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Looking beyond structure: membrane phospholipids of skeletal muscle mitochondria

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Abstract

Skeletal muscle mitochondria are highly dynamic and capable of tremendous expansion to meet cellular energetic demands. Such proliferation in mitochondrial mass requires a synchronized supply of enzymes and structural phospholipids. While transcriptional regulation of mitochondrial enzymes has been extensively studied, there is limited information on how mitochondrial membrane lipids are generated in skeletal muscle. Herein we describe how each class of phospholipids that constitute mitochondrial membranes are synthesized and/or imported, and summarize genetic evidence indicating that membrane phospholipid composition represents a significant modulator of skeletal muscle mitochondrial respiratory function. We also discuss how skeletal muscle mitochondrial phospholipids may mediate the effect of diet and exercise on oxidative metabolism.

Keywords

Membrane phospholipids; mitochondria; skeletal muscle; phospholipid biosynthesis; aerobic capacity

Linking Aerobic Capacity to Skeletal Muscle Mitochondrial Phospholipids

Low **aerobic capacity** (see Glossary) is a stronger risk factor for all-cause mortality than hypertension, type 2 diabetes, or smoking [1]. Animals bred for low intrinsic maximal aerobic capacity have a greater emergence of complex, chronic diseases and reduced longevity compared to animals bred for high intrinsic maximal aerobic capacity [2]. Skeletal muscle mitochondrial respiration is a major contributor to whole-body respiration and overall energy expenditure [3]. Thus, factors that affect skeletal muscle mitochondrial

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function have the potential to substantially impact health and longevity. For example, persistent metabolic overload, via its impact on bioenergetics is implicated in pathogenesis of insulin resistance as well as an array of metabolic and cardiovascular disorders [4], and loss of muscle function and mass in sarcopenia and cachexia [5].

Skeletal muscle mitochondria demonstrate extraordinary plasticity in response to metabolic stressors. Indeed, both aerobic exercise training and short-term high fat diets (HFD) activate mitochondrial biogenesis and increase the capacity for substrate oxidation [6, 7]. Conversely, physical inactivity decreases mitochondrial enzyme activity [8]. Although a great deal of attention has been devoted to deciphering the mechanisms regulating the expression of mitochondrial enzymes, less attention has been given to understanding the mechanisms regulating mitochondrial membrane phospholipid composition despite its recognition as an important determinant of the activity of the **electron transport system (ETS)** complexes, and thus aerobic capacity [9].

This review summarizes the current understanding of the synthesis and function of mitochondrial **phospholipids**, examples of mouse and human genetic defects in enzymes of phospholipid biosynthesis on skeletal muscle metabolism (Table 1), and the effect of exercise or diet on skeletal muscle mitochondrial phospholipids.

Composition of Skeletal Muscle Mitochondrial Membranes

The phospholipid profile of skeletal muscle mitochondria is different compared to that of other organelles or the sarcolemmal membrane. Skeletal muscle mitochondria consist of ~40% phosphatidylcholine (PC), ~30% phosphatidylethanolamine (PE), ~15% cardiolipin (CL), ~7% phosphatidylinositol (PI), ~3% phosphatidylserine (PS), ~3% lyso-PC, and ~2% sphingomyelin [10, 11]. In comparison, liver mitochondria consist of 34–55% PC, 19–36% PE, 12–23% CL, 5–8% PI, 1% PS, 1–2% lyso-phospholipids, 1–3% sphingomyelin, and 1–2% phosphatidic acid (PA) [12]. The large variability in reported data, potentially resulting from differences in mitochondrial isolation procedures, makes it difficult to conclude whether there are meaningful differences in mitochondrial membrane lipids from muscle and liver. It is unknown whether there is a tissue-dependent relationship between membrane phospholipid composition and mitochondrial functions.

Mitochondria consist of two phospholipid bilayers including the outer mitochondrial membrane (OMM) and the inner mitochondrial membrane (IMM). The OMM is lipid rich, smooth, and highly fluid whereas the IMM is protein-rich, extensively folded, and highly compartmentalized. Imbedded within the IMM are the ETS enzymes that carry out oxidative phosphorylation. The phospholipid composition of the OMM and IMM of skeletal muscle mitochondria has not been described, but liver OMM contains 44–59% PC, 20–35% PE, 5–20% PI, with remaining phospholipid pool consisting of PS, PA, CL, and lyso-phospholipids [12, 13]. By contrast, the IMM consists of 38–45% PC, 32–39% PE, 14–23% CL, 2–7% PI, and ~3% PS, PG and lysophospholipids. Below we provide summary on the four most abundant mitochondrial phospholipids including PC, PE, CL, and PI.

Phosphatidylcholine

Despite being the most abundant phospholipid in both the OMM and IMM, PC is not synthesized within mitochondria. Instead PC is imported from the circulation or synthesized at the endoplasmic reticulum (ER) via the cytidine diphosphate (CDP) choline pathway (or **Kennedy pathway**) (Figure 1A) [14], by the enzyme phosphatidylethanolamine N-methyltransferase (PEMT) from PE (Figure 1B) [15], by the reversible enzyme phosphatidylserine synthase 1 (PSS1) from PS (Figure 1B) [16, 17], or through phospholipid remodeling via the Lands cycle [18]. The CDP-choline pathway involves three sequential reactions including: 1) choline kinase (CK) catalyzing the synthesis of phosphocholine from ATP and choline, 2) CTP:phosphocholine cytidylyltransferase (CT) converting CTP and phosphocholine to CDP-choline (rate-limiting step), and 3) diacylglycerol (DAG) molecule displacing the cytidine nucleotide to form PC via the enzyme choline phosphotransferase (CPT). PEMT tri-methylates PE to form PC, though this enzyme appears to have limited activity in skeletal muscle. PSS1-dependent synthesis of PC is also thought to be quantitatively minimal. The Land's cycle only strictly remodels phospholipid fatty acids (FAs) catalyzed by phospholipases and lyso-phospholipid acyltransferases. These PC molecules are then trafficked into the mitochondria through the **mitochondria associated membrane (MAM)** and between the OMM and IMM through contact sites (Figure 1) [13, 19, 20].

Although PC biosynthesis can occur through multiple pathways, acquired or inherited defects in the CDP-choline pathway appear to be particularly detrimental to mitochondrial membrane lipid composition and function. For example, choline deficiency reduces membrane PC content in cultured C2C12 muscle cells and leads to increased triglyceride accumulation, membrane fragility, and increased susceptibility to apoptosis [21, 22]. Mice that lack CKα die during embryogenesis [23]. A defect in CKβ promotes muscular dystrophy in mice [24, 25]. Skeletal muscle mitochondria from these mice are swollen and have lower membrane potential, suggesting mitochondrial dysfunction. A rare congenital muscular dystrophy disease in humans is caused by homozygous or compound heterozygous mutations in the CKβ gene [26]. These individuals have reduced skeletal muscle PC, abnormally enlarged mitochondria that are located in the periphery of muscle fibers, and early-onset muscle wasting, muscle weakness, and hypotonia. Absence of CTα is also embryonically lethal [27], whereas loss of CTβ results in viable mice with no apparent effect on skeletal muscle phenotype [28]. Excess membrane PC also appears to have detrimental effects on mitochondria, as fusing unilamellar vesicles composed of dioleoyl-PC to mitochondria from cardiac muscle increases PC content and reduces ETS complex I, II, and IV activity [29]. Together, these observations highlight the functional importance of PC in regulating skeletal muscle mitochondrial function.

Phosphatidylethanolamine

PE can be synthesized via the CDP-ethanolamine pathway (Figure 1A) [14], from PS by the enzyme phosphatidylserine decarboxylase (PSD) (Figure 1C) [30], or the reversible enzyme phosphatidylserine synthase-2 (PSS2) (Figure 1B) [31], and the Land's cycle via a lyso-PE acyltransferases [18], although the latter two routes are generally considered to be

quantitatively minor in mammalian muscles. Mitochondrial PE is predominantly synthesized by the enzyme PSD, located on the IMM [30, 32]. PE is the second most abundant phospholipid found in mitochondria after PC, but because PC is not synthesized within mitochondria, PE is the most autonomously synthesized phospholipid in mitochondrial membranes. The CDP-ethanolamine pathway or extracellular sources do not appear to substantially contribute to mitochondrial PE, but PE synthesized by PSD can be exported out of mitochondria [32].

Whole-body deletion of the PSD enzyme is embryonically lethal [30]. Mitochondria from embryonic fibroblasts of these mice are swollen, rounded, and fragmented, suggesting mitochondrial PE is important for cristae development and the elongated shape of mitochondria. In CHO cells, reducing PSD expression promotes increases in mitochondrial fission and membrane potential, and decreases the activities of ETS complex I, II, and IV, protein stability, supercomplex formation and ATP synthesis [33]. Whole-body ablation of the CTP:phosphoethanolamine cytidylyltransferase (ET) enzyme is also embryonically lethal [34]. Skeletal muscle-specific ablation of the CDP-ethanolamine pathway (Figure 1A) has been induced by targeting ET and choline/ethanolamine phosphotransferase-1 (CEPT1). In mice with skeletal muscle-specific knockout of ET, reduction in cellular PE coincided with increased **skeletal muscle mitochondrial biogenesis** and exercise endurance [35]. In contrast, muscle-specific deletion of CEPT1 had no apparent effect on skeletal muscle mitochondria but promoted muscle weakness [36]. Differences in mitochondrial phenotype promoted by deletion of PSD, ET, or CEPT1 demonstrate the complexity of skeletal muscle phospholipid metabolism. These differences are at least partly explained by distinct subcellular localization of these enzymes.

Cardiolipin

CL is a tetra-acyl phospholipid that is mostly uniquely present in the IMM. The synthesis of CL mainly occurs on the IMM (Figure 1D) by cardiolipin synthase (CLS) and tafazzin [9]. CLS catalyzes the synthesis of a nascent CL molecule from phosphatidylglycerol (PG) and CDP-diacylglycerol (CDP-DAG). Nascent CL molecules subsequently become trans-acylated with long-chain FAs by tafazzin to yield the final mature CL. Alternatively to tafazzin, nascent CL molecules can be de/re-acylated by phospholipase A_2 (PL-A_2) and monolysocardiolipin acyltransferase-1 (MLCLAT-1). Some studies also suggest that re-acylation of MLCL can occur at the ER by the enzyme acyl-CoA: lysocardiolipin acyltransferase-1 (ALCAT-1) (Figure 1E) [37]. CL has several important functions including providing osmotic stability to mitochondrial membranes, maintaining the mitochondrial membrane potential, stabilizing ETS enzymatic activity and supercomplexes, Ca^{2+} homeostasis, mitophagy, and apoptosis [38–40].

There are two human genetic defects attributed to enzymes of mitochondrial CL metabolism. **Barth syndrome** is an X-linked genetic disorder that is caused by a mutation in the tafazzin gene [41]. These individuals lack mature CL and have abnormal mitochondrial cristae and reduced oxidative capacity in heart and skeletal muscle. Patients with Barth syndrome experience muscle weakness and exercise intolerance due to impaired skeletal muscle O_2 extraction and utilization [42]. In a mouse model of Barth syndrome, exercise

capacity is reduced [43]. Skeletal muscle from these mice is characterized by reduced complex IV activity and lower intracellular pH after electrical stimulation, suggesting metabolic abnormalities in response to contraction [44]. **Sengers syndrome** is an autosomal-recessive disorder caused by a mutation in the mitochondrial enzyme acylglycerol kinase (AGK) [45, 46]. Individuals with Sengers syndrome suffer from congenital cataracts, hypertrophic cardiomyopathy, skeletal myopathy, exercise intolerance, and lactic acidosis. It is thought that the mutation leads to a loss of AGK enzymatic activity, decreasing the synthesis of mitochondrial PA, an important precursor for CL synthesis [46]. Loss of AGK also leads to a decreased pyruvate/succinate metabolism and ATP deficiency, consistent with mitochondrial dysfunction representing the primary defect.

Increased mitochondrial CL content alone may be sufficient to improve mitochondrial respiratory function. Skeletal muscle ATP citrate lyase appears to orchestrate a coordinated response to elevate mitochondrial CL synthesis, as muscle-specific overexpression of this enzyme results in increased abundance and activities of ETS complexes [47]. However, the effect of CL to improve mitochondrial function is likely limited to mature CL. Deletion of group VIA or VIB PL-A_2 (iPL$A_2\beta$ or iPL$A_2\gamma$) that would be expected to result in accumulation of nascent CL impair skeletal muscle FA oxidation [48, 49]. Indeed, in iPL$A_2\gamma$ knockout mice, skeletal muscle mitochondria are larger but contain greater heterogeneity of CL subspecies [49]. These findings highlight a critical role that mitochondrial CL plays on skeletal muscle oxidative metabolism.

Phosphatidylinositol

The enzyme PI synthase catalyzes the formation of PI from the substrates CDP-DAG and myo-inositol (Figure 1F) [50]. The synthesis of PI is thought to occur predominately on the ER membrane (ERM) [51]. The majority of mitochondrial PI is located within the OMM [19]. PI often plays a role as a lipid signaling molecule to regulate cellular vesicular trafficking and ion channel activity, but the role of PI in mitochondria has not been well characterized. Masking or removing PI(4,5)-bisphosphate from the OMM increases mitochondrial fission, mitophagy, and fragmentation, suggesting PI may be an important signaling molecule for regulating mitochondrial morphology [52].

Saturation of Phospholipids

Skeletal muscle mitochondrial phospholipids contain lower amounts of polyunsaturated FAs (PUFAs) compared with whole muscle phospholipids [11], and slow-twitch soleus muscles contain less PUFAs in mitochondrial PC, PE, and CL compared to fast-twitch plantaris muscles [10]. The impact that FA saturation of skeletal muscle mitochondrial phospholipids has on mitochondrial function is not completely clear, but skeletal muscle mitochondrial phospholipid PUFA content appears to be inversely correlated with longevity across multiple mammalian species [53, 54]. For example, naked-mole rats live exceptionally long (> 28 yrs) and have low PUFA-containing mitochondrial phospholipids [55]. The inverse relationship between mitochondrial PUFA content and longevity might be due to greater propensities for these molecules to become peroxidized in response to reactive oxygen species [56, 57]. It has been postulated that these peroxidized phospholipids promote release

of mitochondrial cytochrome c to induce apoptosis [58]. However, lifespan is determined by complex interactions of health in multiple tissues, and it is unlikely that muscle mitochondrial PUFA level can be used as a single reliable predictor of longevity. There are mixed reports on whether diet with low PUFA content extends lifespan [59, 60]. Whether direct manipulation of skeletal muscle mitochondrial phospholipid FA profile is sufficient to alter metabolic health is also unknown.

Exercise/Inactivity and Skeletal Muscle Mitochondrial Phospholipids

Exercise training increases skeletal muscle aerobic capacity and mitochondrial density [6, 8]. Biogenesis of mitochondria induced by exercise training would be predicted to require additional membrane phospholipid molecules (Figure 2). Likewise, a reduction in mitochondrial content with inactivity would require corresponding removal of these lipids. Because proliferation or removal of mitochondrial phospholipids must occur while maintaining ionic gradients of membranes and activities of ETS enzymes to maintain substrate oxidation, it is expected that synthesis/import/export of mitochondrial phospholipids occurs in a highly regulated and coordinated fashion. Furthermore, it is reasonable to suspect that changes in muscle contractile activity may induce a remodeling of mitochondrial membrane phospholipid composition to adapt to changing energetic demands imposed by regular exercise or physical inactivity. Indeed, exercise training alters skeletal muscle total phospholipid content in rodents and humans [61–64], but very few studies have reported responses that occur specifically in mitochondrial phospholipids. Data on total cellular phospholipids are informative, but they do not necessarily reflect changes that occur in mitochondria. Treadmill exercise training in rats appears to increase mitochondrial PC without affecting PE, CL or PI [65], although the findings from this study were not conclusive. Because CL is mostly unique to mitochondria, abundance of this molecule has been used as a marker for mitochondrial density. Indeed, total CL content increases with exercise training in rodents and in humans [47, 66]. Intriguingly, time-course experiments suggest that while CL content and ETS enzyme activity increase synchronously in response to exercise training, detraining promotes a decrease in total CL content that precedes a decrease in ETS enzyme activity [67]. It is unclear whether this represents accelerated removal of all classes of phospholipids (PC, PE, PI, etc.) induced by inactivity, or simply an observation unique to CL. Additional studies on exercise- or physical-inactivity induced adaptation of mitochondrial phospholipids are needed to more rigorously examine how the abundance of each class of phospholipids becomes altered, and in turn affect mitochondrial respiratory function.

Mechanisms that drive exercise-stimulated generation of skeletal muscle mitochondrial membranes are also not clearly understood. Exercise training increases abundance of enzymes of CL and PI synthesis [47, 68]. It is not clear how exercise training increases synthesis/import of other mitochondrial phospholipids, but such a mechanism(s) likely involves an action of peroxisome proliferator-activated receptor gamma coactivator 1α (PGC1α). Skeletal muscle-specific overexpression of PGC1α is sufficient to increase PC and PE, while its absence results in ablation of exercise-induced increases in cellular PC and PE [63, 69]. PGC1α may also play a role in CL synthesis [68], though Akt and AMP-activated protein kinase (AMPK) are also likely involved [47, 70].

Impact of Diet on Skeletal Muscle Mitochondrial Phospholipids

Short-term HFDs induces skeletal muscle mitochondrial biogenesis [7], an effect that presumably coincides with an increase in mitochondrial phospholipid biosynthesis/import. Alteration in diet (composition or total caloric intake) also affects cellular processes that control mitochondrial quality such as fusion, fission, and mitophagy [71], each requiring complex reorganization of mitochondrial membrane phospholipids [72]. There is a surprising lack of studies on the effect of diet on mammalian skeletal muscle mitochondrial phospholipid composition. Total cellular phospholipids appear to decrease in response to HFD, even in the presence of mitochondrial biogenesis [73]. One study reported increased saturated and polyunsaturated FAs and decreased monounsaturated FAs in skeletal muscle mitochondrial phospholipids, changes that coincided with enhanced capacity of the ETS [74]. Another study reported increased cellular CL content with HFD, with increased heterogeneity of CL molecular species that coincided with reduction in substrate oxidation [49]. It remains unclear how short- or long-term HFDs alter skeletal muscle mitochondrial phospholipids, and how such changes may mediate a shift in mitochondrial phenotype induced by these interventions.

The effect of PUFA-containing diets on skeletal muscle mitochondrial phospholipids has been more extensively studied across various animal models. In trout, a diet with high levels of PUFAs leads to modest changes in abundance of skeletal muscle mitochondrial PC and PE [75], without having an effect on skeletal muscle O_2 consumption or activity of ETS complexes [76]. In rats, PUFA-containing diets do not alter head-groups of skeletal muscle mitochondrial phospholipids, but increase PUFAs in mitochondrial CL. Mitochondrial CL PUFA content is in turn positively correlated with cytochrome c oxidase activity [77]. In humans, some studies suggest that PUFA-containing diets induce mitochondrial biogenesis [78], while other studies provide evidence that some functions of mitochondrial respiration might be improved [79]. In all cases, the effect of a PUFA-containing diet on mammalian muscle mitochondrial phospholipids appears to be mostly limited to their FA composition and not on the classes of phospholipids. These studies provide evidence for how diet can influence skeletal muscle mitochondrial phospholipids and respiratory function.

Concluding Remarks

Cellular organelles are separated by hydrophilic cytosol, and as such, compositions of membrane phospholipids are highly compartmentalized, providing unique physiochemical environments ideal for their specific physiological processes. In turn, phospholipid molecules are often thermodynamically unfavorable to transport from one organelle to another. Thus, subcellular localization of the enzymes of phospholipid biosynthesis must have significant biological importance (Figure 1). Although an understanding of the biochemistry of phospholipid biosynthesis has come a long way, the complex roles of phospholipids in regulating cellular homeostasis remain under appreciated and under studied. Thus, a challenge moving forward is to decipher the physiological implications of these reactions in the context of the three-dimensional arena of the cell.

The strongest predictor of all-cause mortality is low aerobic capacity, which is highly dependent on skeletal muscle mitochondrial respiration. Across all organisms, activities of the respiratory enzymes are modulated by composition of membrane phospholipids in which they reside, but there is little information regarding the role that these lipids play in modulating skeletal muscle mitochondrial function. In skeletal muscle, lipid fate towards oxidation or lipid droplet has been extensively studied, but it is unclear how new membrane phospholipids are generated for the highly dynamic and proliferative mitochondria (Figure 2). Diet and exercise are known stimuli that promote skeletal muscle lipid influx, each promoting unique adaptive responses to mitochondrial substrate handling [80]. We speculate that some of these adaptive responses are mediated by alteration in composition of mitochondrial membrane phospholipids.

Genetic studies in mice and in humans provide evidence that alterations in mitochondrial phospholipids can have significant, often detrimental effects on skeletal muscle respiration. Altered concentrations of PC, PE, CL, and PI all appear to have unique effects on mitochondrial phenotype. The functional importance of these phospholipids extends well beyond maintaining the structural integrity of mitochondria, and includes such processes as maintaining and regulating membrane potential, substrate transport, Ca^{2+} homeostasis, activity of ETS enzymes, and gross morphological changes to mitochondria (Figure 2). Mitochondrial phospholipid desaturation is inversely correlated to lifespan, an effect that may be mediated by the ability of the unsaturated fatty acids to become peroxidized. With tools such as mass spectrometry, tissue-specific gene-targeting and high resolution respirometry, we are now better equipped to study how these phospholipid molecules affect mitochondrial function, and aerobic capacity of skeletal muscle (see Outstanding Questions).

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Glossary

Aerobic capacity (VO_2max)
Maximal rate of oxygen consumed during all-out aerobic exercise, usually assessed by indirect calorimetry during a graded treadmill running test. Aerobic capacity is the best predictor of all-cause mortality. The largest contributor of VO_2max is mitochondrial oxidative phosphorylation in skeletal muscle

Barth syndrome
An X-linked genetic disease caused by a mutation in tafazzin, a cardiolipin (CL) remodeling enzyme. This condition results in impaired synthesis of mature CL, an essential membrane phospholipid for mitochondrial function. Individuals with Barth syndrome suffer from cardiomyopathy, neutropenia, underdeveloped skeletal muscles, muscle weakness, and exercise intolerance

Electron transport system (ETS)

Located within the inner mitochondrial membrane (IMM), ETS consists of five enzyme complexes including Complex I (NADH dehydrogenase), Complex II (succinate dehydrogenase), Complex III (cytochrome bc_1 complex), Complex IV (cytochrome c oxidase), and Complex V (ATP Synthase). Redox reactions by complexes I–IV promote the transfer of protons across the IMM, creating an electrochemical proton gradient used to drive ATP synthesis by complex V

Kennedy Pathway

First identified by Eugene Kennedy in 1956, CDP-choline and CDP ethanolamine pathways are the predominant mechanisms by which mammalian cells synthesize phosphatidylcholine (PC) and phosphatidylethanolamine (PE)

Mitochondria Associated Membrane (MAM)

The MAM is a specialized compartment of the endoplasmic reticulum (ER) that directly interacts with mitochondria. The MAM is the main site of phospholipid import/export between the ER and mitochondria

Phospholipids

Cellular membranes are largely composed of phospholipid molecules. The basic phospholipid structure consists of a glycerol backbone with the sn-1 and sn-2 positions occupied by two fatty acid chains and the sn-3 position occupied by a phosphate head group. The phospholipids are classified according to molecules attached to the head group, such as PC, PE, phosphatidylserine (PS), phosphatidylinositol (PI), phosphatidylglycerol (PG), and CL. Conically-shaped phospholipids such as PE and CL form membrane curvature and are more highly concentrated at cristae compared to cylindrically-shaped phospholipids such as PC, PS, PG and PI

Sengers syndrome

An autosomal-recessive disorder caused by a mutation in the gene encoding mitochondrial acylglycerol kinase (AGK). Clinical manifestations of the disease resemble that of Barth syndrome and include cardiomyopathy, congenital cataracts, myopathy, and lactic acidosis. The skeletal muscle of these individuals has defects in mitochondrial ATP synthesis

Skeletal Muscle Mitochondrial Biogenesis

Energetic demands such as exercise, promotes proliferation of skeletal muscle mitochondria. This process involves a highly coordinated and synchronized supply of mitochondrial enzymes and phospholipids. Transcriptional regulation of skeletal muscle mitochondrial biogenesis appears to be highly regulated by PGC1α

References

1. Myers J, et al. Exercise capacity and mortality among men referred for exercise testing. The New England journal of medicine. 2002; 346:793–801. [PubMed: 11893790]
2. Wisloff U, et al. Cardiovascular risk factors emerge after artificial selection for low aerobic capacity. Science. 2005; 307:418–420. [PubMed: 15662013]
3. Zurlo F, et al. Skeletal muscle metabolism is a major determinant of resting energy expenditure. The Journal of clinical investigation. 1990; 86:1423–1427. [PubMed: 2243122]

4. Muoio DM, Neufer PD. Lipid-induced mitochondrial stress and insulin action in muscle. Cell metabolism. 2012; 15:595–605. [PubMed: 22560212]
5. Carter HN, et al. Mitochondria, muscle health, and exercise with advancing age. Physiology. 2015; 30:208–223. [PubMed: 25933821]
6. Holloszy JO. Biochemical adaptations in muscle. Effects of exercise on mitochondrial oxygen uptake and respiratory enzyme activity in skeletal muscle. The Journal of biological chemistry. 1967; 242:2278–2282. [PubMed: 4290225]
7. Hancock CR, et al. High-fat diets cause insulin resistance despite an increase in muscle mitochondria. Proceedings of the National Academy of Sciences of the United States of America. 2008; 105:7815–7820. [PubMed: 18509063]
8. Henriksson J, Reitman JS. Time course of changes in human skeletal muscle succinate dehydrogenase and cytochrome oxidase activities and maximal oxygen uptake with physical activity and inactivity. Acta physiologica Scandinavica. 1977; 99:91–97. [PubMed: 190867]
9. Mejia EM, Hatch GM. Mitochondrial phospholipids: role in mitochondrial function. Journal of bioenergetics and biomembranes. 2015
10. Stefanyk LE, et al. Skeletal muscle type comparison of subsarcolemmal mitochondrial membrane phospholipid fatty acid composition in rat. The Journal of membrane biology. 2010; 234:207–215. [PubMed: 20336283]
11. Tsalouhidou S, et al. Mitochondrial phospholipids of rat skeletal muscle are less polyunsaturated than whole tissue phospholipids: implications for protection against oxidative stress. Journal of animal science. 2006; 84:2818–2825. [PubMed: 16971584]
12. Daum G. Lipids of mitochondria. Biochimica et biophysica acta. 1985; 822:1–42. [PubMed: 2408671]
13. Daum G, Vance JE. Import of lipids into mitochondria. Progress in lipid research. 1997; 36:103–130. [PubMed: 9624424]
14. Kennedy EP, Weiss SB. The function of cytidine coenzymes in the biosynthesis of phospholipides. The Journal of biological chemistry. 1956; 222:193–214. [PubMed: 13366993]
15. Vance DE, Vance JE. Physiological consequences of disruption of mammalian phospholipid biosynthetic genes. Journal of lipid research. 2009; 50(Suppl):S132–137. [PubMed: 18955728]
16. Arikketh D, et al. Defining the importance of phosphatidylserine synthase-1 (PSS1): unexpected viability of PSS1-deficient mice. The Journal of biological chemistry. 2008; 283:12888–12897. [PubMed: 18343815]
17. Sousa SB, et al. Gain-of-function mutations in the phosphatidylserine synthase 1 (PTDSS1) gene cause Lenz-Majewski syndrome. Nature genetics. 2014; 46:70–76. [PubMed: 24241535]
18. Hishikawa D, et al. Discovery of a lysophospholipid acyltransferase family essential for membrane asymmetry and diversity. Proceedings of the National Academy of Sciences of the United States of America. 2008; 105:2830–2835. [PubMed: 18287005]
19. Horvath SE, Daum G. Lipids of mitochondria. Progress in lipid research. 2013; 52:590–614. [PubMed: 24007978]
20. Vance JE. MAM (mitochondria-associated membranes) in mammalian cells: lipids and beyond. Biochimica et biophysica acta. 2014; 1841:595–609. [PubMed: 24316057]
21. Michel V, et al. The impact of choline availability on muscle lipid metabolism. Food & function. 2011; 2:53–62. [PubMed: 21773586]
22. da Costa KA, et al. Elevated serum creatine phosphokinase in choline-deficient humans: mechanistic studies in C2C12 mouse myoblasts. The American journal of clinical nutrition. 2004; 80:163–170. [PubMed: 15213044]
23. Wu G, et al. Early embryonic lethality caused by disruption of the gene for choline kinase alpha, the first enzyme in phosphatidylcholine biosynthesis. The Journal of biological chemistry. 2008; 283:1456–1462. [PubMed: 18029352]
24. Wu G, et al. Understanding the muscular dystrophy caused by deletion of choline kinase beta in mice. Biochimica et biophysica acta. 2009; 1791:347–356. [PubMed: 19236939]
25. Sher RB, et al. A rostrocaudal muscular dystrophy caused by a defect in choline kinase beta, the first enzyme in phosphatidylcholine biosynthesis. The Journal of biological chemistry. 2006; 281:4938–4948. [PubMed: 16371353]

26. Mitsuhashi S, et al. Muscle choline kinase beta defect causes mitochondrial dysfunction and increased mitophagy. Human molecular genetics. 2011; 20:3841–3851. [PubMed: 21750112]
27. Wang L, et al. Early embryonic lethality in mice with targeted deletion of the CTP:phosphocholine cytidylyltransferase alpha gene (Pcyt1a). Molecular and cellular biology. 2005; 25:3357–3363. [PubMed: 15798219]
28. Jackowski S, et al. Disruption of CCTbeta2 expression leads to gonadal dysfunction. Molecular and cellular biology. 2004; 24:4720–4733. [PubMed: 15143167]
29. Shaikh SR, et al. Increasing mitochondrial membrane phospholipid content lowers the enzymatic activity of electron transport complexes. Biochemistry. 2014; 53:5589–5591. [PubMed: 25145682]
30. Steenbergen R, et al. Disruption of the phosphatidylserine decarboxylase gene in mice causes embryonic lethality and mitochondrial defects. The Journal of biological chemistry. 2005; 280:40032–40040. [PubMed: 16192276]
31. Bergo MO, et al. Defining the importance of phosphatidylserine synthase 2 in mice. The Journal of biological chemistry. 2002; 277:47701–47708. [PubMed: 12361952]
32. Shiao YJ, et al. Evidence that phosphatidylserine is imported into mitochondria via a mitochondria-associated membrane and that the majority of mitochondrial phosphatidylethanolamine is derived from decarboxylation of phosphatidylserine. The Journal of biological chemistry. 1995; 270:11190–11198. [PubMed: 7744750]
33. Tasseva G, et al. Phosphatidylethanolamine deficiency in Mammalian mitochondria impairs oxidative phosphorylation and alters mitochondrial morphology. The Journal of biological chemistry. 2013; 288:4158–4173. [PubMed: 23250747]
34. Fullerton MD, et al. Developmental and metabolic effects of disruption of the mouse CTP:phosphoethanolamine cytidylyltransferase gene (Pcyt2). Molecular and cellular biology. 2007; 27:3327–3336. [PubMed: 17325045]
35. Selathurai A, et al. The CDP-Ethanolamine Pathway Regulates Skeletal Muscle Diacylglycerol Content and Mitochondrial Biogenesis without Altering Insulin Sensitivity. Cell metabolism. 2015; 21:718–730. [PubMed: 25955207]
36. Funai K, et al. Skeletal Muscle Phospholipid Metabolism Regulates Insulin Sensitivity and Contractile Function. Diabetes. 2016; 65:358–370. [PubMed: 26512026]
37. Cao J, et al. A novel cardiolipin-remodeling pathway revealed by a gene encoding an endoplasmic reticulum-associated acyl-CoA:lysocardiolipin acyltransferase (ALCAT1) in mouse. The Journal of biological chemistry. 2004; 279:31727–31734. [PubMed: 15152008]
38. Fry M, Green DE. Cardiolipin requirement for electron transfer in complex I and III of the mitochondrial respiratory chain. The Journal of biological chemistry. 1981; 256:1874–1880. [PubMed: 6257690]
39. Zhang M, et al. Gluing the respiratory chain together. Cardiolipin is required for supercomplex formation in the inner mitochondrial membrane. The Journal of biological chemistry. 2002; 277:43553–43556. [PubMed: 12364341]
40. Paradies G, et al. Role of cardiolipin peroxidation and Ca2+ in mitochondrial dysfunction and disease. Cell calcium. 2009; 45:643–650. [PubMed: 19368971]
41. Barth PG, et al. An X-linked mitochondrial disease affecting cardiac muscle, skeletal muscle and neutrophil leucocytes. Journal of the neurological sciences. 1983; 62:327–355. [PubMed: 6142097]
42. Spencer CT, et al. Impaired cardiac reserve and severely diminished skeletal muscle O(2) utilization mediate exercise intolerance in Barth syndrome. American journal of physiology. Heart and circulatory physiology. 2011; 301:H2122–2129. [PubMed: 21873497]
43. Powers C, et al. Diminished Exercise Capacity and Mitochondrial bc1 Complex Deficiency in Tafazzin-Knockdown Mice. Frontiers in physiology. 2013; 4:74. [PubMed: 23616771]
44. Soustek MS, et al. Endurance training ameliorates complex 3 deficiency in a mouse model of Barth syndrome. Journal of inherited metabolic disease. 2015; 38:915–922. [PubMed: 25860817]
45. Sengers RC, et al. Congenital cataract and mitochondrial myopathy of skeletal and heart muscle associated with lactic acidosis after exercise. The Journal of pediatrics. 1975; 86:873–880. [PubMed: 1168700]

46. Mayr JA, et al. Lack of the mitochondrial protein acylglycerol kinase causes Sengers syndrome. American journal of human genetics. 2012; 90:314–320. [PubMed: 22284826]
47. Das S, et al. ATP citrate lyase improves mitochondrial function in skeletal muscle. Cell metabolism. 2015; 21:868–876. [PubMed: 26039450]
48. Carper MJ, et al. Skeletal muscle group VIA phospholipase A2 (iPLA2beta): expression and role in fatty acid oxidation. Biochemistry. 2008; 47:12241–12249. [PubMed: 18937505]
49. Song H, et al. Mice deficient in group VIB phospholipase A2 (iPLA2gamma) exhibit relative resistance to obesity and metabolic abnormalities induced by a Western diet. American journal of physiology. Endocrinology and metabolism. 2010; 298:E1097–1114. [PubMed: 20179248]
50. Gardocki ME, et al. Phosphatidylinositol biosynthesis: biochemistry and regulation. Biochimica et biophysica acta. 2005; 1735:89–100. [PubMed: 15967713]
51. Kim YJ, et al. A highly dynamic ER-derived phosphatidylinositol-synthesizing organelle supplies phosphoinositides to cellular membranes. Developmental cell. 2011; 21:813–824. [PubMed: 22075145]
52. Rosivatz E, Woscholski R. Removal or masking of phosphatidylinositol(4,5)bisphosphate from the outer mitochondrial membrane causes mitochondrial fragmentation. Cellular signalling. 2011; 23:478–486. [PubMed: 21044681]
53. Cortie CH, et al. Of mice, pigs and humans: An analysis of mitochondrial phospholipids from mammals with very different maximal lifespans. Experimental gerontology. 2015; 70:135–143. [PubMed: 26315290]
54. Hulbert AJ. Metabolism and longevity: is there a role for membrane fatty acids? Integrative and comparative biology. 2010; 50:808–817. [PubMed: 21558243]
55. Mitchell TW, et al. Membrane phospholipid composition may contribute to exceptional longevity of the naked mole-rat (Heterocephalus glaber): a comparative study using shotgun lipidomics. Experimental gerontology. 2007; 42:1053–1062. [PubMed: 18029129]
56. Hulbert AJ, et al. The exceptional longevity of an egg-laying mammal, the short-beaked echidna (Tachyglossus aculeatus) is associated with peroxidation-resistant membrane composition. Experimental gerontology. 2008; 43:729–733. [PubMed: 18586080]
57. Gonzalez-Freire M, et al. Reconsidering the Role of Mitochondria in Aging. The journals of gerontology. Series A, Biological sciences and medical sciences. 2015; 70:1334–1342.
58. Nomura K, et al. Mitochondrial phospholipid hydroperoxide glutathione peroxidase inhibits the release of cytochrome c from mitochondria by suppressing the peroxidation of cardiolipin in hypoglycaemia-induced apoptosis. The Biochemical journal. 2000; 351:183–193. [PubMed: 10998361]
59. Lopez-Dominguez JA, et al. The Influence of Dietary Fat Source on Life Span in Calorie Restricted Mice. The journals of gerontology. Series A, Biological sciences and medical sciences. 2015; 70:1181–1188.
60. Valencak TG, Azzu V. Making heads or tails of mitochondrial membranes in longevity and aging: a role for comparative studies. Longevity & healthspan. 2014; 3:3. [PubMed: 24588808]
61. Goto-Inoue N, et al. Lipidomics analysis revealed the phospholipid compositional changes in muscle by chronic exercise and high-fat diet. Scientific reports. 2013; 3:3267. [PubMed: 24253370]
62. Mitchell TW, et al. Exercise alters the profile of phospholipid molecular species in rat skeletal muscle. Journal of applied physiology. 2004; 97:1823–1829. [PubMed: 15208292]
63. Senoo N, et al. PGC-1alpha-mediated changes in phospholipid profiles of exercise-trained skeletal muscle. Journal of lipid research. 2015; 56:2286–2296. [PubMed: 26438561]
64. Liang MT, et al. Effects of exercise training and anabolic steroids on plantaris and soleus phospholipids: a 31P nuclear magnetic resonance study. The International journal of biochemistry. 1993; 25:337–347. [PubMed: 8462725]
65. Dohm GL, et al. Changes in muscle mitochondrial lipid composition resulting from training and exhaustive exercise. Life sciences. 1975; 17:1075–1080. [PubMed: 1195997]
66. Toledo FG, et al. Effects of physical activity and weight loss on skeletal muscle mitochondria and relationship with glucose control in type 2 diabetes. Diabetes. 2007; 56:2142–2147. [PubMed: 17536063]

67. Takahashi M, Hood DA. Chronic stimulation-induced changes in mitochondria and performance in rat skeletal muscle. Journal of applied physiology. 1993; 74:934–941. [PubMed: 8458817]
68. Ostojic O, et al. The effects of chronic muscle use and disuse on cardiolipin metabolism. Journal of applied physiology. 2013; 114:444–452. [PubMed: 23221956]
69. Lai L, et al. A role for peroxisome proliferator-activated receptor gamma coactivator 1 (PGC-1) in the regulation of cardiac mitochondrial phospholipid biosynthesis. The Journal of biological chemistry. 2014; 289:2250–2259. [PubMed: 24337569]
70. Athea Y, et al. AMP-activated protein kinase alpha2 deficiency affects cardiac cardiolipin homeostasis and mitochondrial function. Diabetes. 2007; 56:786–794. [PubMed: 17327449]
71. Romanello V, Sandri M. Mitochondrial Quality Control and Muscle Mass Maintenance. Frontiers in physiology. 2015; 6:422. [PubMed: 26793123]
72. Mayr JA. Lipid metabolism in mitochondrial membranes. Journal of inherited metabolic disease. 2015; 38:137–144. [PubMed: 25082432]
73. de Wilde J, et al. Short-term high fat-feeding results in morphological and metabolic adaptations in the skeletal muscle of C57BL/6J mice. Physiological genomics. 2008; 32:360–369. [PubMed: 18042831]
74. Hoeks J, et al. High fat diet-induced changes in mouse muscle mitochondrial phospholipids do not impair mitochondrial respiration despite insulin resistance. PloS one. 2011; 6:e27274. [PubMed: 22140436]
75. Martin N, et al. Dietary fatty acid composition and the homeostatic regulation of mitochondrial phospholipid classes in red muscle of rainbow trout (Oncorhynchus mykiss). Journal of experimental zoology. Part A, Ecological genetics and physiology. 2015; 323:60–71.
76. Martin N, et al. Dietary lipid quality and mitochondrial membrane composition in trout: responses of membrane enzymes and oxidative capacities. Journal of comparative physiology. B, Biochemical, systemic, and environmental physiology. 2013; 183:393–408.
77. Fajardo VA, et al. Cardiolipin linoleic acid content and mitochondrial cytochrome c oxidase activity are associated in rat skeletal muscle. Chemistry and physics of lipids. 2015; 187:50–55. [PubMed: 25727371]
78. Lanza IR, et al. Influence of fish oil on skeletal muscle mitochondrial energetics and lipid metabolites during high-fat diet. American journal of physiology. Endocrinology and metabolism. 2013; 304:E1391–1403. [PubMed: 23632634]
79. Herbst EA, et al. Omega-3 supplementation alters mitochondrial membrane composition and respiration kinetics in human skeletal muscle. The Journal of physiology. 2014; 592:1341–1352. [PubMed: 24396061]
80. Funai K, Semenkovich CF. Skeletal muscle lipid flux: running water carries no poison. American journal of physiology. Endocrinology and metabolism. 2011; 301:E245–251. [PubMed: 21558546]

Outstanding Questions Box

- What are the mechanisms by which phospholipids are imported into or exported out of mitochondria? Do class-specific mechanisms exist for phospholipid exchanges between the OMM and IMM, and at MAM?
- Mitochondrial phospholipids modulate the activity of enzymes of the ETS. Does exercise training or diet induce a remodeling of existing skeletal muscle mitochondrial membranes that results in an alteration in respiratory function, capacity or substrate preference?
- What are the mechanisms that mediate membrane phospholipid biosynthesis with exercise- or diet-induced skeletal muscle mitochondrial biogenesis?
- What is the fate of phospholipids in degraded mitochondria? Are they recycled to make new mitochondria or other organelle membranes?

Trends Box

- The strongest predictor of all-cause mortality is aerobic capacity, which is highly dependent on skeletal muscle mitochondrial function.
- Phospholipid composition of mitochondrial membrane phospholipids modulates respiration through its effects on the activity of enzymes of the electron transport system (ETS), ultrastructure of cristae, and signaling for mitophagy, fusion, and fission.
- Clinical and experimental evidence links disruption of skeletal muscle mitochondrial phospholipid synthesis to functional defects and abnormal morphology, leading to myopathy, exercise intolerance, and shortened lifespan.
- The transcriptional control of mitochondrial enzymes has been well studied, but there is limited information on mechanisms that regulate mitochondrial phospholipid synthesis.

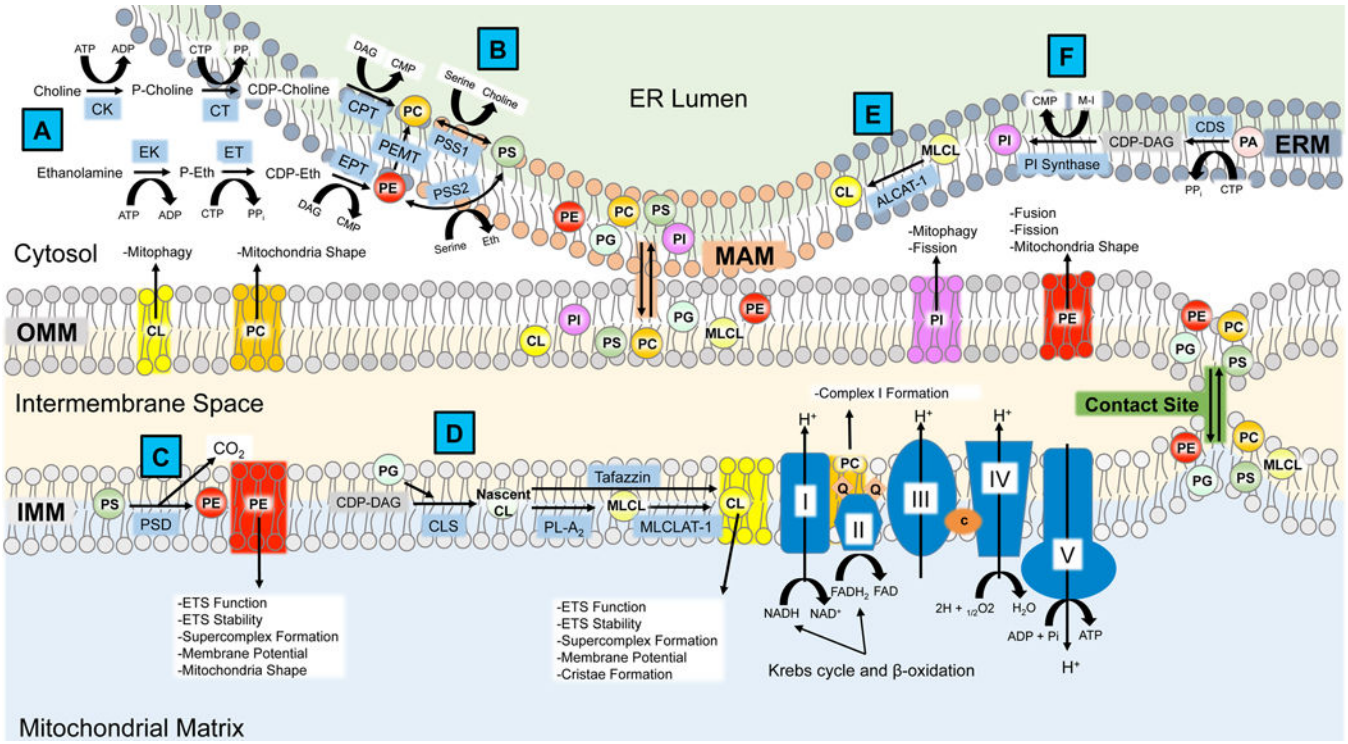

Figure 1. Biosyntheses and functional relevance of mitochondrial membrane phospholipids

A. PC and PE syntheses by the Kennedy pathway. Choline or ethanolamine undergoes sequential reactions with ATP, CTP, and DAG to produce PC or PE. The first two reactions are mediated by cytosolic enzymes, while the final steps involving DAG are mediated by enzymes that are embedded in the ERM.

B. Phospho-headgroup modifications of PC, PE, and PS mediated by PEMT, PSS1, and PSS2. PEMT is located at ERM/MAM, whereas PSS1/PSS2 are located at MAM.

C. Mitochondrial synthesis of PE by PSD at IMM.

D. Mitochondrial CL synthesis by CLS, tafazzin, PL-A_2, and MLCLAT-1. Nascent CL molecules produced by CLS are subsequently de/re-acylated to form mature and functional CL.

E. Small quantity of CL can be synthesized on the ERM by ALCAT-1.

F. PI synthesis by CDS and PI synthase on the ERM. Molecules synthesized in ERM, OMM and IMM can be transported at MAM or intra-mitochondrial contact site.

Abbreviations: ALCAT-1 (acyl-CoA: lysocardiolipin acyltransferase-1), c (cytochrome c oxidase), CDS (CDP-DAG synthase), CK (choline kinase), CL (cardiolipin), CLS (cardiolipin synthase), CPT (CDP-choline phosphotransferase), CT (CTP:phosphocholine cytidylyltransferase), DAG (diacylglycerol), Eth (ethanolamine), EK (ethanolamine kinase), ERM (endoplasmic reticulum membrane), EPT (CDP-ethanolamine phosphotransferase), ET (CTP:phosphoethanolamine cytidylyltransferase), ETS (electron transport system), IMM (inner mitochondrial membrane), MAM (mitochondrial associated membrane), M-I (myo-inositol), MLCLAT-1 (monolysocardiolipin acyltransferase-1), OMM (outer mitochondrial membrane), PA (phosphatidic acid), PC (phosphatidylcholine), PE (phosphatidylethanolamine), PEMT (phosphatidylethanolamine N-methyltransferase), PG

(phosphatidylglycerol), PI (phosphatidylinositol), PL-A_2 (phospholipase-A_2), PS (phosphatidylserine), PSD (phosphatidylserine decarboxylase), PSS1/2 (phosphatidylserine synthase 1/2), Q (co-enzyme Q10).

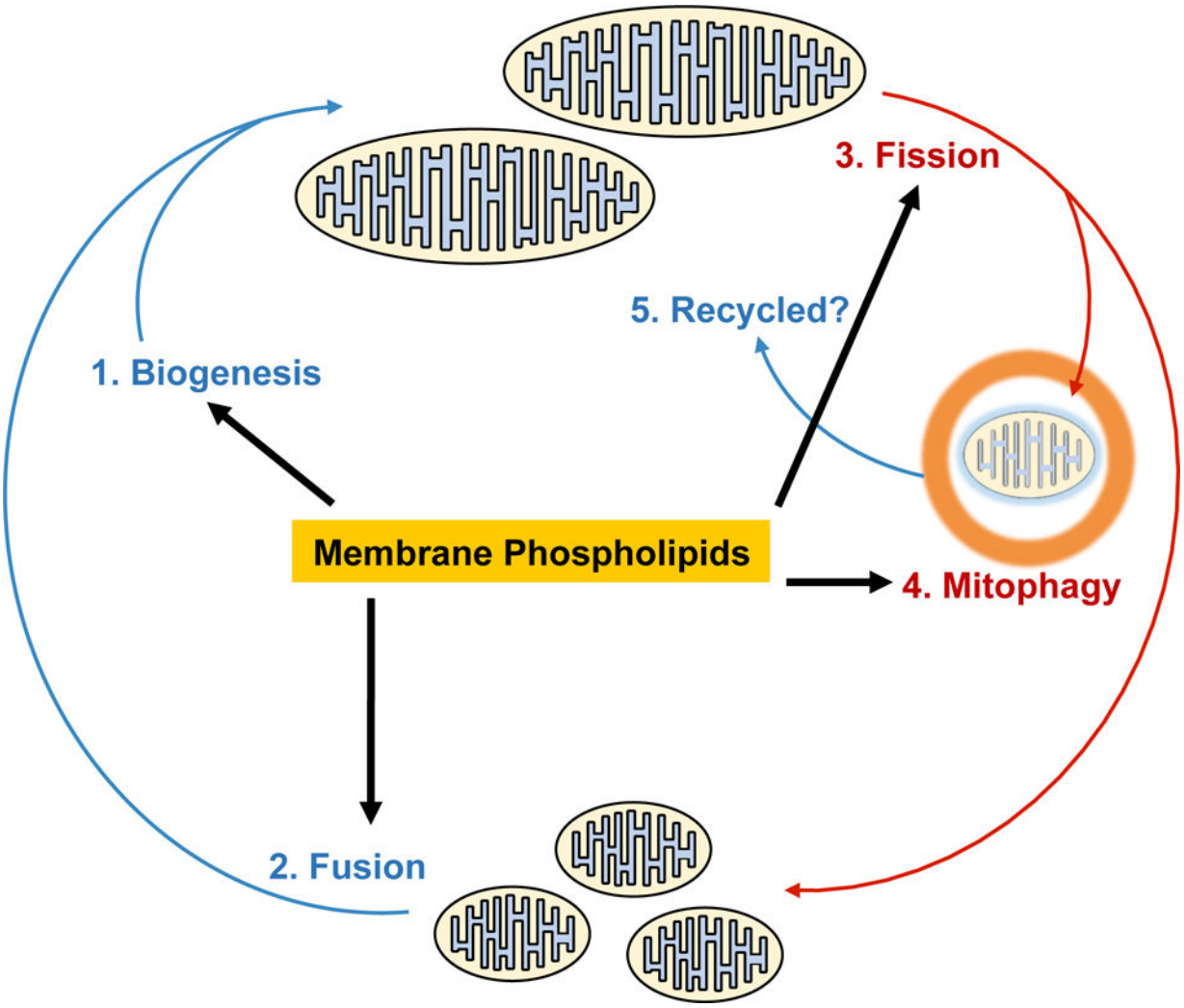

Figure 2. Membrane phospholipids in the regulation of mitochondrial mass and morphology

1. Mitochondrial biogenesis requires a synchronous supply of enzymes and membrane phospholipids while maintaining electrochemical gradient across the IMM.

2. Mitochondrial fusion is in part regulated by phospholipids that reside in the OMM. Membrane phospholipid composition immediately around the fusion site is likely rearranged for membrane stalk formation.

3. Conversely, mitochondrial fission may be signaled by phospholipids in the OMM. Phospholipid composition immediately around the fission site is also likely reorganized.

4. Mitophagy can be promoted by membrane phospholipids in the OMM. Some evidence suggests that autophagosomes preferentially target smaller mitochondria produced by mitochondrial fission.

5. It is unknown whether phospholipid molecules derived from lysosomal degradation are recycled to form new membranes.

Table 1

Summary of studies examining how skeletal muscle metabolism is affected by disruptions in genes of phospholipid biosynthesis.

Lipid	Enzyme	Mutation	Species	Phenotype	Reference
PC	CKα	−/−	Mouse	-Embryonically lethal	[23]
	CKβ	−/−	Mouse	-Hindlimb muscular dystrophy -Large mitochondria with reduced inner mitochondrial membrane potential	[24, 25]
		−/− or compound +/−	Human	-Congenital muscular dystrophy -Early-onset muscle wasting -Large mitochondria in periphery	[26]
	CTα	−/−	Mouse	-Embryonically lethal	[27]
	CTβ	−/−	Mouse	-No apparent muscle phenotype	[28]
	PSS1	−/−	Mouse	-No apparent phenotype	[16]
		Gain of function	Human	-Lenz-Majewski syndrome -No apparent muscle phenotype	[17]
PE	PSD	−/−	Mouse	-Embryonically lethal -Fragmented mitochondria	[30]
	ET	−/−	Mouse	-Embryonically lethal	[34]
		Muscle-specific −/−	Mouse	-Increased exercise endurance -Smaller muscle mass -Muscle mitochondrial biogenesis	[35]
	CEPT1	Muscle-specific −/−	Mouse	-Muscle weakness -Increased insulin sensitivity	[36]
	PSS2	−/−	Mouse	-No apparent muscle phenotype	[31]
CL	Tafazzin	−/−	Human	-Barth syndrome -Reduced mitochondrial respiration -Increased intramuscular fat -Lactic acidosis/exercise intolerance	[41, 42]
		−/−	Mouse	-Reduced mitochondrial respiration -Exercise intolerance	[43, 44]
	AGK	−/−	Human	-Sengers syndrome	[45, 46]

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Lipid	Enzyme	Mutation	Species	Phenotype	Reference
				-Mitochondrial myopathy -Lactic acidosis/exercise intolerance	
	ACL	Muscle over-expression	Mouse	-Increased CL synthesis -Increased mitochondrial respiration	[47]
	$iPLA_2\beta$	−/−	Mouse	-Reduced FA oxidation	[48]
	$iPLA_2\gamma$	−/−	Mouse	-Large mitochondria -Heterogeneity in CL subspecies -Reduced fatty acid oxidation	[49]