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Review

Mitochondrial metabolism in aging: Effect of dietary interventions

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ABSTRACT

Mitochondrial energy metabolism and mitochondrially-derived oxidants have, for many years, been recognized as central toward the effects of aging. A body of recent work has focused on the relationship between mitochondrial redox state, aging and dietary interventions that affect lifespan. These studies have uncovered mechanisms through which diet alters mitochondrial metabolism, in addition to determining how these changes affect oxidant generation, which in itself has an impact on mitochondrial function in aged animals. Many of the studies conducted to date, however, are correlative, and it remains to be determined which of the energy metabolism and redox modifications induced by diet are central toward lifespan extent. Furthermore, dietary interventions used for laboratory animals are often unequal, and of difficult comparison with humans (for whom, by nature, no long-term sound scientific information on the effects of diet on mitochondrial redox state and aging is available). We hope future studies will be able to mechanistically characterize which energy metabolism and redox changes promoted by dietary interventions have positive lifespan effects, and translate these findings into human prevention and treatment of age-related disease.

1. Introduction

The importance of energy metabolism in aging has been recognized since 1928, when Pearl proposed that rates of aerobic metabolism correlated with aging. Rate of living is well demonstrated today *not* to be directly correlated with lifespan, in particular when oxidative metabolism is corrected for animal mass (Barja, 2002). However, the idea that aerobic metabolism (and in particular mitochondrial metabolism) is central toward the development of the aging phenotype has not only stood the test of time, but is gaining further strength in recent years. Indeed, it is to be expected that mitochondria, as the site of oxidative phosphorylation and providing the vast majority of high energy phosphates the cell requires to function, are strongly involved in aging effects. This concept is further supported by the very early finding that the restriction of dietary calories (calorie restriction, CR), an intervention which clearly alters mitochondrial metabolism, extends rodent lifespan (McCay et al., 1989 (reprinted from 1935)).

In addition to their central role in energy metabolism, mitochondria were found to be a source of free radicals and other reactive oxygen species (ROS) in the 1960s (Hinkle et al., 1967). Based on his earlier proposal that oxidative damage was a limiting factor in lifespan (Harman, 1956) and the experimental evidence that mitochondria were a significant source of intracellular oxidants, Harman revisited his free radical theory of aging to suggest mitochondria were centrally involved in aging (Harman, 1972; reviewed in Sohal and Weindruch, 1996; Wallace, 2005).

Indeed, CR decreases mitochondrial ROS formation and results in a decrease in levels of markers of oxidized biomolecules (revised in Sohal and Weindruch, 1996; Merry, 2004; Gredilla and Barja, 2005; Kowaltowski, 2011), evidence that supports the mitochondrial/free radical theory of aging (Barja, 2002; Yu et al., 2008). Despite this correlative experimental support, the concept that global oxidative damage is limiting in lifespan is but in check by results showing that the manipulation of antioxidant enzyme expression and use of chemical antioxidants failed to extend lifespans in most models (Seto et al., 1990; Huang et al., 2000; Van Remmen and Jones, 2009; Alexeyev, 2009; Jang and Van Remmen, 2009; Perez et al., 2009, 2012). However, one must remember that antioxidants cannot be expected to remove all types of ROS from the large variety of biological microenvironments. Mitochondrially-targeted antioxidant interventions seem to be more successful in terms of impact on lifespan compared to less specific antioxidants (Schriner et al., 2005; Wanagat et al., 2010; Anisimov et al., 2011). In addition, mildly uncoupling mitochondria, which among other effects decreases ROS generation by this organelle, extends lifespans of yeast and mice (Barros et al., 2004; Caldeira da Silva et al., 2008; Mookerjee et al., 2010; Cunha et al., 2011). Together, these findings suggest that mitochondrial oxidant levels are a more important limiting factor in lifespan than overall oxidative damage.

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Oxidants are not all equal, and recently nitric oxide radical ($NO^{\bullet}$) levels have been shown to be increased by CR (Nisoli et al., 2005; Cerqueira and Kowaltowski, 2010; Cerqueira et al., 2011a,b). $NO^{\bullet}$ is a poorly reactive and highly diffusible species (Moncada and Higgs, 1993) associated with multiple signaling pathways (Patel et al., 1999), but which can also react with superoxide radicals ($O_2^{\bullet-}$) generating the powerful nitrating agent peroxynitrite ($ONOO^-$) (Beckman et al., 1990; Radi et al., 1991; Jessup et al., 1992; Radi, 2004).

Interestingly, $NO^{\bullet}$ generated by the endothelial nitric oxide synthase (eNOS) has recently been shown to activate PGC1-α (Nisoli et al., 2003), resulting in an increase in mitochondrial mass (Nisoli et al., 2005; Cerqueira et al., 2011b; Civitarese et al., 2007). Since mitochondrial mass decreases during aging (Oberley et al., 2008; Picard et al., 2010), the stimulation of mitochondrial biogenesis and maintenance of mitochondrial energetic metabolism over time may be a key mechanism through which CR acts.

It is thus clear that the interplay between diet, mitochondrial energy metabolism, levels of intracellular oxidants and healthy aging is complex. This review will cover aspects of these factors and their interrelationships.

2. Mitochondrial changes during aging

It is well established that animals accumulate oxidatively modified DNA as they age, and that this accumulation is more substantial in mitochondria (reviewed in Shigenaga et al., 1994; Balaban et al., 2005; Kim et al., 2007). This has lead to the idea that a vicious cycle may occur, in which damaged mtDNA leads to the production of defective respiratory chains which generate more ROS, resulting in further damage to these mitochondria, although it should be noted that the vicious cycle hypothesis is not a necessary condition for the mitochondria/free radical theory of aging to be true.

In support of the vicious cycle hypothesis, mice deficient in mtDNA proofreading exhibit shortened lifespans and a phenotype of premature aging (Trifunvic et al., 2004). On the other hand, evidence that electron transport activity is affected by aging is not clear cut (reviewed by Maklashina and Ackrell, 2004). This may be an artifact of isolating mitochondria for these studies, a process that removes damaged organelles (Picard et al., 2010). Furthermore, damaged mitochondria are eliminated *in vivo* through mitochondrial autophagy (mitophagy, reviewed by Cuervo et al., 2005), which may also explain the lack of a consistent decline in mitochondrial function over time. Indeed, aging is associated with enhanced mitophagy (Oberley et al., 2008) and total mitochondrial mass decreases with age (Byrne and Dennett, 1992; Müller-Höcker et al., 1992). Interestingly, CR (see Table 1) increases both mitophagy (Cuervo et al., 2005) and mitochondrial biogenesis (Cerqueira et al., 2011b; Civitarese et al., 2007; Nisoli et al., 2005), possibly resulting in higher organelle turnover and a quantitatively and qualitatively healthier mitochondrial pool. This idea is well in line with the emerging concept that spare respiratory capacity of mitochondria, above the normal energetic demands of the cell, is a key feature ensuring cellular survival under stressful conditions (Nicholls, 2009; Dranka et al., 2010).

Another dietary intervention that enhances lifespans in laboratory animals is the restriction of methionine (Richie et al., 1994; Miller et al., 2005; Pamplona and Barja, 2006). Interestingly, methionine restriction (see Table 1) also enhances mitochondrial biogenesis (Naudí et al., 2007; Perrone et al., 2010), may enhance mitophagy (Hipkiss, 2008) and decreases mitochondrial ROS generation and oxidative damage (Sanz et al., 2006).

3. Mitochondrial ROS and reactive nitrogen species (RNS) metabolism

While larger respiratory capacities seem to be beneficial during aging, and are a possible mechanism through which CR delays the aging phenotype, it is certainly undesirable for this larger mitochondrial mass to be associated with larger levels of oxidant production, leading to enhanced oxidative damage. That does not appear to be the case in CR, since it not only regulates mitochondrial mass, but also oxidant levels.

Mitochondria continuously reduce a small quantity of oxygen by one electron, generating $O_2^{\bullet-}$ (reviewed by Kowaltowski et al., 2009). $O_2^{\bullet-}$, a reasonably reactive ROS, is dismutated to more stable H_2O_2 through the activity of matrix Mn-superoxide dismutase (Mn-SOD) as well as Cu,Zn-superoxide dismutase (Cu,Zn-SOD) in the intermembrane space. H_2O_2 is removed by antioxidant enzymes present redundantly in mitochondria and the cytosol, including glutathione peroxidase, catalase and thioredoxin peroxidase (reviewed by Kowaltowski et al., 2009).

H_2O_2 can produce highly potent hydroxyl radicals ($HO^{\bullet}$; Sies, 1993), in the presence of transition metals. Some criticism exists regarding the availability of free copper and iron to generate $HO^{\bullet}$ *in vivo*, but evidence for its formation exists based on the presence of oxidatively damaged biomolecules (Burkitt and Mason, 1991; Halpern et al., 1995). In this regard, iron and copper-rich mitochondria, which generate substantial levels of H_2O_2, are the most feasible intracellular site for site $HO^{\bullet}$ generation *in vivo*.

$O_2^{\bullet-}$ also reacts with $NO^{\bullet}$ producing peroxynitrite ($ONOO^-$) a strong and biologically relevant oxidant, known to react with DNA bases, tyrosine residues and unsaturated fatty acids (reviewed by Radi, 2004). Since both SOD and $NO^{\bullet}$ react with $O_2^{\bullet-}$ at diffusion controlled rates (Iwabu et al., 2010), they essentially complete as $O_2^{\bullet-}$ reactants. $ONOO^-$ and carbon dioxide (CO_2), which is in equilibrium with intracellular HCO_3^-, can react, resulting in the production of carbonate ($CO_3^{\bullet-}$) and nitrogen dioxide ($^{\bullet}NO_2$) radicals (Augusto et al., 2002; Szabó et al., 2007). $^{\bullet}NO_2$ nitrates cystein and tyrosin residues in proteins, lipids and 2′-deoxyguanine. $CO_3^{\bullet-}$ oxidizes a wide range of aminoacids as well as 2′-deoxyguanine (Augusto et al., 2002; Kalyanaraman et al., 2012). Lipid peroxides produced from $ONOO^-$, $^{\bullet}NO_2$; $O_2^{\bullet-}$ or $HO^{\bullet}$ are an abundant product of biomolecule oxidation. Lipid peroxides generate highly oxidative intermediates such as aldehydes, which can amplify oxidative reactions.

Evidence demonstrating a mitochondrial source of $NO^{\bullet}$ is controversial (see Giulivi, 2003; Brookes, 2004; Ghafourifar and Cadenas, 2005), but this radical is certainly diffusible enough to affect mitochondria even if generated at significant distances (Cardoso et al., in press). Three NOS isoforms have been identified: endothelial (eNOS), neural (nNOS) and inducible (iNOS). All are expressed in most mammalian cells, and are located in the cytoplasm, plasma membrane and cytoplasmatic vesicles (Pollock et al., 1993). In mitochondria, $NO^{\bullet}$ at nanomolar concentrations inhibits cytochrome c oxidase (Brown and Borutaite, 2001) by binding to its metal centers forming metal nitrosyls (Brown and Borutaite, 2001; Brown, 1995). Thiol nitrosylation promoted by $NO^{\bullet}$ regulates enzymatic activities (Cooper, 1999; Foster et al., 2009) and is reversible (Sengupta and Holmgren, in press). In excess, S-nitrosylation can lead to protein missfolding and aggregation found in age-related diseases (reviewed by Nakamura and Lipton, 2011).

4. Damaging effects of oxidants during aging

A rough inverse correlation has been observed between the rate of $O_2^{\bullet-}$ or H_2O_2 production and maximum life span potential in different species (Sohal et al., 1989, 1990; Barja, 1998; Lambert