeric phospholipid specific to mitochondria and an essential component of mitochondrial membranes, is involved in mitochondrial protein transport, maintaining structural organization of mitochondrial membranes, cellular signaling, regulating enzymes involved in β -oxidation of fatty acids, and facilitating normal electron transport chain (ETC) function and generation of ATP. The fatty acid species composition of cardiolipin is critical to mitochondrial bioenergetics, as cardiolipin affects membrane biophysical properties, binds and stabilizes ETC protein complexes, and shapes the curvature of the mitochondrial cristae. Tetra-linoleoyl cardiolipin (18:2)₄ comprises ~80% of cardiolipin in mitochondria in normal human skeletal and cardiac muscle and is optimal for effective ETC function and ATP generation. Aging is associated with a decrease in cardiolipin content, decrease in tetra-linoleoyl cardiolipin (18:2)₄ and replacement of linoleic acid (18:2) with other fatty acids in cardiolipin composition, decline of ETC function, and increased generation of reactive oxygen species in muscle. Together, these findings from the literature prompt the hypothesis that depletion of the cardiolipin (18:2)₄ species may be at the root of mitochondrial dysfunction with aging, in turn leading to sarcopenia. Corroboration of the tetra-linoleoyl cardiolipin depletion hypothesis suggests new leads for the

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prevention and treatment of sarcopenia by enhancing the biosynthesis, accretion, and integrity of tetra-linoleoyl cardiolipin.

Keywords

aging; cardiolipin; lipids; mitochondria; sarcopenia

Introduction

Sarcopenia is the progressive loss of muscle mass, strength, and physical performance that occurs during aging [1,2]. Sarcopenia is estimated to affect 10% of community-dwelling adults 60 years worldwide [3] and ~20–70% of chronically ill older adults [4]. From 20 to 80 years of age, humans generally lose ~20–40% of both skeletal muscle mass and strength [5,6]. Aging is accompanied by loss of muscle fibers and a reduction in the size of muscle fibers [7]. Low muscle mass is associated with low strength [8], decreased lower extremity performance [9], functional impairment [10], falls [11,12], and physical disability [10,13,14]. The decline of muscle strength with aging is greater than would be predicted by loss of muscle mass alone [15]. Other factors such as muscle quality [16], neuromuscular junction and motor unit size may contribute to the age-related loss of muscle strength [17,18]. Longitudinal studies show that by around 75 years of age, muscle strength is lost at a rate of 3–4% per year in men and 2.5–3% per year in women [19]. Low muscle strength predicts disability [20,21] and mortality [22,23]. Major risk factors for sarcopenia are age, physical inactivity, inadequate nutrition, alteration in sex hormones, and the age-related pro-inflammatory state [1,2,24,25].

Biological pathways that have been implicated in sarcopenia include hormonal changes [26], anorexia and malnutrition [27,28], reduced satellite cell function [29], altered proteostasis [30], impairment of neuromuscular function [17], increased inflammation [31], and mitochondrial dysfunction [32]. Mitochondria are essential for fatty acid and glucose metabolism [33], energy production [32], regulation of intracellular calcium homeostasis [34], reactive oxygen species (ROS) signaling [35], mitophagy [36,37], and apoptosis in skeletal muscle [32,38] and are a nexus in the pathway of some of these proposed pathways to sarcopenia.

Aging and sarcopenia are associated with a loss of bioenergetic capacity

Progressive mitochondrial dysfunction is a central hallmark of aging [39–44]. Skeletal muscle mitochondrial protein synthesis, biogenesis, respiratory capacity, coupling control, ATP production, and calcium-handling capacity declines with age [44–48]. The relationship of aging with mitochondrial energetics has been studied invasively using direct measurement of oxygen consumption by respirometry in isolated mitochondria or permeabilized muscle fibers from skeletal muscle biopsies [48] or non-invasively in humans using phosphorus magnetic resonance spectroscopy (^{31}P -MRS). ^{31}P -MRS measures the post exercise recovery rate of phosphocreatine (PCr), k_{PCr} , which reflects the capacity of muscle mitochondria to synthesize ATP [48]. ^{31}P -MRS correlates well with *in vitro* measurements of mitochondrial oxidative capacity [49,50]. Low k_{PCr} is independently associated with slower gait speed in

adults [51]. Impaired mitochondrial oxidative function, as assessed by ^{31}P -MRS is associated with insulin resistance in older adults without diabetes [52].

Hypothesis: Depletion of tetra-linoleoyl cardiolipin plays a major role in the pathogenesis of sarcopenia

Cardiolipin is a unique dimeric phospholipid specific to mitochondria and an essential component of mitochondrial membranes [53]. Cardiolipin consists of a glycerol headgroup, two phosphatidyl moieties, and four fatty acid chains (Figure 1). The four fatty acid chains can differ in length and saturation. Cardiolipin is distinct from other phospholipids in that it contains two phosphatidyl moieties bound to a single glycerol group, a feature which results in a small, relatively rigid head group and a large rigid hydrophobic tail [54]. The functions of cardiolipin include mitochondrial protein transport, maintaining structural organization of the inner mitochondrial membrane, cellular signaling, regulating enzymes involved in β -oxidation of fatty acids, and facilitating electron transport chain (ETC) function and generation of ATP [55–58]. Alterations in cardiolipin could play a major role in the impairment of mitochondrial function and decline of muscle mass, strength, and physical performance with aging. A conceptual model for the hypothesis that depletion of tetra-linoleoyl cardiolipin plays a central role in sarcopenia is shown in Figure 2.

Synthesis and remodeling of cardiolipin

Cardiolipin is synthesized in a pathway involving lysophosphatidic acid (LPA), phosphatidic acid (PA), and other intermediates (Figure 3). LPA can be generated from the addition of an acyl group from acyl-CoA to the *sn*-1 position of glycerol-3-phosphate (G3P) by glycerol-3-phosphate acyltransferase (GPAT) [59]. There are four isoforms of GPAT, coded by separate genes, with localization to the outer mitochondrial membrane (GPAT1, GPAT2) and endoplasmic reticulum (ER) (GPAT3, GPAT4) [60]. LPA can also be generated through the hydrolysis of LPC by ectonucleotide pyrophosphatase/phosphodiesterase family member 2 (ENPP2) (also known as autotaxin), a glycoprotein widely expressed in tissues and present in ER, cytoplasm, and plasma [61–63]. LPC is present in mitochondria and ER membranes [64–67] and intracellular lipid droplets, which are located adjacent to mitochondria in skeletal muscle [68–70]. The role of lipid droplets in skeletal muscle lipid metabolism is not well understood. Lipid droplets consist of neutral lipids surrounded by a phospholipid monolayer containing proteins involved in lipid synthesis and remodeling, lipid droplet clustering and fusion, and scaffold proteins [68–71]. LPC can be generated from membrane phospholipids by phospholipase A₂ enzymes, a step in the Lands' cycle of lipid remodeling [72]. Peroxiredoxin 6 (PRDX6) is localized in the cytoplasm and has PLA₂ activity to generate LPC from phosphatidylcholine (PC) [73,74].

Most of the enzymes involved in cardiolipin synthesis have been localized to ER or a specialized ER region known as the mitochondrial-associated membrane (MAM) where the ER membrane and outer mitochondrial membrane are in close proximity [59]. The precise locations of the different enzymes involved in phospholipid synthesis, the transfer of lipid species between ER and mitochondria, and the potential role of autophagosomes are not completely known [59,75]. The MAM is enriched with terminal enzymes of different lipid

synthesis pathways, suggesting MAM is a hub for lipid synthesis [59]. PA, the central precursor for cardiolipin synthesis, can be generated via pathways within ER or in the outer mitochondrial membrane, but the relative contribution of each source for cardiolipin synthesis has not been established [54]. Acylglycerol-3-phosphate acyltransferase (AGPAT) catalyzes the acylation of LPA at the *sn*-2 position to form PA. AGPAT3 is located in the ER membrane [76]. AGPAT4 and AGPAT5 are located on the outer mitochondrial membrane [77,78]. Subsequently, PA is converted in the ER to cytidinediphosphate-1,2-diacyl-*sn*-glycerol (CDP-DAG) by CDP-diacylglycerol synthase, also known as Tam41 [79]. An example of the intersection and overlap of different lipid pathways in the MAM is CDP-DAG, which can be converted to phosphatidyl glycerol phosphate (PGP) by PGP synthase as the committed step in the cardiolipin synthesis pathway, or can enter synthesis pathways for PC (Kennedy pathway), phosphatidylethanolamine (PE), monoacylglycerol, or triacylglycerol [80]. PGP synthase transfers the activated phosphatidyl group from CDP-DAG to the *sn*-1 position of G3P to yield PGP. PGP is then hydrolyzed by protein tyrosine phosphatase mitochondrion 1 (PTPMT1) to form phosphatidylglycerol (PG) [81]. In the final step of the cardiolipin synthesis pathway, which occurs in the inner mitochondrial membrane, cardiolipin synthase (CLS) transfers a phosphatidyl group from another CDP-DAG molecule to PG to form nascent cardiolipin [80].

Nascent cardiolipin contains four fatty acid chains but then undergoes structural remodeling in which cardiolipin acquires a new set of fatty acids. The final fatty acid composition of the four acyl chains of mature cardiolipin is significantly different from nascent cardiolipin [53]. The remodeling of cardiolipin is catalyzed by tafazzin (TAZ), an enzyme that transfers fatty acids between phospholipids and lysophospholipids [53]. After cycles of deacylation and reacylation, remodeled cardiolipin contains predominantly unsaturated fatty acids. TAZ is generally considered to have no substrate preference and is not specific for fatty acid chains; substrate availability, physical properties of lipids, and thermodynamics are considered to influence the final form of cardiolipin in mitochondrial inner membranes [57,82]. Cardiolipin is relatively long-lived and has a half-life that is several times longer than the half-life of other phospholipids [53,83–85].

The fatty species composition of cardiolipin is critical to mitochondrial bioenergetics, as cardiolipin affects membrane biophysical properties and shapes the curvature of the mitochondrial cristae [57,86]. Cardiolipin is cone-shaped and generates negative curvature elastic stress in lipid bilayers [54,87]. PE is also cone-shaped with similar membrane properties. Together cardiolipin and PE comprise ~50% of phospholipid mass in the inner mitochondrial membrane [Ikou 2017]. The conical shape of these lipids gives stability to the tight curvature of the crista membranes, with the small glycerol head groups of cardiolipin and PE facing the cristae lumen. The curvature and properties of the membrane affect the assembly and function of the ETC complexes [88] and mitochondrial metabolic signaling [58]. Cardiolipin directly binds to complexes I [89,90], III [89,91–93], IV [94,95], and V [96] and the ADP/ATP carrier (ATC) [97,98] of the ETC. Cardiolipin is an integral component to ETC proteins and is critical to their folding, configuration, and function [88]. Disruption of cardiolipin biosynthesis results in impaired ETC complex formation [99,100], increased proton leak [101], and altered cristae morphology [102]. Reduction of cardiolipin impairs the activity of cytochrome c oxidase (COX), or ETC complex IV [103,104].

Tetra-linoleoyl cardiolipin (18:2)₄ is the dominant species in skeletal muscle and heart

The cardiolipin that comprises ~80% of cardiolipin in mitochondria from normal human skeletal and cardiac muscle is tetra-linoleoyl cardiolipin (18:2)₄ (Figure 1) [57]. There are many different possible combinations of fatty acids that could comprise the four fatty acid chains found in cardiolipin. With fourteen major fatty acids, eukaryotes could potentially produce 38,416 distinct cardiolipin species [105], but it is important to note that most cardiolipin in skeletal muscle and heart consists of tetra-linoleoyl cardiolipin (18:2)₄ [57,106]. This highly symmetrical conical configuration of cardiolipin and cardiolipin binding to membrane proteins optimizes function of the ETC in the inner mitochondrial membrane [88].

The evidence that cardiolipin (18:2)₄ is critical for mitochondrial function comes from studies in which cardiolipin fatty acid composition was modified by dietary intervention and observations from human heart failure. Reduction of cardiolipin (18:2)₄ content in mitochondria is associated with impairment of mitochondrial function. Feeding rats with a diet extremely low or deficient in linoleic acid (18:2) resulted in decreased cardiolipin (18:2)₄ in cardiac mitochondria and a large reduction in COX activity [107–109]. In a rat model of heart failure, a diet rich in linoleic acid increased cardiolipin (18:2)₄ in cardiac mitochondria, improved mitochondrial function, and reduced mortality [110,111]. Rats fed a diet rich in oleic acid (18:1) showed increased cardiolipin (18:1)₂, (18:2)₂ and decreased cardiolipin (18:2)₄ in liver mitochondria, with comitant decrease in mitochondrial state 3 respiration [112]. In a rat model, lower cardiolipin (18:2)₄ content of skeletal muscle mitochondria was significantly associated with decreased COX activity [113]. In a mouse model, a diet rich in docosahexaenoic acid (DHA) (22:6) led to replacement of cardiolipin (18:2)₄ with tetra-docosahexaenoyl (22:6)₄ cardiolipin in the heart, with impaired activity of ETC complexes I, IV, V, and I+III [114]. Mitochondrial respiratory activity was recovered with reintroduction of linoleic acid via fusion of phospholipid vesicles to mitochondria isolated from DHA-fed mice [114]. In humans, the relationship of aging with cardiolipin species in tissues remains a major gap in knowledge. In patients with heart failure, the tetra-linoleoyl cardiolipin (18:2)₄ content of mitochondria in left ventricle is significantly lower than those without heart failure [115,116].

Additional evidence for the importance of tetra-linoleoyl cardiolipin (18:2)₄ in mitochondrial function comes from studies of aging in animal models and Barth syndrome, as discussed in further detail below.

The cardiolipin content of mitochondria decreases with aging

Aging is associated with a decrease in the cardiolipin content of mitochondria and changes in the fatty acid composition of cardiolipin in a variety of tissues. The cardiolipin content of mitochondria decreases with age in rat heart [115,117–125], mouse heart [126], fish heart [127], rat brain [128–130], monkey brain [131]; rat liver [132–136], rat kidney [132], mouse skeletal muscle [137], and fish skeletal muscle [138]. The cardiolipin content of mitochondria decreases with age in human skin [139]. Animal studies show that the age-

related decrease in mitochondrial cardiolipin content is accompanied by a decrease in tetra-linoleoyl cardiolipin (18:2)₄ accompanied by substitution of other fatty acids in place of linoleic acid (18:2) in the heart, such as arachidonic and docosahexaenoic acids [115,126]. Other aging changes that have been described in the mitochondria of rat heart include a decline in complex I activity, state 3 respiration [140], and complex IV activity (cytochrome c oxidase) [115,121], and decreased carnitine-acyltransferase (CAT) activity [120,141,142]. Whether the loss of tetra-linoleoyl cardiolipin (18:2)₄ with age is due to decreased availability of linoleic acid or increased degradation of tetra-linoleoyl cardiolipin is not clear.

Tetra-linoleoyl cardiolipin as a substrate for peroxidation

Cardiolipin is susceptible to peroxidation due to its high content of polyunsaturated fatty acid chains containing one or more 1,4 *cis, cis* pentadiene structures in close proximity to sites where ROS are produced. Peroxidation of cardiolipin leads to a conformation change in both the glycerol headgroup and fatty acid chains, inducing a tilt to the molecule and loss of symmetry and change in thickness of the lipid bilayer [143]. Aging is associated with an increase in oxidized cardiolipin species in the rat heart [140,144] and fish heart [127]. Higher oxidized cardiolipin content is associated with impaired ETC function [140].

Linoleic acid in cardiolipin is a substrate for cytochrome c-mediated peroxidation [145], generating multiple esterified oxidized linoleic acid species including hydroperoxy-, hydroxy- and keto-octadecadienoic acids and epoxy-octadecenoic acids [146,147]. Peroxidation of linoleic acid moieties in cardiolipin is reported to release cytochrome c to the cytoplasm where it plays a key role in triggering apoptosis [148–150]. Oxidized linoleic acid species in cardiolipin are also substrates for enzyme-catalysed hydrolysis, releasing bioactive unesterified acids [145]. While specific actions and relevance of these oxidized lipids in skeletal muscle have not been identified, these products are reported to evoke inflammation and nociceptive responses in other tissues [151,152]. Thus, cardiolipin linoleic acid peroxidation could potentially impact sarcopenia by depleting tetra-linoleoyl cardiolipin with accompanying alterations in inflammatory signaling and apoptosis.

Implications of Barth syndrome for sarcopenia

Barth syndrome is a rare, X-linked recessive disorder due to mutations in the tafazzin (*TAZ*) gene locus. Barth syndrome is characterized by impaired cardiolipin synthesis and remodeling, altered cardiolipin species, decreased cardiolipin content of mitochondria, and abnormal mitochondrial structure with a clinical phenotype of cardiomyopathy, skeletal muscle weakness, neutropenia, and organic aciduria [153–155]. The cardiolipin alterations are associated with abnormalities of the mitochondrial inner membrane, impaired ETC function, decreased ATP production, inhibition of the tricarboxylic acid (TCA) cycle, disrupted β -oxidation of fatty acids, decreased plasma LPC, elevated plasma acylcarnitines, and accumulation of organic acids [155–157]. Skeletal muscle from Barth syndrome patients shows extremely low content of cardiolipin, primarily due to deficiency of tetra-linoleoyl cardiolipin (18:2)₄ [158]. In mouse models of Barth syndrome that involve *TAZ* knockdown or depletion, there is a large decrease in tetra-linoleoyl cardiolipin (18:2)₄ from cardiac and skeletal muscle, accumulation of cardiolipin species with aberrant fatty acid chains, and

disrupted cristae in mitochondria [159,160]. Under normal conditions, cardiolipin has slow turnover [53,83,84,161], however, in Barth syndrome, cardiolipin loses its association with membrane proteins and supercomplexes and is rapidly degraded [161]. Patients with Barth syndrome have severe exercise intolerance and greatly reduced skeletal muscle O₂ utilization [162]. Studies using ³¹P-MRS show that children, adolescents, and young adults with Barth syndrome have impaired skeletal muscle mitochondrial oxidative capacity [163].

Barth syndrome has some salient features in common with sarcopenia and aging, such as a reduction in cardiolipin content, decrease in tetra-linoleoyl cardiolipin (18:2)₄, impaired β-oxidation of fatty acids, reduced exercise tolerance, reduced skeletal muscle O₂ utilization, impaired skeletal muscle mitochondrial oxidative capacity, and a plasma metabolomic profile characterized by increased plasma acylcarnitines and decreased plasma LPC.

The tetra-linoleoyl cardiolipin depletion hypothesis and other pathways implicated in sarcopenia

Since cardiolipin plays a fundamental role in mitochondrial function, alterations in cardiolipin could affect other hypothesized pathways in the pathogenesis of sarcopenia such as anorexia of aging, altered proteostasis, and inflammation. The insufficient dietary intake that occurs with anorexia of aging [28,164] could involve qualitative changes in diet and an inadequate intake of essential fatty acids and other factors required for cardiolipin and optimal mitochondrial function. Alterations in cardiolipin could impair the role that cardiolipin plays in protein folding and stabilization and impair proteostasis [165,166]. Inadequate cardiolipin content and composition are associated with impairment of ETC function and contribute to excessive generation of ROS and inflammation [31,32].

Cardiolipin as a potential therapeutic target in sarcopenia

The cardiolipin content and composition of skeletal muscle could potentially be targeted through changes in lifestyle such as exercise, changes in diet or through weight loss, and by pharmacological intervention. In the rat model, exercise increased cardiolipin content in heart and enhanced cytochrome oxidase activity in mitochondria [167,168]. Endurance training increased cardiolipin content in red gastrocnemius muscle in the rat [169]. Twelve weeks of exercise training increased the cardiolipin content of vastus lateralis muscle in older sedentary adults [170]. In older overweight to obese individuals, 16 weeks of moderate exercise increased cardiolipin content in vastus lateralis muscle, while both 16 weeks of either caloric restriction or exercise induced cardiolipin remodeling in muscle with a significant increase in tetra-linoleoyl cardiolipin (18:2)₄ [171]. Twelve weeks of daily moderate-intensity exercise combined with weight loss increased mitochondrial cardiolipin content and mitochondrial oxidative enzymes in vastus lateralis muscle in patients with type 2 diabetes [172]. In patients who underwent bariatric surgery, six months of moderate exercise induced cardiolipin remodeling with an increase of tetra-linoleoyl cardiolipin (18:2)₄ content in vastus lateralis muscle [173].

In rodent models, the tetra-linoleoyl cardiolipin (18:2)₄ content of heart can be decreased by restricting linoleic acid and increasing oleic acid [112] or docosahexaenoic acid in the diet

[114]. Conversely the tetra-linoleoyl cardiolipin (18:2)₄ content of heart can be increased by a diet rich in linoleic acid [110,111]. Humans are unable to synthesize linoleic acid (18:2n-6), thus, this essential omega-6 fatty acid must be obtained from the diet. Linoleic acid is widely available in a concentrated form from plant seed oils such as soybean, corn, and cottonseed oils, and is the primary polyunsaturated fatty acid in the US diet [174]. Among US adults, mean linoleic acid intake tends to decrease with age and is lower among those aged > 70 years compared with those aged 20–70 years [174]. However, current U.S. LA intakes are several-fold higher than can be achieved from pre-industrial diets without added liquid vegetable oils [175].

Recently, a family of small, synthetic cell-penetrating peptides, also known as Szeto-Schiller (SS) peptides, were described that selectively target cardiolipin in the inner mitochondrial membrane [176,177]. These peptides bind to cardiolipin and increase coupling efficiency while reducing generation of ROS [177]. The SS peptide, SS-31 (elamipretide) has shown promise in rejuvenating mitochondrial bioenergetics in phase I-II trials for heart failure [178] and primary mitochondrial myopathy [179]. There are over a dozen enzymes involved in the lipid pathways leading to cardiolipin synthesis and remodeling; these enzymes are potential therapeutic targets for modulating cardiolipin content in skeletal muscle and other tissues.

Gaps in knowledge and unanswered questions

While we have hypothesized that cardiolipin plays a major role in the pathogenesis of sarcopenia, there are many unanswered questions. Do the content and fatty acid composition of cardiolipin change in human skeletal muscle with aging? Does the susceptibility to cardiolipin to peroxidation increase in human skeletal muscle with sarcopenia? What is the pathway by which cardiolipin fatty acid composition is modulated in humans by diet and physical activity? What is the optimal range of linoleic acid intake to maintain tetra-linoleoyl cardiolipin across the lifespan? Is the content and fatty acid composition of cardiolipin in human skeletal muscle related to muscle strength or physical performance? What is the relationship between cardiolipin fatty acid content and composition in human skeletal muscle with mitochondrial oxidative capacity? Addressing these questions may reveal novel therapeutic targets for the prevention of age-related sarcopenia.

Conclusion

In summary, we propose the hypothesis that the depletion of tetra-linoleoyl cardiolipin plays a major role in the pathogenesis of sarcopenia. This is a testable hypothesis, that if corroborated, will provide new leads for the prevention and treatment of sarcopenia.

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Figure 1.

The structure of cardiolipin includes a glycerol headgroup, two phosphatidyl moieties, and four fatty acid chains. The example shown is tetra-linoleoyl cardiolipin (18:2)₄ with the four fatty acid chains consisting of linoleic acid.

Figure 2.

A conceptual model for the hypothesis that depletion of tetra-linoleoyl cardiolipin plays a central role in sarcopenia.

Figure 3.

Synthesis pathway of cardiolipin from lysophosphatidic acid and lysophosphatidylcholine.

Abbreviations: acylglycerol-3-phosphate acyltransferase (AGPAT), autotaxin (ATX), cardiolipin synthase (CLS1), cytidine diphosphate (CDP), CDP-diacylglycerol synthase (CDS), cytidine monophosphate (CMP), cytidine triphosphate (CTP), endothelial lipase (EL), glycerol-3-phosphate acyltransferase (GPAT), phosphatidylcholine-sterol acyltransferase (LCAT), phosphatidylglycerophosphate and protein-tyrosine phosphatase 1 (PTPMT1), phosphatidylglycerophosphate synthase (PGP synthase), phospholipase A2 (PLA2), pyrophosphate (PPi), tafazzin (TAZ).