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COMMENTARY

Stress turns on the heat: Regulation of mitochondrial biogenesis and UCP1 by ROS in adipocytes

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ABSTRACT

Reactive oxygen species (ROS) production and oxidative stress (OS) in adipose tissue are associated with obesity and insulin resistance (IR). The nature of this relationship i.e., cause and effect or consequence has not been clearly determined. We provide evidence that elevated mitochondrial ROS generated by adipocytes from mice with diet-induced obesity (DIO) represents an adaptive mechanism that precipitates fatty acid oxidation, mitochondrial biogenesis, and mitochondrial uncoupling in an effort to defend against weight gain. Consistent with that, mice with adipocyte-specific deletion of manganese superoxide dismutase (MnSOD) exhibit increased adipocyte superoxide generation and are protected from weight gain and insulin resistance which otherwise develops in wild-type (WT) mice that consume an obesogenic diet. The defense mechanism displayed by MnSOD-deficiency in fat cells appears to be mediated by a dual effect of ROS on inefficient substrate oxidation through uncoupling of oxidative phosphorylation and enhanced mitochondrial biogenesis. The aim of this commentary is to summarize and contextualize additional evidence supporting the importance of mitochondrial ROS in the regulation of mitochondrial biogenesis and the modulation of uncoupling protein 1 (UCP1) expression and activation in both white and brown adipocytes.

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Mitochondria are the major sites of substrate oxidation in mammalian cells and a major source of ROS.[1-3] Mitochondria can generate superoxide and hydrogen peroxide (H_2O_2) from at least 11 different oxidoreductases associated with substrate catabolism and the electron transport chain (ETC).[4] ROS generation by mitochondria is a tightly controlled process i.e., low levels for cellular signaling are allowed whereas high levels that can damage the cellular milieu are mitigated by endogenous antioxidant enzymes. Importantly, there is substantial uncertainly regarding the different mechanisms controlling increased mitochondrial ROS generation *in vivo*, as well as the type of ROS species that are relevant for signaling in different aspects of physiology. Superoxide is the short-lived proximal ROS generated by mitochondrial oxidoreductases, and is rapidly converted to H_2O_2 by manganese superoxide dismutase (MnSOD) in the mitochondrial matrix.[5] Superoxide that is generated and released in the mitochondrial intermembrane space by complex III and the enzyme p66Shc is converted to H_2O_2 by the Cu,ZnSOD enzyme.[6-8] Matrix H_2O_2 is degraded by a glutathione-dependent system catalyzed by glutathione peroxidases and the peroredoxin-thioredoxin system,[9] whereas cytosolic degradation of H_2O_2 is mainly controlled by catalase, cytosolic peroxidases, and peroxiredoxins.[10] Distinct from other antioxidant enzymes, MnSOD is important due to its localization in the mitochondrial matrix, and to its high affinity for superoxide in that compartment.[5] The physiologic relevance of MnSOD was demonstrated by the robust phenotype of mice lacking the *in vitro Sod2* gene (encoding MnSOD). In this regard, *Sod2*-deficient mice die within 1–18 d from dilated cardiomyopathy and neurodegenerative abnormalities, highlighting the importance of this enzyme in the maintenance of organ function.[11]

Obesity is a growing epidemic driving a rise in the incidence of type 2 diabetes, cardiovascular diseases and cancer.[12] Obesity results from an energy imbalance wherein storage of fat in white adipose tissue (WAT) exceeds energy expenditure. Recent studies have associated systemic OS with obesity-related complications[13] as enhanced adipose OS is linked to inflammation,

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adipokine dysregulation and insulin resistance.[14-16] While these studies demonstrated a *correlation* exists between increased adipose OS and the incidence of obesity and insulin resistance, a *cause and effect* has not been demonstrated *in vivo*. Furthermore, the use of knockout or transgenic animals targeting antioxidant enzymes at the whole body level makes it difficult to precisely define the contribution to weight gain from adipose tissue OS *per se*. In addition, ROS, in the form of H_2O_2, stimulate adipogenesis *in vitro*,[17] and could explain why fat accumulation is increased in mouse models with whole body deletion of certain antioxidant enzymes.[15,16,18,19] Thus, defining the precise role of adipocyte ROS in the regulation of its metabolism and function is crucial for developing novel therapeutic strategies to combat obesity.

AdSod2 KO increases energy expenditure and protects against DIO

To define the specific contribution of mitochondria-generated ROS in adipocytes to the pathogenesis of obesity and insulin resistance, we generated the first mouse model wherein MnSOD could be specifically deleted in adipocytes i.e., AdSod2 KO mice. We originally hypothesized that AdSod2 KO mice would develop obesity due to superoxide-mediated damage to mitochondrial components, leading to reduced substrate oxidation and lipid accumulation in adipocytes. Instead, increased superoxide generation by adipocytes from AdSod2KO vs. WT mice stimulated fatty acid (FA) oxidation and increased mitochondrial biogenesis in adipocytes, and these effects were most prominent in mice that consumed high-fat diet (HFD) vs. standard chow.[20] Specifically, enhanced FA oxidation in white adipose tissue (WAT) and brown adipose tissue (BAT) of AdSod2 KO mice increased metabolic rate and energy expenditure and protected the mice from weight gain and insulin resistance that otherwise developed in WT mice that consumed HFD. Elevated energy expenditure observed in AdSod2 KO mice occurred despite BAT atrophy, and is not secondary to shivering-induced thermogenesis. Moreover, energy expenditure was still elevated even when adrenergic input to BAT and shivering-induced thermogenesis were blocked by housing the AdSod2 KO mice at thermoneutrality. Therefore, AdSod2 KO was sufficient to drive increased adipose energy expenditure in the absence of systemic adrenergic cues. Most importantly, when adipose progenitors were isolated from these mice and induced to differentiate *in vitro*, a marked increase in mitochondrial proton leak was observed in cells isolated from AdSod2 KO vs. WT mice, supporting a cell autonomous effect. This evidence for increased mitochondrial uncoupling in adipocytes from the mutant mice was associated with a robust induction of uncoupling protein 1 (UCP1) expression in WAT and BAT. Collectively, these data clearly demonstrate that enhanced mitochondrial ROS triggers an adaptive mechanism that protects against diet-induced fat accrual through the stimulation of FA oxidation, enhanced mitochondrial proliferation, and the induction of mitochondrial uncoupling (Fig. 1).

Adipocyte OS induced by SOD2 KO signals to drive mitochondrial biogenesis in WAT

Studies in mice have shown that during the development of DIO, there is an early increase in ROS levels in WAT followed by a later induction of mitochondrial biogenesis.[21] Mitochondrial biogenesis is orchestrated by a transcriptional cascade involving peroxisome proliferator-activated receptor γ coactivators 1α (PGC-1α), PGC-1β, PGC-1-related coactivator (PRC), estrogen-related receptor α (ERRα), nuclear respiratory factors 1 (NRF1) and 2 (NRF2) and mitochondrial transcription factor A (Tfam).[22] Mitochondrial biogenesis was enhanced in WAT of the AdSod2 KO mice and was associated with increased transcription of PGC-1α, PGC-1β and nuclear and mitochondrial-encoded genes, implying a causal role for ROS in the induction of mitochondrial biogenesis. Lack of both PGC-1α and PGC-1β impairs differentiation-induced mitochondria biogenesis and mitochondrial respiration in brown preadipocytes.[23] Mice lacking PGC-1α in fat cells revealed a role for this co-activator in the orchestration of the transcription of mitochondrial and FA oxidation genes and the regulation of glucose homeostasis and insulin sensitivity in response to high fat feeding.[24] Indeed, during acute or chronic cold acclimation in rodents, an elevation in ROS generation and a shift toward a more oxidized environment was observed in BAT and was associated with mitochondrial proliferation.[25-28] Furthermore, agents known to deplete glutathione (GSH) such as buthionine sulfoximine (BSO) increased the expression of PGC-1α both in WAT and in BAT.[29] In addition, ROS in the form of H_2O_2 was shown to directly induce the expression of PGC-1α and PGC-1β in brown-fat fibroblasts through a mechanism involving the Cre-binding protein (CREB).[30] Whether the induction of mitochondrial biogenesis observed in WAT of the AdSod2 KO mice is CREB-mediated is unknown, but studies in our laboratory are actively investigating this possibility.

The induction of mitochondrial biogenesis in WAT of AdSod2 KO mice was only observed when the animals were maintained on a high fat diet (HFD). Moreover, while AdSod2 KO appeared to increase basal superoxide levels, HFD was sufficient to drive superoxide higher but

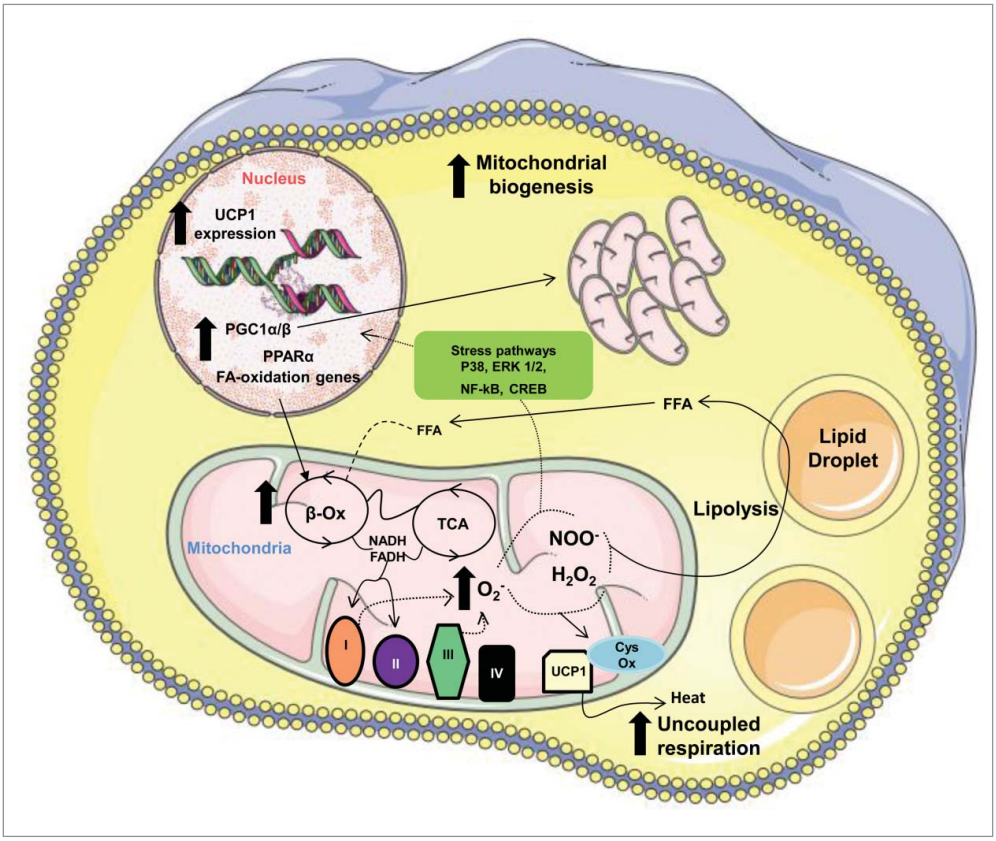

Figure 1. Schematic diagram of the effects of MnSOD deletion in adipocytes. The free fatty acids (FFA) released after lipolysis are oxidized in the mitochondria through an inefficient process involving active UCP1. The inefficient FA oxidation is supported by ROS-mediated transcriptional regulation of mitochondrial biogenesis and UCP1 expression.

to similar extents in both WT and KO mice.[20] However, it is unknown if the levels of other ROS species such as H_2O_2 or peroxynitrite, previously shown to drive mitochondrial proliferation.[30,31] are different in AdSod2 KO vs. WT mice on HFD, and this is a topic for future study. Furthermore, the lack of difference in superoxide levels in WAT between KO and WT mice fed HFD could be explained by the higher mitochondrial uncoupling capacity and ROS mitigation in the KO mice. Further studies using mitochondrial-targeted antioxidant, and mitochondrial uncoupling inhibitors, and redox proteomic methods[32] are required to define the role of ROS in the promotion of mitochondrial biogenesis in AdSod2 KO mice.

Another important finding in the AdSod2 KO mouse model is the induction of UCP1 expression both in WAT and BAT, and the increase in mitochondrial uncoupling in WAT in mice that consumed HFD. Interestingly, despite elevated UCP1 expression in BAT, AdSod2 KO mice were cold-intolerant, probably due to the significant atrophy and loss of lipid stores in this depot. The mechanisms underlying BAT atrophy in AdSod2 KO mice are not known, but similar finding was reported in the aP2-*Ucp* transgenic mice.[33,34] Despite the atrophy of BAT, UCP1 content was preserved in aP2-*Ucp* transgenic mice and increased in AdSod2 KO mice, suggesting that atrophy is caused by a reduction in the total number of cells or a depletion in the lipid stores. In the AdSod2 KO model, the reduction in cellularity in BAT could be caused by reduced proliferation or increased cell death as a result of excessive UCP1-mediated mitochondrial uncoupling and a depletion of ATP necessary for these processes. UCP1 induction in WAT of AdSod2 KO mice occurred independently of the diet and does not correlate with the induction of mitochondrial biogenesis, suggesting ROS as the underlying trigger. Indeed, H_2O_2 treatment induced UCP1 expression in brown-fat fibroblasts even when PGC-1α was ablated, thus separating UCP1 induction from mitochondrial biogenesis.[30] Moreover, the peroxisome proliferator-activated receptor γ (PPAR γ) agonist rosiglitazone was shown to induce UCP1 expression along with mitochondrial biogenesis in WAT, but the abundance of UCP1 mRNA was greater than any mitochondrial transcripts, indicating specific activation of this gene.[35] Consistent with this idea, recent studies demonstrated that ROS are required for both the induction of UCP1 expression in BAT and WAT, as well as increased UCP1-dependent

respiration. In this regard, overexpression of the antioxidant molecule Sestrin2 resulted in a reduction of UCP1 expression in BAT, an effect similar to the use of the compounds that modulate cellular redox status such as butylated hydroxyanisole (BHA) and N-Acetylcysteine (NAC) in mice.[36] In this study, it was shown that cold-induced ROS-mediated UCP1 expression in BAT was mediated through p38 mitogen-activated protein kinase (p38MAPK) pathway. Moreover, mice deficient in nuclear factor-erythroid 2-related factor 2 (NRF2), which controls expression of antioxidant genes, display a 2-fold induction of Ucp1 mRNA in WAT and in isolated fibroblasts vs. WT mice, and this effect is mitigated when mutant cells are treated with antioxidants.[18] Surprisingly, MnSOD deficiency in skin tissue, which enhanced superoxide levels, also induced UCP1 expression through a PPARα-dependent mechanism.[37] On balance, when our data are taken together with what currently is known in the literature, it appears that a common ROS-dependent mechanism for the induction of UCP1 expression exists in WAT and BAT. Future investigations from our laboratory will define the signaling pathways involved in the induction of UCP1 transcription in both WAT and BAT of the AdSod2 KO mice.

AdSOD2 deletion increases uncoupled respiration in cells and *in vivo*

In addition to the induction of UCP1 transcription in WAT and BAT of AdSod2 KO mice, we showed that this resulted in increased energy expenditure at both the whole body and adipocyte-autonomous level. Specifically, HF-fed but not chow-fed AdSod2 KO mice exhibited elevation in energy expenditure *in vivo*, enhanced mitochondrial uncoupling, and increased leak respiration in isolated preadipocytes *in vitro*. In interpreting these findings it is important to note that increased UCP1 gene and protein expression alone are not sufficient to explain increased energy expenditure in the AdSod2KO model. Although UCP1 facilitates uncoupled respiration, in the cellular milieu, it is maintained in a purine-nucleotide bound state,[38] which in the absence of FA renders it inactive.[39] So, current evidence suggests that in the native mitochondrial and adipocyte environment, UCP1 does not drive uncoupled respiration basally, instead requiring activation (for example by adrenergic stimulus or increased local FA). Therefore, diet-induced initiation of mitochondrial uncoupling in Sod2 KO adipocytes presumably involves modulation in the activation status of UCP1 and UCP1-dependent respiration. This is further supported by the observation that, in AdSod2 KO adipocytes, leak respiration specifically is enhanced while chemically uncoupled maximal respiration is unaffected. So, enhancement of respiration is attributable to a specific effect on the ATP synthase uncoupled component.

How OS stimulates UCP1-dependent respiration in AdSod2 KO mice is an outstanding question. More generally, the role of ROS and related redox-active signaling modalities in regulation of UCP1 activity has been the subject of longstanding studies. The importance of superoxide.[38,40-42] and other redox-active species (e.g. 4-HNE[43,44]) in modulating UCP1 function is the subject of debate. Relatedly, a role for thiol redox status (which depends on local ROS modulation) has been suggested in BAT mitochondrial UCP1 uncoupling.[26] Studies on the role of OS and UCP1 activation have relied largely on the use of isolated mitochondria and reconstituted *in vitro* systems. Therefore, the extent to which physiologically relevant ROS production, redox tone, and local UCP1 environment are faithfully recapitulated is unclear. An advantage of the AdSOD2 KO model is that OS-dependent effects on leak respiration and UCP1-dependent thermogenesis are monitored under native conditions (both in adipocytes and *in vivo*).

Indeed, recent observations have suggested that redox regulation of UCP1-dependent respiration is particularly relevant when studied in adipocytes and *in vivo* where both redox homeostasis and free fatty-acid concentrations are subject to strict endogenous regulation; parameters that are necessarily divergent from *in vitro* conditions. Specifically, a role was recently demonstrated for mitochondrial ROS induction and protein thiol redox tone in supporting UCP1-dependent thermogenesis upon adrenergic stimulus *in vivo*.[28] In this study, the role of mitochondrial ROS in supporting increased energy expenditure was shown to genetically require UCP1 *in vivo* and in brown adipocytes, and involve direct modification by ROS of a functional cysteine residue (Cys253) on UCP1. Interestingly, mutagenesis of this redox-sensitive site on UCP1 renders it less sensitive to acute activation by adrenergic stimulus in adipocytes, suggesting that this ROS-modified site acts as a rheostat to tune UCP1 activity. More generally, this study demonstrated that numerous BAT mitochondrial metabolic enzymes are similarly modified by ROS *in vivo* during thermogenesis and could be similarly functionally regulated by these modifications. It would therefore be of great interest to examine ROS-mediated post-translational modifications of UCP1 and other metabolic targets in genetic models of increased OS such as the AdSod2 KO model, to determine whether these modifications play a role in driving increased energy expenditure. More generally, the AdSod2KO model now provides an elegant system with which to examine the molecular mechanisms of ROS-mediated activation of thermogenic gene programs

and active thermogenic respiration on a cellular and organismal level.

Disclosure of potential conflict of interest

No potential conflicts of interest were disclosed.

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