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Facts and controversies in our understanding of how caloric restriction impacts the mitochondrion

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ABSTRACT

Caloric restriction (CR) has pronounced benefits in promoting healthy aging. Amongst the most frequently implicated physiological mechanisms implicated in this benefit is altered mitochondrial function. Whereas a reduction in mitochondrial reactive oxygen species (ROS) production is a widely consistent effect of CR, an increase in mitochondrial biogenesis, which is accepted by many as fact, is contradicted on several levels, most critically by a lack of increase in mitochondrial protein synthesis rate in vivo. Furthermore, an increase in PGC-1α protein and markers of mitochondrial content with CR is a highly variable observation between studies. On the other hand, deacetylation of several mitochondrial proteins by the sirtuin, Sirt3, is an increasingly reported observation and at least so far, this observation is consistent between studies. Notwithstanding this point, the controversies evident in the published literature underscore the significant questions that remain in our understanding of how CR impacts the mitochondrion and suggest we have yet to fully understand the complexities herein.

1. Introduction

Caloric restriction (CR) is amongst the very few non-genetic interventions known to increase both median and maximal lifespan, a positive effect that has been demonstrated in several species, including yeast, worms and rodents (Fontana et al., 2010). Although CR-induced lifespan extension in humans is currently debated, the impact of CR in attenuating age-related disease continues to be a consistent finding (Colman et al., 2009; Fontana et al., 2010; Mattison et al., 2012). Further to this point, metabolic alterations secondary to CR are widely reported, with mitochondrial adaptations featuring prominently in these effects (Anderson and Weindruch, 2010; Palacios et al., 2009; Rodgers et al., 2005). Indeed, since mitochondria are implicated in causing the deterioration of cellular function and cellular loss with aging (the mitochondrial theory of aging) (Loeb et al., 2005), and because mitochondria act as a central regulator of numerous cellular homeostatic signals including nutrient intake (Scarpulla, 2012), mitochondrial alterations are likely to play a key role in the health-promoting effects of CR (Raffaello and Rizzuto, 2011). As will be detailed below, however, not only are there important differences between mitochondrial functional alterations with normal aging versus that which is predicted by some aspects of the mitochondrial theory of aging, but some of the most widely accepted effects of CR on mitochondria are contradicted by credible contrary evidence, and in other cases no clear consensus has yet emerged. These controversies underscore the fact that further research is needed to clarify the impact of CR on mitochondrial function and thus, clarify the role mitochondrial alterations play in the health-promoting effects of CR. Furthermore, these controversies are likely amplified by virtue of the many different means by which CR can be imposed, including variations in the caloric intake (ranging from 20 to 50% reductions between studies), variation in frequency in feeding (e.g., every other day feeding), and variations in diet composition (e.g., specifically restricting the intake of one amino acid such as methionine). Thus, future studies should also aim for better standardization of the method by which CR is imposed and at the very least, draw comparisons to other studies using similar approaches to implement CR.

2. Impact of aging on mitochondrial function

To help put the impact of CR on mitochondria in context, it is important to first discuss the impact of aging on the mitochondria and the mechanisms responsible. Many of the arguments made in favor of mitochondria being involved with aging stemmed from the work of Denham Harman, beginning with his seminal paper in 1956 wherein several hypotheses related to the production of reactive oxygen species (ROS) and their role in causing damage with aging were first put forth (Harman, 1956). Harman (1972) and many others (Bejma and Ji, 1999; Richter et al., 1988; Schwarze et al., 1995) expanded upon this initial work to build a case in support of the idea that cellular aging

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is caused by accumulation of mitochondrial damage induced by ROS. Specifically, it had been suggested that mitochondria might accumulate damage arising from the ROS production inherent to mitochondrial energy production and that this damage, by impairing multiple aspects of mitochondrial function, underpinned aging itself, a point that seems consistent with the fact that mitochondria are a major cellular site of reactive oxygen species (ROS) production (Balaban et al., 2005). This theory received experimental support in many initial investigations (Ku et al., 1993; Orr and Sohal, 1994; Sohal et al., 1985), but in recent years has seen that an increasing number of studies rise to challenge the central tenant that ROS are inherently damaging in the context of aging (Kuwahara et al., 2010; Labunskyy and Gladyshev, 2012; Lapointe et al., 2009; Lewis et al., 2012; Tweedie et al., 2011). Thus, whereas the significance of ROS in causing aging per se is now seriously in question, excessive ROS production is still considered to play an important role in pathology and disease (Barker and Traber, 2007; Jang et al., 2010; Roberts and Sindhu, 2009) and appears to be involved in some aspects of deteriorating function with aging (Kuwahara et al., 2010).

Another aspect of the mitochondrial theory of aging posits that there is progressive accumulation of mitochondrial DNA (mtDNA) damage with aging, due in part to the fact that mtDNA is located within the mitochondria next to the source of ROS production, resulting in synthesis of abnormally functioning mitochondria. Whereas a lack of protective histone proteins may contribute to mtDNA being more susceptible to damage, mitochondrial transcription factor A (TFAM) at least partially compensates for this by protecting and maintaining mtDNA integrity (Alexeyev, 2009; Campbell et al., 2012; Kang et al., 2007). Notwithstanding this point, as predicted by the mtDNA damage aspect of the mitochondrial theory of aging, a mouse engineered with a proof-reading deficient version of mtDNA polymerase gamma (PolG mouse) accumulates mtDNA damage rapidly beginning in early adulthood (Vermulst et al., 2007, 2008), and exhibits a markedly shortened lifespan characterized by numerous 'aging-like' traits such as alopecia, cardiac hypertrophy, bone fragility (Kujoth et al., 2005; Trifunovic et al., 2004, 2005), muscle atrophy (Hiona et al., 2010), and muscle weakness (Yamada et al., 2012). Interestingly, this model diverges from the oxidative stress theory of aging in that mitochondrial hydrogen peroxide production and markers of cellular oxidative stress are not elevated in PolG mice compared to wild type mice (Kujoth et al., 2005). Similarly, no sign of increased reactive species production is seen in mouse embryonic fibroblasts cultured from PolG mice (Trifunovic et al., 2005). Finally, it is also important to appreciate that many of the phenotypes seen in this PolG mouse do not conform to those seen with normal aging. For example, the nature of the muscle atrophy is qualitatively different in the PolG mouse which exhibits preferential type I fiber atrophy (Hiona et al., 2010), whereas aging muscle typically exhibits greater type II fiber atrophy (Lexell et al., 1988), until advanced age when both type I and type II fibers atrophy similarly (Purves-Smith et al., 2012). In addition, the muscle mitochondrial phenotypes seen in the PolG mouse are characterized by a reduced expression of electron transport chain subunit proteins and reduced reactive oxygen species (ROS) production in fast twitch muscle (Hiona et al., 2010), whereas normally aging fast twitch muscle exhibits higher expression of electron transport chain subunit proteins and greater ROS production (Picard et al., 2010, 2011a). Finally, whereas PolG mouse muscle is more fatigable (Yamada et al., 2012), normally aged muscle is less fatigable (Hepple et al., 2004; Lanza et al., 2004), except at high contraction velocities when it is sometimes seen to be more fatigable (Callahan and Kent-Braun, 2011). Since many phenotypes seen with normal muscle aging are not consistent with the established impact of mtDNA damage accumulation, the applicability of this version of the mitochondrial theory of aging remains, at best, unclear.

A third aspect of the mitochondrial theory of aging posits that the rate of mitochondrial turnover declines with aging, due to failed removal of damaged organelles, resulting in the accumulation of mitochondria with aberrant function (Brunk and Terman, 2002; Terman and Brunk, 2004; Terman et al., 2010). This is consistent with the accumulation of so-called "giant mitochondria" with aging which are proposed to accumulate due to failed mitochondrial autophagy (Navratil et al., 2008). Since mitochondrial fission is necessary for mitochondrial autophagy (Bess et al., 2012; Parone et al., 2008; Romanello et al., 2010; Twig et al., 2008), imbalance between mitochondrial fission and fusion events seems a likely cause of both the accumulation of "giant mitochondria" and inadequate mitochondrial turnover with aging. Supporting the idea that impaired mitochondrial autophagy leads to accumulation of dysfunctional organelles with aging, inhibition of autophagy leads to mitochondrial dysfunction characterized by a sensitization to mitochondrial permeability transition (Kim et al., 2008), a trait that is shared with aged mitochondria in skeletal muscle (Chabi et al., 2008; Picard et al., 2010, 2011a), heart (Ljubicic et al., 2010; Picard et al., 2012), and some regions of the brain (LaFrance et al., 2005). This aging-related sensitization to mitochondrial permeability transition is believed to play important role in the aging process of post-mitotic tissues, such as skeletal muscle, since it is believed to lead to the release of pro-apoptotic factors from mitochondrial inner membrane space and in turn, to trigger apoptosis (Marzetti et al., 2010). In addition, inhibition of mitochondrial autophagy through the Pink/Parkin pathway increases mitochondrial ROS generation (Chu, 2010), which is another trait commonly reported in aged mitochondria (Capel et al., 2004; Chabi et al., 2008; Mansouri et al., 2006). As such, in contrast to the mtDNA damage version of the mitochondrial theory of aging, the inadequate mitochondrial renewal version has more empirical support in that the anticipated consequences of reduced mitochondrial renewal are in fact seen with normal aging.

In summary, whereas the role of free radicals and mtDNA mutation in causing aging remains in doubt, there are clearly alterations in mitochondrial function with aging and some of these changes are at least qualitatively similar to the impact of impaired autophagic removal of mitochondria. Another question yet to be resolved is whether there may be tissue-specific involvement of impaired mitochondrial function secondary to reduced autophagy versus mitochondrial dysfunction secondary to accumulated mtDNA damage. Further study is required to address this question. Notwithstanding this point, even if changes in mitochondrial function do not cause aging per se, there remains strong support for their contribution to deteriorating function with aging and thus, they remain an important therapeutic target to promote healthy aging, and in explaining the improvements in health seen with CR.

3. Impact of caloric restriction on mitochondrial function

CR has numerous effects on mitochondrial function, many of which are thought to contribute to the health benefits seen in CR animals. Amongst the most clearly established effects is a reduction in ROS emission, resulting in a lower rate of oxidative damage and downstream effects such as less apoptosis. A reduction in ROS production, assessed by the indirect monitoring of mitochondrial H_2O_2 release, by CR has been documented in mitochondria from rat liver (Hagopian et al., 2005; Lambert and Merry, 2004), rat skeletal muscle (Bevilacqua et al., 2005), mouse liver (Faulks et al., 2006), and rat brain (Sanz et al., 2005), amongst other examples. CR appears to specifically reduce ROS emission from complex I of the mitochondrial electron transport system (Gredilla et al., 2001; Hagopian et al., 2005, 2011; Lopez-Torres et al., 2002) (Fig. 1). In addition to an impact of CR on ROS emission in juvenile or adult animals, CR also seems to attenuate the increase in ROS normally seen with aging (Asami et al., 2008; Lanza et al., 2012). Importantly, these prior studies appear to have examined mitochondrial ROS generation under only state I or state II conditions and thus, the impact of CR on mitochondrial ROS generation under varying levels of mitochondrial respiration that correspond to the range of metabolic rate seen in vivo remains unclear. In addition, because of the inherent limitations

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