Editorial

What You Eat Affects Your Shape

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Mitochondria are no longer considered to be static structures that just make ATP. Emerging data show that mitochondrial form or shape is intimately related to mitochondrial function.[1,2] It, therefore, follows that changes in mitochondrial substrate selection and metabolism might lead to altered mitochondrial dynamics. A recent study in *Circulation Research* examines this issue[3]

Diabetic cardiomyopathy is associated with cardiac lipotoxicity and mitochondrial dysfunction,[4] and a better understanding of the mechanisms involved are needed. To examine the mechanisms linking lipid overload and diabetic cardiomyopathy, Tsushima et al[3] studied a mouse model with overexpression of ACSL1 (long-chain acyl-CoA synthetase 1) in cardiomyocytes (ACS-transgenic [Tg]). ACSL1 was under the control of α-myosin heavy chain promoter, and the gene was, therefore, turned on shortly before birth. Wild-type (WT) hearts had little or no ACSL1 mRNA expression at postnatal day zero (P0), whereas ACS-Tg hearts expressed mRNA but no protein at this time (P0). An increase in ACSL1 protein was observed in the ACS-Tg hearts at ≈1 week, and by 12 weeks, there was >10-fold increase in ACSL1 in the transgenic hearts. Normally, after birth, there is an increase in mitochondrial dimensions, and the mitochondria become larger. Interestingly, the postnatal increase in mitochondrial dimensions is blunted in the ACS-Tg hearts. Two-dimensional electron microscopy showed that in WT hearts, mitochondrial dimensions doubled by 3 weeks after birth; however, this postnatal increase was not observed in ASC-Tg mice. To better assess mitochondrial differences, 3-dimensional transmission electron microscopy tomography was performed, and data collected at 8 weeks showed that ACS-Tg mitochondria were narrower and more elongated than mitochondria from WT hearts. Thus, a role for alterations in proteins that regulate mitochondrial dynamics, the mitochondrial fission and fusion proteins, were considered.

As indicated by their names, these proteins regulate mitochondrial fission and fusion, but perhaps more important for a nondividing myocyte, they regulate mitochondrial structure, cristae formation, bioenergetics, and metabolism.[5,6] Fission is initiated by DRP1 (dynamin-related protein 1) translocation to the outer mitochondrial membrane, and this process is regulated, at least in part, by post-translational modifications.[6] DRP1 is phosphorylated on S637 (human sequence numbers) by PKA (protein kinase A) or Pim1 (proto-oncogene serine/threonine-protein kinase) leading to DRP1 inhibition. Calcineurin has been reported to dephosphorylate S637 and activate DRP1 and fission. DRP1 is activated by phosphorylation at S616, which is mediated by CaMKII (calmodulin dependent kinase II), Cdk1 (cyclin-dependent kinase 1; which is important in dividing cells), and MAP (mitogen-activated protein) kinase. Fusion is mediated by the outer mitochondrial membrane proteins Mfn1/2 (mitofusin 1/2) and by the inner mitochondrial membrane protein OPA1 (optic atrophy 1). OPA1 is regulated by proteolytic cleavage; the long form, L-OPA1, promotes fusion, but the cleaved short form, S-OPA1, is generally thought to inhibit fusion; however, this maybe more complicated.[7]

Tsushima et al[3] tested the hypothesis that lipid overload alters mitochondrial dynamics. Consistent with the increase in mitochondrial dimensions that occur in WT mitochondria after birth, WT hearts show an increase in phosphorylation of the mitochondrial fission protein DRP1 at serine 637, which inhibits fission and a decrease at serine 616 (serine 616 phosphorylation activates fission) with the net result that fission is normally blunted after birth. Interestingly, in the ACS-Tg hearts, the postnatal increase in DRP1 phosphorylation at S637 was not observed. These data suggest that the increase in palmitate oxidation in some way alters the remodeling that normally occurs after birth. Clearly, mitochondria are not static, and they alter their shape in response to environmental and dietary cues.

Tsushima et al explored the mechanism responsible for the alteration in DRP1 phosphorylation. AKAP121 (A-kinase anchoring protein 121) is a scaffold protein that brings together DRP1 and PKA at the outer mitochondrial membrane thereby enhancing phosphorylation of DRP at S637. AKAP121 was decreased in ACS-Tg mice relative to WT, and this decrease was because of increased ubiquitination and degradation by the proteasome as it was blocked by the proteasome inhibitor, MG 132. Alterations in OPA1 processing were also observed in the ACS-Tg hearts. Specifically, an increase in the ratio of short (fusion incompetent) to long (fusion competent) OPA1 isoforms was observed.

The authors further demonstrate an increase in reactive oxygen species (ROS) in mitochondria from ACS-Tg hearts but only with succinate or palmitoyl-carnitine in the absence of rotenone. There was no increase in ROS in these ACS-Tg mitochondria with glutamate and malate as substrate or in the presence of rotenone. These findings suggest a role for reverse electron transport as a possible mechanism for the increase in ROS in the ACS-Tg mitochondria. It might be interesting to investigate whether lipid overload leads to post-translational modifications (perhaps fatty acid–mediated modifications such

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as palmitylation or succinylation or acetylation) that might be involved in enhancing reverse electron transport. A major finding of the article is that reducing the increase in ROS in ACS-Tg hearts by overexpression of SOD (superoxide dismutase) partially rescued the altered mitochondrial shape and also rescued the postnatal increased phosphorylation of DRP S637 and the altered OPA1 processing observed in the ACS-Tg hearts.

The findings that overexpression of ACSL1 at birth alters the postnatal changes in mitochondrial morphology are interesting in light of recent studies from the Dorn lab,[2] which reported that the mitochondrial remodeling which occurs after birth requires mitophagy induced by PINK1–Mfn2–Parkin.[2] It is unclear whether autophagy or Mfn2 were altered in the ACS-Tg hearts or whether the lipid overload interferes with the mitochondrial remodeling via autophagy that occurs postnatally. Furthermore, because an increase in fatty acid oxidation occurs after birth, one might expect increasing lipid metabolism in the ACS-Tg hearts to enhance rather than retard the mitochondrial remodeling that occurs after birth. Perhaps, there is a sweet spot for fatty acid oxidation such that an increase in fatty acid oxidation stimulates remodeling—but if the levels are too high, they might impair some aspects of remodeling. In any case, it is becoming increasingly clear that mitochondrial morphology is not static and that it is altered by mitochondrial substrate selection, oxidative phosphorylation rates, and ROS generation.

Lewandowski lab[8] has reported sex differences in long-chain acyl-CoA metabolism. Thus, it would be interesting to examine whether there are sex differences in mitochondrial remodeling. Furthermore, in this study, ACSL1 is overexpressed at birth. It would also be of interest to determine whether lipid overload would have a similar effect on adult cardiomyocytes. The perinatal period is a time of mitochondrial reorganization, and it is unclear whether lipid overload is just blocking the mitochondrial remodeling that occurs postnatally or whether it would alter morphology in adult myocardium. This is an important question because type 2 diabetes mellitus typically occurs in adults.

As with most studies, the novel data in this article raise many questions. The details of how increasing lipid metabolism alters phosphorylation of DRP at S616 and proteolytic processing of OPA1 are areas for future study. Tsushima et al show alterations in the isoforms of OPA1, but the details of the mechanism are not clear. OPA1 has 2 cleavage sites: S1 is cleaved by Oma1 (overlapping with m-AAA protease) and S2 is cleaved by Yme1L (yeast mitochondrial escape protein 1 like). The details of OPA1 cleavage and how the different cleavage products regulate fusion is still debated and is reviewed elsewhere.[5,6] At baseline, OPA1 has a membrane-anchored long form (OPA-L) and cleaved soluble form (OPA-S). A decrease in mitochondrial membrane potential has been reported to increase Oma1-dependent cleavage and to inhibit fusion. Interestingly, a recent article showed that an increase in oxidative phosphorylation increased fusion and was associated with an increase in OPA1 cleavage.[7] Thus, the details of how OPA1 initiates fusion are still somewhat unclear. However, increased lipid oxidation has been frequently shown to decrease mitochondrial membrane potential, and whether this or a change in oxidative phosphorylation might be involved in altering OPA1 cleavage products remains to be seen. Precisely, how changes in oxidative phosphorylation lead to

cleavage of OPA1 is also an area for future study. The details of how lipid overload leads to an increase in phosphorylation of DRP1 at S616 also need additional study. S616 of DRP1 is phosphorylated by CaMKII,[9] MAP kinases, and Cdk1. Changes in ROS have been shown to lead to changes in calcium, which could activate CaMKII. Furthermore, oxidation of CaMKII leads to its activation. It is tempting to speculate that perhaps an ROS-mediated increase in CaMKII is involved in the increased phosphorylation.

Perhaps the most interesting question is defining the relationship between mitochondrial morphology and function. It is likely in postmitotic cardiomyocytes that fission/fusion proteins primarily function to regulate mitochondrial morphology to optimize mitochondrial respiration and function.[5] There is considerable emerging data showing that mitochondrial fusion and OPA1 cleavage is sensitive to oxidative phosphorylation activity.[7] There is also a link between mitochondrial morphology and metabolism. Mice with deletion of Oma1 (Oma1-knockout) have altered metabolism and develop obesity and defects in thermogenesis.[10] Alterations in mitochondrial fission and fusion proteins can alter mitochondrial cristae formation and possibly alter organization of electron transport complexes. This is an exciting area for future research.

In summary, the data in the article provide strong support for the model that overexpression of ACSL1 and the resultant lipid overload leads to altered mitochondrial shape: more elongated, thinner mitochondria. This altered mitochondrial shape was associated with changes in phosphorylation of DRP1 and altered processing of OPA1. The authors further show a role for increased ROS in leading to the changes in DRP1 and OPA1 and the changes in mitochondrial shape. These data suggest that an increase in ROS, possibly by enhancing reverse electron transport through complex I, leads to alterations in phosphorylation of DRP1 and alterations in OPA1 processing and that these changes in the mitochondrial fission and fusion proteins lead to an alteration in mitochondrial shape. This study by Tsushima et al provides novel food for thought and for regulating mitochondrial dynamics.

Disclosures

None.

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