Cardiac mitochondrial bioenergetics, oxidative stress, and aging

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Judge S, Leeuwenburgh C. Cardiac mitochondrial bioenergetics, oxidative stress, and aging. Am J Physiol Cell Physiol 292: C1983–C1992, 2007; doi:10.1152/ajpcell.00285.2006.—Mitochondria have been a central focus of several theories of aging as a result of their critical role in bioenergetics, oxidant production, and regulation of cell death. A decline in cardiac mitochondrial function coupled with the accumulation of oxidative damage to macromolecules may be causal to the decline in cardiac performance with age. In contrast, regular physical activity and lifelong caloric restriction can prevent oxidative stress, delay the onset of morbidity, increase life span, and reduce the risk of developing several pathological conditions. The health benefits of life long exercise and caloric restriction may be, at least partially, due to a reduction in the chronic amount of mitochondrial oxidant production. In addition, the available data suggest that chronic exercise may serve to enhance antioxidant enzyme activities, and augment certain repair/removal pathways, thereby reducing the amount of oxidative tissue damage. However, the characterization of age-related changes to cardiac mitochondria has been complicated by the fact that two distinct populations of mitochondria exist in the myocardium: subsarcolemmal and interfibrillar mitochondria. Several studies now suggest the importance of studying both mitochondrial populations when attempting to elucidate the contribution of mitochondrial dysfunction to myocardial aging. The role that mitochondrial dysfunction and oxidative stress play in contributing to cardiac aging will be discussed along with the use of lifelong exercise and caloric restriction as countermeasures to aging.

superoxide anion; longevity; postmitotic; calorie restriction; subsarcolemmal, interfibrillar, exercise

AGING IS INEVITABLE, and is characterized by a progressive deterioration in physiological processes, ultimate leading to morbidity and mortality. The free radical theory of aging (5, 30, 38) proposes that free radicals [specifically reactive oxygen species (ROS)], by-products of normal metabolism, cause oxidative damage to macromolecules, whose accumulation causes cellular dysfunction with age and eventually cell death. Over time, the free radical theory has been further refined to reflect the fact that mitochondria are at the same time major sources and targets of ROS (76, 80). According to the mitochondrial theory of aging, ROS produced via mitochondrial respiration attack mitochondrial constituents. In particular, accumulation of oxidant-induced somatic mutations in mitochondrial DNA (mtDNA) is believed to be the underlying cause of the decline in physiological function with age (61, 127). Mitochondrial respiratory complex function may be altered as a result of mtDNA mutations, leading to increased ROS production and further damage to mtDNA, as well as other macromolecules (73). The age-related increase in oxidative damage to DNA, lipids, and proteins has been well documented (9, 118), along with evidence supporting increased mtDNA deletions (20, 39) and mitochondrial dysfunction with age (75, 113). According to the hypothesized central role of mitochondria in the aging process, tissues that exhibit a high rate of oxygen consumption throughout an individual’s lifetime, such as the heart, may be especially prone to oxidative damage.

Numerous experimental interventions designed to delay the aging process have been attempted. To date, the only intervention that has consistently been shown to slow the rate of aging and to increase mean and maximum lifespan in a variety of species is calorie restriction (CR) (118, 133, 137). However, overexpression of antioxidant enzymes (91, 111), antioxidant supplemented diets (131), and lifelong voluntary wheel running (32, 43–46) have had some degree of success in increasing mean, but not maximal, lifespan. In this review, we will discuss how mitochondrial dysfunction and oxidative stress contribute to cardiac aging and how CR and lifelong exercise counteract this process.

MITOCHONDRIAL THEORY OF AGING

While there are numerous endogenous producers of ROS, including peroxisomes, NADPH oxidase, and cytochrome $P-450$, mitochondria appear to be responsible for producing the majority of oxidants. Classic in vitro studies in the 1970s showed that during mitochondrial respiration, 1–2% of the oxygen consumed was converted to $H_2O_2$ (14). Although this may overestimate in vivo rates of oxidant production, mitochondria continue to be considered significant sources of oxi-
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Mitochondrial Bioenergetics and Aging

Electrons (active form) and H$_2$O$_2$ (a two-electron reduced product) and H$_2$O$_2$ (a two-electron reduced product) and H$_2$O$_2$ (a two-electron reduced product) and H$_2$O$_2$ are generated in the liver and is a strong inflammatory marker. We recently showed that CR and exercise + CR attenuate the age-related increase in plasma CRP level (53), supporting a possible link between a decrease in oxidative stress, attenuated NF-$\kappa$B activity in liver cells, and lowered CRP levels. Furthermore, in some instances, ROS can act as a second messenger downstream of the specific ligands, such as transforming growth factor-1 (136) and endothelin (109) and activator protein-1 (56). However, it is clear that during normal aging conditions the mitochondria appear to be responsible for producing the majority of oxidants, which likely have local effects within the mitochondria or in their very close proximity.

AGE-RELATED ALTERATIONS IN CARDIAC MITOCHONDRIAL FUNCTION

The heart is primarily a postmitotic tissue and exhibits a highly aerobic metabolism due to the abundance of large mitochondria. These features implicate two consequences for normal organ function: dependence on healthy mitochondria and on healthy cells. Therefore, the physiology and bioenergetics of cardiomyocytes with age has been an important research area for many years. Interpretation of age-related changes in mitochondrial function is made difficult by the fact that the heart contains two structurally similar but biochemically different mitochondrial populations (94). Subsarcolemmal mitochondria (SSM) are located beneath the plasma membrane, and interfibrillar mitochondria (IFM) are arranged in parallel rows between the myofibrils (27). Citrate synthase and succinate dehydrogenase activities were higher in IFM compared with SSM, and IFM oxidized substrates quicker than SSM. Later, the same group reported that state 3 respiration, content of respiratory cytochromes, and activities of electron transport chain complexes were higher in IFM compared with SSM (93). However, despite this knowledge the evaluation of presently available data is complicated because most isolation procedures yield either SSM alone or a mixed population of SSM and IFM (27, 50, 74, 93). In addition, detecting bioenergetic changes due to aging is problematic not only because of the presence of different mitochondrial populations but also because cells with extremely dysfunctional mitochondria will likely die via apoptosis or necrosis (so that only relatively healthy mitochondria are obtained upon isolation) (73).

Until recently, there has been little agreement in the literature as to whether mitochondrial oxidative phosphorylation is effectively impaired with age, since some laboratories have reported a decline in oxygen consumption with age (24, 134) while others reported no changes (19, 79). Fannin et al. (27) found that when SSM and IFM were isolated from adult (6 mo old) and old (24 and 28 mo old) rat hearts and analyzed individually, only the IFM exhibited age-related declines in...
protein yield and oxidative phosphorylation rates. These results have recently been confirmed in our laboratory (50). Since the amount of IFM in a mixed population of mitochondria would be expected to vary, this may help explain the lack of consistency regarding age-related changes in oxidative phosphorylation.

There is also little agreement regarding changes in various electron transport complex activities with age. Of the five respiratory chain complexes, Complex I (NADH-ubiquinone reductase or NADH dehydrogenase) appears to be the most susceptible to age-related declines in activity (3, 16, 72, 122, 126), although some studies have reported no significant changes in Complex I activity with age (63, 81). Physiologically relevant ROS generation occurs at the flavin mononucleotide group (FMN) of complex I through reversed electron transfer using complex II substrate succinate (77). Given that 7 of the 13 mtDNA encoded polypeptides in the respiratory chain complex are found in complex I, it is not surprising that this complex would be most greatly affected with aging if the mitochondrial theory of aging proves to be true (72). In addition, many studies report age-associated declines in the activities of complex III (ubiquinol-cytochrome c reductase) (16, 74) and complex IV (cytochrome c oxidase) (3, 16, 27, 63, 122), which also contain mtDNA encoded proteins, while the activity of complex II (succinate dehydrogenase) appears unaffected (81, 122, 126) or even increased (16, 63). This finding is noteworthy, since mtDNA does not encode for any of the polypeptides in complex II (92). In further support of the idea that complexes I–IV are negatively affected with age, mRNA levels of NADH dehydrogenase subunit 1, cytochrome c oxidase subunit 3, and cytochrome b are reduced in old compared with young mouse hearts (12). In addition, protein levels of complexes III–V (ATP synthase) were decreased in left ventricular mitochondria obtained from old monkeys (20 yr) compared with young monkeys (6 yr) (134).

Attempts to identify some of the mechanisms responsible for these declines in activity indicate that the reduction in complex III activity may be due to alterations in the cytochrome c binding site (74), while complex IV activity may be decreased due to changes in cardiolipin content or composition (97–99). However, at least one group has reported that aging does not alter cardiolipin content or composition in either SSM or IFM isolated from rat heart (82). Complex V activity also declines during aging in the heart (22, 34, 135) and oxidative modification of the β-polypeptide of the F1 complex of this enzyme by malondialdehyde may be at least partially responsible for this phenomenon (135). Other oxidative modifications to this enzyme may also contribute to the decline in function as an age-related increase in 3-nitrotyrosine has also been reported (55). In conclusion, substantial evidence supports an age-related decline in cardiac mitochondrial function, but future studies are required to determine the mechanisms contributing to these changes, and to further characterize differential effects of age upon the SSM and IFM populations in the heart. Moreover, isolation of mitochondrial complexes using affinity chromatography and the determination of specific enzyme activities and composition using proteomics may shed light on some of the mechanisms contributing to the age-related decline in function.

MITOCHONDRIAL OXIDATIVE PRODUCTION IN THE AGING HEART

Soon after it was reported that mitochondria were sources of oxidants (14), Nohl and Hegner (89) found that heart mitochondria isolated from old rats generated more H2O2 than did mitochondria from young animals. Since then, numerous studies have been published supporting an age-related increase in cardiac mitochondrial oxidant production (50, 85, 116, 117, 123, 124), while others have reported no changes (25, 33, 37). It is likely that the type and concentration of substrate used to determine the rate of H2O2 production may account for some of these discrepancies (37). In addition, since SSM and IFM are differently affected by aging (27, 50, 74, 93), it is possible that they may also generate different amounts of H2O2 with age and this issue has recently been addressed (50, 123). Data from our laboratory indicate that H2O2 production from SSM, but not IFM, increases with age (50). These results appear to be in contrast to previous findings that oxidant production increased with age in IFM, but not SSM, isolated from heart (123). However, Suh and colleagues (123) did not directly measure H2O2 production from isolated mitochondria, but used the rate of oxidation of 2′,7′-dihydrodichlorofluorescein (DCFH) in isolated mitochondria as an indicator of total mitochondrial oxidant production. DCFH is able to cross the mitochondrial membranes and acts as an indicator of oxidant production (including H2O2 and NO·) inside of the mitochondria (125), whereas our assay measures the amount of H2O2 released from intact mitochondria. The fluorescent probe DCFH detects a variety of oxidants, such as NO·since mtNOS has been characterized in the mitochondrial membrane of the heart (54).

In IFM from the same animals, we also detected highly increased antioxidant enzyme activity (superoxide dismutase, glutathione peroxidase, catalase), reduced glutathione levels, and increased oxidative damage (50), supporting the notion that oxidant production within the matrix of old IFM was greater than that in young IFM, which is in agreement with Suh and colleagues (123).

We also determined whether SSM and IFM from same-aged animals produced different amounts of H2O2 (50). In agreement with Suh et al. (123), we found that SSM and IFM from young rats produced similar amounts of H2O2 (50). However, in old rats, H2O2 production was significantly lower in IFM compared with SSM. Similarly, state 4 oxygen consumption was also significantly reduced in IFM from old rats, compared with SSM from the same animals. Mitochondrial oxidant production is higher in state 4 than in state 3 due to greater reduction of the respiratory chain in state 4 (78). Since we measure H2O2 production under state 4 conditions decreased H2O2 production may be directly related to the decline in state 4 O2 consumption. Further research is clearly required to characterize age-related differences in oxidant production in IFM and SSM.

MITOCHONDRIAL OXIDATIVE DAMAGE IN THE AGING HEART

As a major source of oxidants, mitochondria themselves are believed to be especially susceptible to oxidative damage. Indeed, there is a great deal of evidence showing an increase in oxidative damage to mitochondrial DNA with age (8, 23, 39, 86). Oxidants produced by mitochondria can damage DNA
(both mitochondrial and nuclear), lipids, and proteins if not effectively scavenged. Oxidized DNA may become mutated (35), lipid peroxidation can cause alterations in membrane fluidity (17), and oxidized proteins frequently lose catalytic activity (120). All of these events can negatively impact mitochondrial and cell function and contribute to the decline in physiological function with age. DNA damage in the heart (and other postmitotic tissues, such as the brain) is more extensive than that observed in mitotic tissues such as the liver (48). This is likely a result of the limited ability of heart cells to undergo mitosis, coupled with the fact that the heart exhibits high oxygen consumption and low antioxidant capacity compared with the liver (41).

While oxidative damage to both nuclear and mitochondrial DNA increases as a function of age (36), Barja and Herrero (8) found that levels of 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodG), an indicator of oxidative DNA damage, were four-fold higher in mtDNA compared with nuclear DNA in heart tissue obtained from eight mammalian species with varying life spans (3.5 to 46 yr). Furthermore, there was a significant inverse correlation between 8-oxodG levels in mtDNA and maximum life span among the different species. This agrees with previous work indicating that long-lived animals produce less mitochondrial superoxide and hydrogen peroxide compared with short-lived animals (60). Since the rate of repair of 8-oxodG is similar in nuclear and mtDNA (4), it has been postulated that the increased level of 8-oxodG observed in mtDNA is related at least partly to the chronic exposure of mtDNA to oxidants by virtue of its location. Furthermore, mtDNA repair activity has actually been shown to increase in the aging heart, further suggesting that the rate of damage to mtDNA is increased with age (119).

In addition to DNA, proteins and lipids within the mitochondria are also susceptible to oxidative modification. The polyunsaturated fatty acids found in membrane lipids are vulnerable to peroxidation by oxidants, and lipid peroxidation has been shown to increase in cardiac mitochondria during aging (19, 49, 50, 100). Chen and Yu (17) reported that lipid peroxidation is a major contributor to the age-related loss of membrane fluidity and that two aldehydic lipid peroxidation products, malondialdehyde (MDA) and 4-hydroxy-2-nonenal (HNE), are primarily responsible for the decrease in membrane fluidity. In cell culture studies, both MDA and HNE have shown cytotoxic effects, possibly because they can readily react with proteins (26). Indeed, although HNE is an end product of lipid peroxidation, it is highly reactive with other biological molecules, especially proteins (90). HNE exerts numerous effects, including inhibition of protein and DNA synthesis and enzyme inactivation, and is believed to play a major role in oxidative stress-induced cellular dysfunction (90). In IMF, the decline in complex IV activity with age has been associated with a fourfold increase in the amount of HNE added to complex IV (123). Furthermore, HNE has been shown to covalently bind to and inhibit proteasome activity (90); and HNE-modified proteins can become resistant to proteolytic degradation (28), and act as noncompetitive inhibitors of the proteasome (28, 29).

Although the measurement of protein carbonyls as a marker of protein oxidative damage has been extensively criticized, it remains the most commonly used method for assessing protein oxidation. Carbonyls can be formed via several mechanisms including site-specific metal-catalyzed oxidation of lysine, arginine, proline, and threonine residues; glycation reactions; and interaction of amino acid side chains with lipid peroxidation products, such as HNE and MDA (1, 120). Accumulation of oxidized proteins is believed to play a key role in the loss of physiological function with age, since oxidized proteins can lose catalytic activity and are also prone to forming large, potentially cytotoxic, protein aggregates (114, 120).

Davies et al. (22) measured α-, and m-tyrosine, along with protein carbonyls, in the mitochondria and cytosol from hearts of young (2 to 3 mo) and old (24 mo) rats, finding that protein oxidative damage was not greater in the mitochondria compared with the cytosol. Furthermore, they found no evidence of an age-related increase in mitochondrial or cytosolic protein oxidation. Conversely, several other studies report an age-related increase in protein carbonyls in heart mitochondria (3, 19, 50) and this may contribute to the decline in mitochondrial function observed with aging.

LONG-TERM VOLUNTARY EXERCISE AS A COUNTERMEASURE TO AGE-RELATED MITOCHONDRIAL DYSFUNCTION AND OXIDATIVE STRESS

The sedentary lifestyle that many individuals lead is detrimental to their health, as indicated by the numerous benefits of consistent, moderate exercise. Regular physical activity can delay the onset of morbidity, and increase mean life span (43, 45, 66). Indeed, the risk of developing coronary heart disease, cerebrovascular disease, hypertension, type 2 diabetes, colon cancer, and osteoporosis is significantly reduced in physically active individuals compared with those who are sedentary (66). Although it is widely agreed that exercise is beneficial for health and longevity, the molecular mechanisms through which exercise exerts these effects are not well understood. However, it has been hypothesized that the health benefits and the extension of average life span of chronic exercise may be at least partially due to a reduction in inflammation, oxidant production, and oxidative damage.

Paradoxically, it was shown more than two decades ago that strenuous acute exercise can actually increase oxidant production and tissue damage (21). Additional evidence has been obtained indicating that acute bouts of exercise in untrained subjects can indeed elevate oxidant production and tissue oxidative damage (10, 11, 62, 69), and it is often assumed that mitochondrial oxidant production rises in direct proportion to tissue oxygen consumption. Therefore, it has been proposed that the increased oxidative stress with acute exercise is a result of the dramatic increases in tissue oxygen consumption (up to 2.3-fold in skeletal muscle and 3.6-fold in the heart) that occur during heavy exercise (42). In contrast to strenuous heavy exercise, moderate adapted exercise seems to have a beneficial effect. Venditti et al. (130) found that 10 wk of swim training significantly reduced the basal rate of mitochondrial H2O2 production in rat gastrocnemius muscle compared with untrained animals. What is the beneficial mechanism of moderate chronic adapted exercise?

First, it was found that mitochondrial oxidant production does not dramatically increase during ADP-supplemented (state 3) respiration, which is reflective of the exercised state (42). Conversely, mitochondrial oxidant production measured in vitro actually decreases quite significantly in state 3 com-
pared with state 4, although there is still a small amount of oxidant production. Although some oxidative damage may occur due to the low level of state 3 oxidant production, this observation helps explain the lack of massive oxidative damage after exercise despite the tremendous increases in oxygen consumption. Thus, electron flow between the various electron complexes is likely more efficient during state 3 respiration, thereby decreasing the possibility for oxygen at the electron complexes to form $O_2^{2-}$.

In addition to an apparently more efficient state 3 respiration where oxidant production is reduced, several investigators have reported increases in antioxidant enzyme activity in skeletal and cardiac muscle after endurance exercise training (67, 70, 101, 102, 129), an adaptation that would help reduce oxidative damage. Kim et al. (58) measured lipid peroxidation and antioxidant enzyme activity in hearts from 20-mo-old male Fischer 344 rats after 18.5 mo of voluntary wheel running. Compared with the sedentary rats, long-term voluntary wheel running resulted in decreased lipid peroxidation and increased catalase activity but no changes in superoxide dismutase or glutathione peroxidase activities. These changes were observed despite the fact that the rats in this study ran, on average, only 60 m per day. Wheel running also resulted in a significant increase in catalase and glutathione peroxidase activities and increased glutathione content in liver (57). Liver microsomal oxidant production was reduced by exercise, although no differences were seen in mitochondrial oxidant production between sedentary and exercised animals. In addition, membrane fluidity was significantly increased in both hepatic microsomes and mitochondria obtained from the wheel running animals. In another study, levels of $o,o'$-dityrosine (a marker of protein oxidation) in urine were significantly reduced in 24-mo-old rats that had undergone 19 mo of voluntary wheel running compared with sedentary control animals (68). Glutathione peroxidase and mitochondrial superoxide dismutase activities were also elevated in skeletal muscle from the exercised animals in this study.

Both reduced oxidant production and an increased antioxidant enzyme activity as a consequence of moderate exercise training would therefore be expected to reduce tissue oxidative damage. Indeed, lipid peroxidation has been shown to be lower in heart and liver of exercise-trained animals compared with sedentary animals (129). A reduction in the oxidative DNA damage marker, 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodG), has been reported in skeletal muscle after exercise training (104, 105); and protein carbonyl content in the heart is significantly lower in trained rats compared with sedentary rats (106). Moderate treadmill exercise for 24 wk also reduced protein carbonyls and lipid peroxidation in hearts from 52-wk-old mice (88).

Exercise also appears to have beneficial effects on repair and removal of oxidized molecules. Radak et al. (104–106) determined the effect of exercise training on DNA repair activity and 20S proteasome activity. Eight weeks of treadmill training in middle-aged (20 mo) and old (30 mo) rats increased 8-oxodG repair activity and chymotrypsin-like proteasome activity in rat skeletal muscle (105). Nine weeks of swim training increased the chymotrypsin-like proteasome activity in rat heart (106) and both chymotrypsin- and trypsin-like activities in gastrocnemius muscle (104). Taken together, the available data suggest that chronic exercise may serve to reduce mitochondrial oxidant production, enhance antioxidant enzyme activities, and augment certain repair/removal pathways, thereby reducing the amount of oxidative tissue damage.

Despite the fact that several studies have examined the effects of long-term voluntary wheel running on survival, little is known about the effects it has on mitochondrial function and oxidative stress. We recently investigated whether long-term voluntary wheel running could reduce mitochondrial oxidant production and oxidative damage in SSM and IFM isolated from hearts of Fischer 344 rats (51). Mitochondrial hydrogen peroxide production was significantly reduced in both SSM and IFM from wheel runners compared with sedentary rats (Fig. 1; Refs. 50 and 51). Moreover, the age-associated increase in oxidant production in isolated mitochondria was reduced by moderate (8%) CR (Fig. 1; Refs. 50 and 51). Since mitochondrial hydrogen peroxide is produced via the dismutation of two superoxide radicals, we speculated that voluntary exercise may have reduced the amount of superoxide being produced during electron transport. Although we did not directly measure superoxide production, the activity of manganese superoxide dismutase (MnSOD), the major enzyme responsible for scavenging superoxide within the mitochondrial matrix, was also decreased in SSM and IFM from wheel runners. Alternatively, it is also possible that an increase in mtNOS removed $O_2^{2-}$, resulting in less $H_2O_2$ leakage and less need for MnSOD.

The recent development of gene expression microarrays allows investigators to monitor changes in mRNA transcript levels in particular tissues under various experimental conditions. Results from these studies indicate that there are numerous age-related changes in gene expression in a variety of tissues (12, 15, 103, 132), and provide a powerful tool by which to study aging. Gene expression analyses have been performed in the hearts of physiologically active and sedentary middle-aged (20 mo) and old (33 mo) mice (15). Mice in the physiologically active group were placed in cages with running wheels at 1 mo of age and were allowed to exercise voluntarily until the time of death. In the sedentary group, 137 genes were significantly different (up- or downregulated by at least 50%) in the old compared with the middle-aged mice and most of these genes were associated with either the inflammatory or stress response. Exercise attenuated age-related changes in 70 Fig. 1. $H_2O_2$ production in subsarcolemmal mitochondria (SSM) mitochondria from young (6 mo), old (24 mo) ad libitum-fed, old (24 mo) caloric-restricted (CR, 8%), and old (24 mo) caloric restricted (CR, 8%) and exercised (E) Fischer 344 rats. Values are means ± SE. *$P < 0.05$, significant difference from young; **$P < 0.05$, significant difference from old; ***$P < 0.05$, significant difference from old CR. Data adapted with permission (50, 51).
of the 137 genes that changed in the old, sedentary mice. It was concluded that the aging heart is subjected to oxidative stress (which leads to a proinflammatory state) and that long-term voluntary exercise can prevent many of these changes. Of interest, it was found that Lon protease expression was significantly increased with age in the exercised but not the sedentary animals. Lon protease is found in the mitochondria and Bota et al. (13) reported that protein levels of this protease decline with age. This is important because Lon protease preferentially degrades oxidized proteins and therefore, may play a crucial role in preventing the accumulation of oxidized proteins in the mitochondria with age.

There remains a great deal of debate surrounding the optimum intensity, frequency, and duration of exercise required to elicit the maximum benefits. However, there is some evidence that moderate and prolonged exercise may provide the most protection against oxidative stress (47). In rodent models, a commonly used method of exercise training involves forced treadmill running. The obvious advantage of this type of training is that the investigator can control the duration, intensity, and frequency of the running. Furthermore, there are generally large improvements in the oxidative capacity of skeletal and cardiac muscle with this type of training (83). On the negative side, forced treadmill running can also cause physiological adaptations that are indicative of chronic stress. Moraska et al. (83) found that 8 wk of treadmill training in male rats resulted in adrenal gland hypertrophy and thymic involution, both of which were attributed to chronic elevations in stress hormones, which can negatively impact health. Additionally, serum levels of the corticosterone carrier protein, corticosteroid binding globulin, were reduced which could result in prolonged elevations in free corticosterone. Finally,

![Fig. 2. Summary of changes in subsarcolemmal (SSM) and interfibrillar (IFM) mitochondria isolated from lifelong wheel runners compared with sedentary controls (top). Items shown in black are parameters we measured while items in gray are probably occurring or measured by others (123). SSM and IFM from wheel runners likely produced less superoxide anion ($\text{O}_2^{-}$) as witnessed by a significant reduction in manganese superoxide dismutase (MnSOD) activity. The decrease in the activity of this enzyme should lead to decreased production of $\text{H}_2\text{O}_2$ inside the mitochondria. Indeed we found a reduced level of $\text{H}_2\text{O}_2$ released from isolated mitochondria. In SSM and IFM, there were no differences between groups in mitochondrial glutathione peroxidase (GPX) and catalase (CAT) activities and therefore, $\text{H}_2\text{O}_2$ released from the mitochondria was lower in wheel runners compared with sedentary animals. There were no significant changes in the levels of mitochondrial glutathione reductase (GR) and reduced glutathione (GSH). Moreover, no changes were found in the levels of 4-hydroxy-2-nonenal (HNE) and thiobarbituric acid-reactive substance (TBARS) in SSM or IFM, but carbonyls increased in SSM from wheel runners. Adapted with permission from Ref. 51.](http://ajpcell.physiology.org/)

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**Mitochondrial adaptations due to lifelong wheel running**

- **CAT** → **GSH** → **TBARS**
- **$\downarrow \text{O}_2^{-}$**
- **$\downarrow \text{MnSOD}$**
- **$\downarrow \text{H}_2\text{O}_2$**
- **$\uparrow \text{Carbonyls}$**
- **$\downarrow \text{O}_2^{-}$**

**Mitochondrial adaptations due to calorie restriction**

- **$\downarrow \text{O}_2^{-}$**
- **$\downarrow 8\text{-oxoG}$**
- **$\downarrow \text{H}_2\text{O}_2$**
- **$\downarrow \text{Carbonyls}$**
- **$\downarrow \text{lipid and protein peroxidation}$**
- **$\downarrow \Delta p$**
- **$\uparrow 8\text{-oxo}-7,8\text{-dihydro}-2'\text{-deoxyguanosine}$**
lymphocyte proliferation and antigen-specific IgM production were suppressed in the treadmill-trained animals, an adaptation that could increase susceptibility to disease. Clearly, there is a need to be aware of the negative stress-induced changes brought on by forced treadmill running, as they impact other physiological parameters being measured and could complicate data interpretation.

An alternative to forced treadmill running is to place rodents in cages with running wheels and allow them to exercise voluntarily for an extended period of time. This mode of training has been found to increase mean lifespan (32, 43–46, 87) but does not alter maximum life span. Thus, this mode of prevention likely affects disease states, but unlikely has an effect on primary aging. Furthermore, body weights of rats allowed to run voluntarily are lower than those of rats forced to run on a treadmill (87). Narath et al. (87) compared survival of rats that were either allowed to run voluntarily in wheels (24 h access) or forced to run on a treadmill (20 min, twice a day, 5 days a week at a speed of 20 m/min) between the ages of 5 and 23 mo. There was a significant decrease in survival of the rats in the forced treadmill running group (10 deaths, n = 32) compared with rats in the voluntary wheel-running group (2 deaths, n = 32). Furthermore, voluntary wheel running does not cause the negative adaptations associated with chronic stress that forced treadmill running does (84). However, the duration, frequency, and intensity of exercise cannot be controlled with this mode of training, and there are large variations between rodents in the total meters run per day.

In summary, one of the mechanisms by which voluntary exercise extends mean lifespan may be via a reduction in mitochondrial oxidant production. A summary of our findings related to changes in oxidant production, antioxidant enzyme defenses, and oxidative stress in response to lifelong, voluntary wheel running are depicted in Fig. 2. This mechanism is also common to another known life-prolonging intervention, CR. We have previously shown that MnSOD activity and H$_2$O$_2$ production are reduced in SSM from hearts of young animals after 8 wk of CR (52). However, since CR increases maximum life span and voluntary exercise does not, additional mechanisms to explain the life-prolonging effects of CR must also exist.

CR AS A COUNTERMEASURE TO AGE-RELATED MITOCHONDRIAL DYSFUNCTION AND OXIDATIVE STRESS

CR is the only experimental intervention that has consistently been shown to slow the rate of aging and increase mean and maximum lifespan in a variety of species (118, 133, 137). The exact mechanisms through which CR extends lifespan have not yet been established, although there is considerable evidence supporting the role of a reduction in oxidative stress as being causally involved in the anti-aging effects of CR. CR attenuates the age-associated increases in mitochondrial O$_2^-$ and H$_2$O$_2$ production (5–7, 117), lipid peroxidation (65), protein oxidation (71, 95, 96, 117), and oxidative damage to DNA (33, 115).

Several studies have reported that CR decreases mitochondrial oxidant production in the heart (33, 52, 64, 117) but the mechanism underlying this observation remains to be determined. We have previously shown that MnSOD activity and H$_2$O$_2$ production were significantly reduced in SSM from hearts of young animals after 8 wk of CR (45). The simplest explanation, therefore, would be that CR reduces mitochondrial respiration, thereby leading to reduced ROS production. Lambert et al. (64) tested this hypothesis and found that state 4 respiration was not different in heart mitochondria isolated from 6.5- and 17-mo-old rats subjected to 55% CR compared with ad libitum-fed controls. In agreement with this, Gredilla et al. (33) also reported no effect of long-term CR on state 4 respiration in heart mitochondria. Since mitochondrial oxidant production is highest during state 4 respiration (78), it appears that another mechanism must be responsible for reduced mitochondrial oxidant production in calorie-restricted rodents. A recent study by Lambert et al. (65) found that CR caused a significant reduction in liver mitochondrial protonmotive force ($\Delta p$) and H$_2$O$_2$ production, despite the fact that no changes in state 4 respiration were observed. It was determined that the reduction in $\Delta p$ in response to CR arose as a result of decreased substrate oxidation and increased proton leak, and it is plausible to hypothesize that a similar phenomenon occurs in the heart. Currently, in several laboratories, studies are under way to determine the extent of oxidant production under physiological oxygen partial pressure using high-resolution respirometry (31). Although mitochondria are exposed to low oxygen levels within tissues, scientists have routinely studied isolated mitochondria under air-saturated conditions, which may lead to hyperoxia and increased oxidative stress. Therefore, there is a void in the literature regarding the rates of respiration, $\Delta p$, and oxidant production at low physiological partial pressure.

Since mitochondrial oxidant production has been reported to be lower in animals subjected to CR, it is not surprising that CR also leads to less oxidative damage to mtDNA, lipids, and proteins. Damage to mitochondrial DNA is believed to play a major role in determining maximum lifespan, with higher levels of oxidative damage to mtDNA being correlated with shorter lifespans (8). Levels of 8-oxodG are lower in heart mitochondria from CR rats compared with ad libitum fed controls (33, 110) and this may be just one way by which CR extends maximum life span. There are also numerous reports that CR reduces oxidative damage to proteins and lipids within the mitochondria (64, 95, 96, 107) and it is likely that this effect contributes to improved cellular function and prolonged life span (summarized in Fig. 2). However, there is little information available regarding whether CR alters the activities of the various respiratory chain complexes or regarding the effects of CR on SSM and IFM function, and these are important areas for future investigation.

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