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Translational

Translational Article

The Mitochondria's Role in the Aging Process

Original Article

Recovery of Indicators of Mitochondrial Biogenesis, Oxidative Stress, and Aging With (–)-Epicatechin in Senile Mice

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Abstract

There is evidence implicating oxidative stress (OS) as the cause of the deleterious effects of aging. In this study, we evaluated the capacity of the flavanol (–)-epicatechin (Epi) to reduce aging-induced OS and restore mitochondrial biogenesis, as well as, structural and functional endpoints in aged mice. Senile (S; 26-month-old) C57BL/6 male mice were randomly assigned to receive either water (vehicle) or 1 mg/kg of Epi via oral gavage (twice daily) for 15 days. Young (Y; 6-month-old) mice were used as controls. In S brain, kidney, heart, and skeletal muscle (compared with Y animals) an increase in OS was observed as evidenced by increased protein-free carbonyls and decreased reduced glutathione levels as well as sirtuin 3, superoxide dismutase 2, catalase, thioredoxin and glutathione peroxidase protein levels. Well-recognized factors (eg, sirtuin 1) that regulate mitochondrial biogenesis and mitochondrial structure- and/or function-related endpoints (eg, mitofilin and citrate synthase) protein levels were also reduced in S organs. In contrast, the aging biomarker senescence-associated β-galactosidase was increased in S compared with Y animals, and Epi administration reduced levels towards those observed in Y animals. Altogether, these data suggest that Epi is capable of shifting the biology of S mice towards that of Y animals.

Key Words: (–)-Epicatechin—Oxidative stress—Mitochondrial biogenesis—Senescence-associated β-galactosidase

Limiting and/or preventing the detrimental effects of aging through safe and effective means have proven to be an elusive goal (1). It is clear that in addition to moderate exercise, a healthy diet can contribute to decreasing aging-related conditions and/or diseases. It is believed that specific components of natural products may underlie such effects (2–4). A notable example for "health-promoting"

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compounds is resveratrol, which is found in the skin of grapes and has been suspected of mediating some of the salutary effects of red wine (5). Other examples are those associated with the effects of olive and fish oils (oleic and omega fatty acids, respectively) (6).

Excess (ie, net) levels of reactive oxygen species (ROS) is thought to be central to the deterioration of cell (ie, organ) function (7,8). When their excess production cannot be modulated by natural antioxidant systems, ROS can alter DNA, lipid, protein, and other molecules and contribute to the loss of their optimal function (9). With aging, the levels of natural buffering systems (eg, intracellular glutathione, thioredoxin [TRX]) can be significantly reduced, thereby limiting the capacity of tissues and organs to buffer excess of ROS (10). Most estimates suggest that the majority of intracellular ROS production is derived from the mitochondria. Mitochondrial respiratory transport chain generates superoxide anion that is released into the mitochondrial matrix, where it is catabolized by superoxide dismutase 2 (SOD2) to oxygen and hydrogen peroxide (11). Hydrogen peroxide is further metabolized into water and oxygen via catalase and glutathione peroxidase (GPx) (11). Crucial to the "buffering" of excess ROS is the maintenance of optimal activity levels of these enzymes and others including TRX an oxidoreductase enzymes (12). Interestingly, recent reports suggest that sirtuin 3 (SIRT) mediates the induction of antioxidant defenses during calorie restriction (13).

Excess ROS can contribute to aging via the accumulation of mitochondrial DNA mutations, which compromise optimal organelle function (7,8). Aging is also associated with a lower renewal of mitochondria (ie, impaired mitochondrial biogenesis [MB]), which further limits the organelle's ability to produce sufficient amounts of ATP so as to maintain optimal cell health (14,15). Aging-associated impaired MB has been associated with reduced levels and activity of key regulatory factors such as sirtuins, peroxisome proliferator-activated receptor γ, coactivator 1 α (PGC1α), nuclear respiratory factor-2 (NRF2), and mitochondrial transcription factor A (TFAM) (16–18). Moreover, age-related alterations in mitochondria have been reported to occur in its internal (ie, mitofilin, complexes I and V) and outer membrane (ie, porin) components, which can have considerable impact on the activity of the organelle (14,19,20). Importantly, the promotion of MB and an adequate function of the organelle are critical to counteract aging.

The consumption of moderate amounts of cocoa products has been linked (thorough epidemiological studies) with important salutary effects. Kuna Indians, which live in the San Blas islands off the coast of Panama, are known to consume on the daily basis a cacao beverage and have very low indices of cardiovascular-, cancer-, and diabetes-associated mortality (21). We recently reported on the unique capacity of flavanol-rich cocoa to facilitate the recovery of skeletal muscle mitochondria structure and function in patients with heart failure and type 2 diabetes mellitus (22). In rodent studies, these effects can be replicated by using the most abundant flavanol present in cocoa, (−)-epicatechin (Epi) (23,24).

Given the aforementioned evidence for beneficial effects in humans and pre-clinical models, the purpose of this study was to compare the levels of ROS buffering systems and MB- and/or structure-related proteins in senile (S) (26-month-old) mice skeletal muscle, heart, kidney, and brain versus young (Y) (6-month-old) animals, and to explore the capacity of 2-weeks treatment of Epi to favorably affect age-related changes in mice.

Materials and Methods

Animal Studies

C57BL/6 male mice were divided into three groups. Group one, Y mice (6-month-old), $n = 5$; group two, S mice (26-month-old), $n = 5$; and group three, S mice treated with Epi, $n = 5$. Groups one and two were treated with vehicle (water). Group three was treated by gavage for 2 weeks with Epi 1 mg/kg of body weight as described previously (23). At the end of the treatment period, frontal cortex (brain), kidney, heart, and quadriceps (SkM) were collected and stored at −80°C until used. All animal procedures were approved by the UCSD's Institutional Animal Care and Use Committee.

Measurement of Reduced (GSH) and Oxidized (GSSG) Glutathione

Tissue samples (25 mg) were homogenized with a polytron in 250 μL of cold buffer (50 mM potassium phosphate, pH 7, containing 1 mM EDTA), centrifuged at 10,000*g* for 15 minutes at 4°C. The supernatants were deproteinated and used to measure glutathione (GSH) and oxidized glutathione (GSSG) using a colorimetric detection assay kit according to the manufacturer's instructions (Cayman Chemicals; intra-assay coefficient of variation of 1.6%). All samples were tested in duplicates and measured at room temperature.

Catalase Activity

Tissue samples (25 mg) were homogenized with a polytron in 250 μL of cold buffer (50 mM potassium phosphate, pH 7.4 containing 1 mM EDTA), centrifuged at 10,000*g* for 15 minutes at 4°C. The supernatants were used to measure catalase activity using a colorimetric kit according to the manufacturer's instructions (Cayman Chemicals; intra-assay coefficient of variation of 3.8%). All samples were tested in duplicates and measured at room temperature.

Citrate Synthase Activity

Tissue samples (25 mg) were homogenized with a polytron in 250 μL of cold extraction buffer (20 mM Tris–HCl, 140 mM NaCl, 2 mM EDTA, and 0.1% sodium dodecyl sulfate) with protease inhibitors (P2714, Sigma-Aldrich), 5 mM Na_3VO_4, and 3 mM NaF. Homogenates were centrifuged at 10,000*g* for 15 minutes at 4°C. Supernatants were recovered and used to measure citrate synthase (CS) as described previously (23), according to the technique of Srere (1969). All samples were tested in duplicates and measured at room temperature.

Protein Carbonylation

Tissue samples (50 mg) were homogenized in 500 μL of cold buffer (50 mM 4-morpholineethanesulfonic acid, pH 6.7, containing 1 mM EDTA). Homogenates were centrifuged at 10,000*g* for 15 minutes at 4°C. Supernatants were recovered and incubated at room temperature for 15 minutes with streptomycin sulfate at a final concentration of 1%. Samples were centrifuged at 6,000*g* for 10 minutes at 4°C. Total protein carbonylation was measured in supernatants using a colorimetric protein carbonyl assay kit according to the manufacturer's instructions (Cayman Chemicals; intra-assay coefficient of variation of 4.7%). All samples were tested in duplicates at room temperature.

Antibodies

The primary antibodies SOD2, TRX, GPx, and PGC1α were from Abcam. Catalase, SIRT3, NRF2, and glyceraldehyde-3-phosphate dehydrogenase were from Cell Signaling. SIRT1 and TFAM were from Sigma-Aldrich. Mitofilin, mitochondrial oxidative phosphorylation complexes I and V as well as porin were from MitoSciences. Senescence-associated β-galactosidase (SA-β-gal) was from Millipore. Anti-rabbit and anti-mouse horseradish peroxidase-conjugated secondary antibodies were from Cell Signaling.

Western Blotting

Tissue samples (25 mg) were homogenized with a polytron in 250 µL of lysis buffer (1% Triton X-100, 20 mM Tris, 140 mM NaCl, 2 mM EDTA, and 0.1% sodium dodecyl sulfate) with protease inhibitors (P2714; Sigma-Aldrich), 5 mM Na_3VO_4, and 3 mM NaF. Homogenates were sonicated for 25 minutes (brain 15 minutes) at 4°C and centrifuged (10,000*g*) for 10 minutes at 4°C. The total protein content was measured in the supernatant using the Bradford method. A total of 40 µg of protein were loaded onto a 4%–15% precast polyacrylamide gels, electrotransferred to a vinyl membrane using a semidry system. Membranes were incubated for 1 hour in blocking solution (5% nonfat dry milk in Tris-buffered saline plus 0.1% Tween 20), followed by 3 hours of incubation at room temperature with primary antibodies. Membranes were washed (3× for 5 minutes) in Tris-buffered saline plus 0.1% Tween 20 and incubated for 1 hour at room temperature in the presence of horseradish peroxidase-conjugated secondary antibodies diluted 1:10,000 in blocking solution. Membranes were again washed 3× in Tris-buffered saline plus 0.1% Tween 20, and the immunoblots were developed using an enhanced chemiluminescence plus detection kit (Amersham-GE). The band intensities were digitally quantified using ImageJ software.

Data and Statistical Analysis

For all blot images, multiple exposures were obtained to ensure that the data obtained from computer-assisted image analysis was within the linear range. Data are expressed as mean ± standard deviation. Statistical analysis of data was performed using one-way analysis of variance followed by the Tukey post hoc test or Student's *t* test, as appropriate. Statistical significance was defined when $p < .05$.

Results

GSH/GSSG and Protein Carbonylation Levels

The ratio of GSH/GSSG levels in the different organs examined was significantly reduced in S compared with Y mice (Figure 1A). In S, Epi treatment completely restored GSH/GSSG levels in kidney and SkM compared with Y. In brain and heart, Epi treatment partially restored GSH/GSSG levels. Total protein carbonylation as a surrogate of oxidized proteins was increased in all organs examined in S (Figure 1B), and Epi treatment reduced significantly protein carbonylation levels in S brain and SkM. In kidney and heart, Epi reduced protein carbonylation with a trend towards significance.

Modulators of Antioxidant Defense: SIRT3, SOD2, Catalase, TRX, and GPx

All the examined protein levels were lower in S compared with Y. In S brain, Epi treatment partially increased SIRT3, TRX, and GPx; on the other hand, SOD2 and catalase protein levels increased to levels found in Y (Figure 2A and B). In kidney, Epi partially increased SIRT3, catalase, TRX, and GPx protein levels with no effects observed in SOD2 (Figure 2A and B). In heart, Epi partially increased SIRT3, SOD2, catalase, and GPx protein levels, with no effects observed in TRX (Figure 2A and B). In SkM, Epi treatment increased SIRT3 levels to those found in Y, whereas SOD2, catalase, and TRX protein levels were partially increased, with no effects observed in GPx (Figure 2A and B).

Modulators of MB: SIRT1, PGC1α, NRF2, and TFAM

In order to study the role of Epi on MB during the aging process, we evaluated well-recognized factors, which regulate the process. In S, all organs examined showed a decrease in SIRT1, PGC1α, NRF2, and TFAM protein levels compared with Y (Figure 3A and B). In S brain, Epi treatment partially increased SIRT1, PGC1α, NRF2, and TFAM protein levels towards Y levels (Figure 3A and B). In kidney, Epi increased SIRT1 and PGC1α to Y levels with a partial increase in NRF2 and TFAM protein levels (Figure 3A and B). In heart, Epi treatment increased SIRT1 and TFAM, with partial increases in PGC1α and NRF2 protein levels (Figure 3A and B). In SkM, Epi treatment increased PGC1α and only partially SIRT1, NRF2, and TFAM protein levels (Figure 3A and B).

Mitochondrial Proteins: Mitofilin, Complexes I and V, and Porin

We investigated the impact that aging and Epi treatment have on mitochondrial proteins by measuring levels of validated markers of the mitochondrial inner (mitofilin, complexes V and I) and outer membrane (porin). The levels of these mitochondrial proteins were reduced in all S organs compared with Y (Figure 4A and B). Porin protein levels were not reduced in heart. In S brain, Epi treatment increased complex V and porin to Y levels, but induced only a partial increase in mitofilin and complex I protein levels (Figure 4A and B). In kidney, Epi treatment increased complex I, complex V, and porin, but it partially increased mitofilin protein levels (Figure 4A and B). In heart, Epi treatment restored complexes I and V protein levels towards those found in Y, and mitofilin was partially increased (Figure 4A and B). In SkM, Epi treatment completely increased complex I protein levels, while mitofilin and complex V were partially increased (Figure 4A and B).

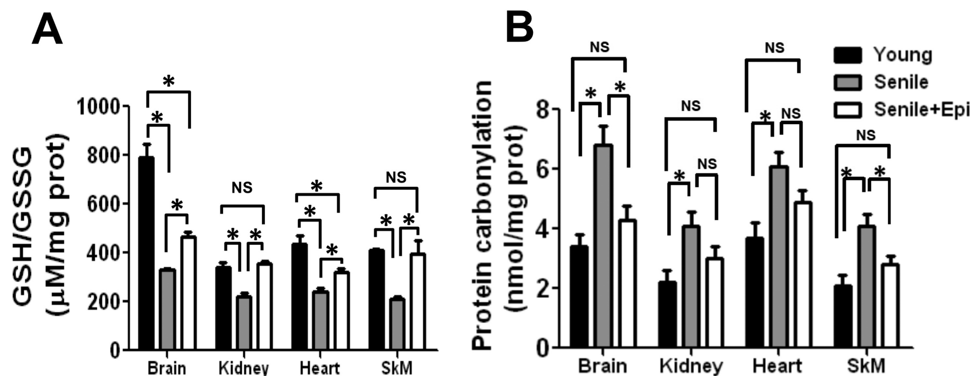

Figure 1. Oxidative stress regulator levels in young, senile, and senile mice treated with (–)-epicatechin (Epi). (**A**) Changes observed in the ratio of reduced glutathione to oxidized glutathione (GSH/GSSG). Lower GSH/GSSG ratios are an indication of increased oxidative stress. (**B**) Changes observed in total protein carbonylation levels. Values were normalized by protein in the samples ($n = 5$/group, $*p < .05$).

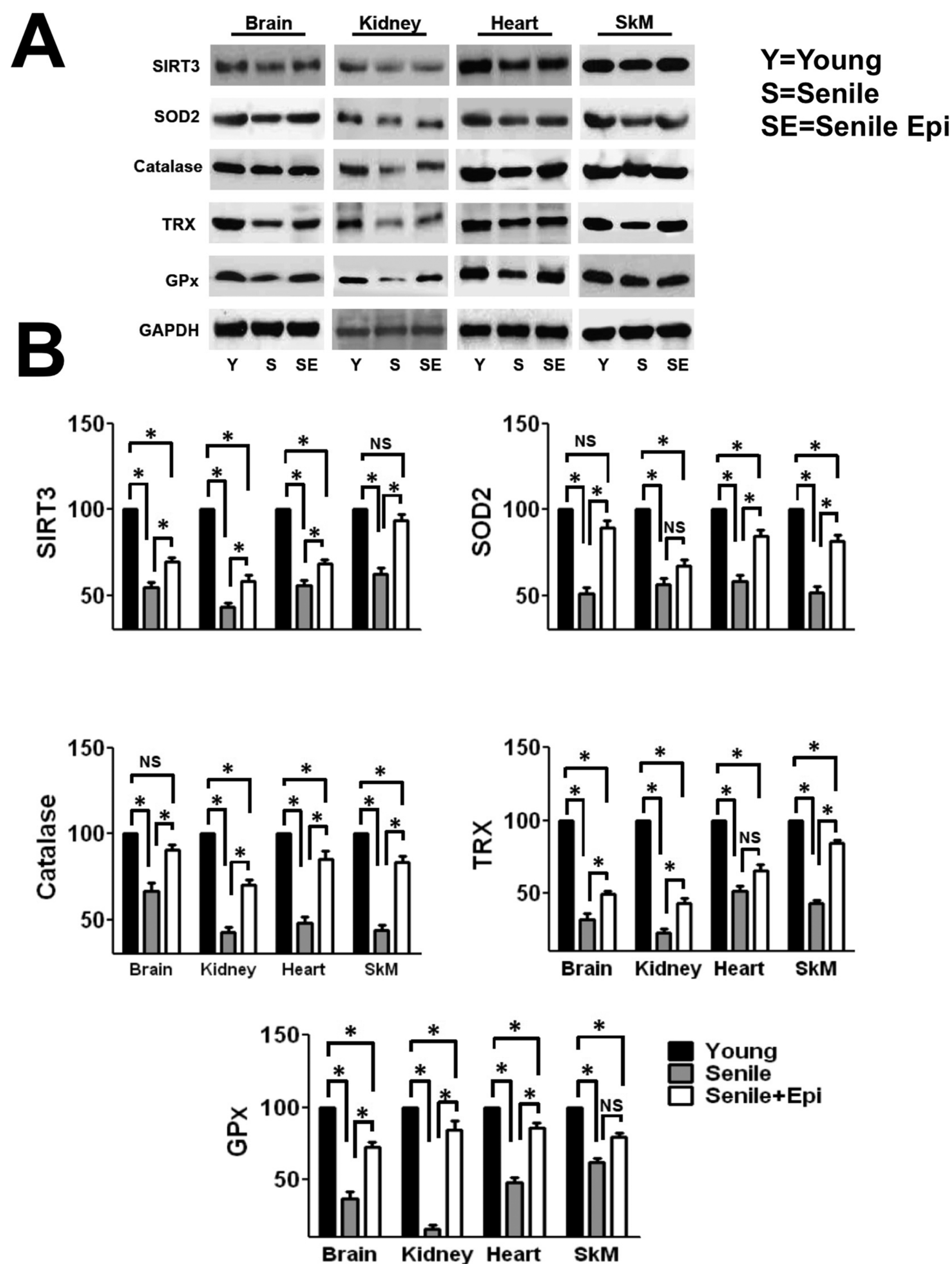

Figure 2. Antioxidant defense-related protein levels in young (Y), senile (S), and S mice treated with (–)-epicatechin (Epi). (**A**) Representative Western blot images observed in SIRT3, SOD2, catalase, thioredoxin (TRX), and glutathione peroxidase (GPx) protein levels. (**B**) Changes observed in proteins of interest in S and Epi-treated S mice compared with Y mice (set as 100%). Protein levels were normalized using glyceraldehyde-3-phosphate dehydrogenase values ($n = 5$/group, $*p < .05$).

CS Activity

We evaluated changes in CS as a surrogate of mitochondrial function. Results demonstrated reduced levels of CS in all S organs compared with Y (Figure 5A). In S mice, Epi treatment partially restored CS in all organs compared with Y (Figure 5A).

Catalase Activity

To assess the negative impact that aging has on antioxidant enzyme activity, we evaluated catalase activity. Catalase activity was reduced in all S organs compared with Y (Figure 5B), recapitulating the observed pattern in catalase protein levels in S mice (Figure 2A and B). In S brain and heart, Epi treatment totally restored enzyme activity levels towards those observed in Y (Figure 5B). In S kidney and SkM, Epi treatment partially restored enzyme activity (Figure 5B).

SA-β-gal

As SA-β-gal is a well-recognized marker of aging, we evaluated its protein levels in the animals studied. In brain, kidney, and heart, SA-β-gal protein levels were increased in S compared with Y (Figure 6). In S, Epi treatment led to a significant reduction in SA-β-gal protein levels in all organs analyzed (Figure 6).

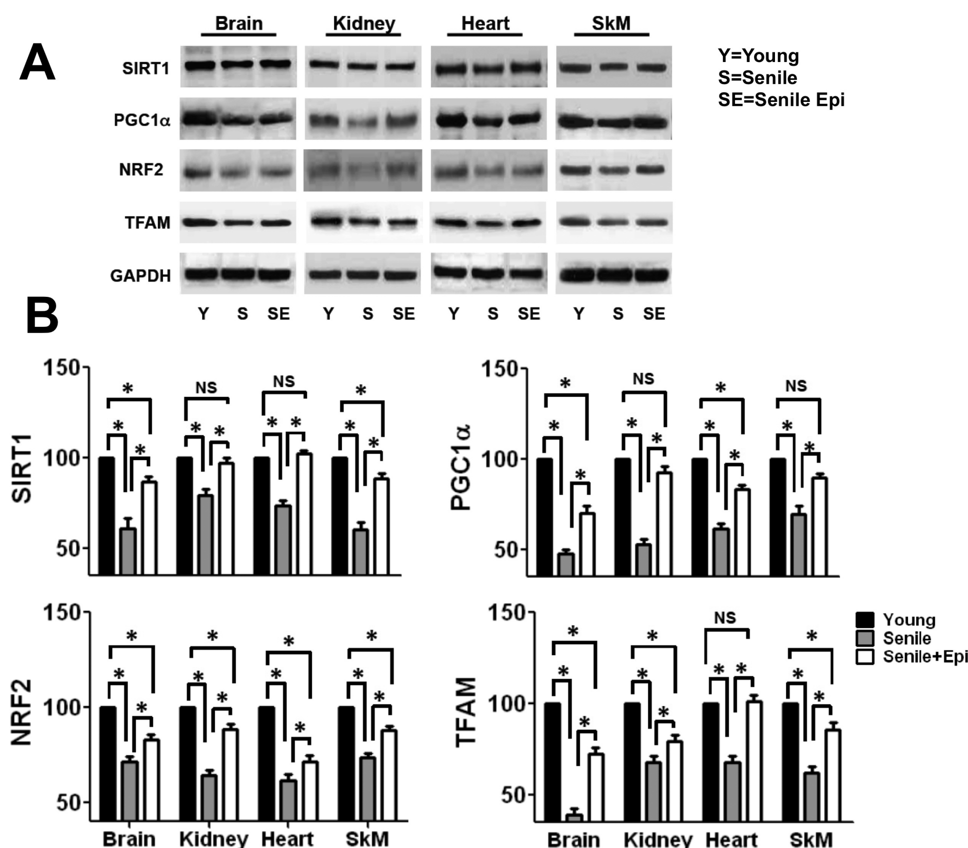

Figure 3. Mitochondrial biogenesis protein-regulator levels in young (Y), senile (S), and S mice treated with (−)-epicatechin (Epi). (**A**) Representative Western blot images observed in SIRT1, peroxisome proliferator-activated receptor γ, coactivator 1 α (PGC1α), nuclear respiratory factor-2 (NRF2), and mitochondrial transcription factor A (TFAM) protein levels. (**B**) Changes observed in proteins of interest in S and Epi-treated S mice compared with Y mice (set as 100%). Protein levels were normalized using glyceraldehyde-3-phosphate dehydrogenase values (n = 5/group, $*p < .05$).

Discussion

Results from this study demonstrate the unique ability of Epi to improve the levels of several key recognized regulators of oxidative stress (OS) in multiple organs of S mice. Epi effects include the improvement of OS surrogates and restoration of ROS buffering system towards those of Y cohorts. Regulatory factors involved in MB and mitochondrial proteins were also restored in most S organs with treatment. Moreover, Epi treatment also improved mitochondrial and antioxidant enzyme activity as measured by CS and catalase activity in S mice. As a result of Epi treatment, a decrease in protein levels of the well-defined indicator of tissue aging SA-β-gal was observed, likely reflecting a "global" shift towards younger organ biology. In particular, novel aspects of this study are that the flavanol Epi is capable of reverting the deleterious effects that aging has on S mice organs and that in comparison with other polyphenols, Epi exerts the aforementioned effects at low doses.

A major goal of modern medicine is to reduce or mitigate aging-induced increases in OS. Together with moderate exercise, healthy eating habits, which include the modest consumption of natural products such as fruits, oils, and wine, appear to offer one of the best solutions to achieve this goal (25). The use of antioxidant supplementation has also been explored. Nonetheless, large-scale clinical trials of "antioxidant" therapies, such as vitamin C, vitamin E, or β-carotene, have not demonstrated satisfactory benefits to patients and in some cases appear to be harmful (26). In contrast, epidemiological studies suggest that regular consumption of products rich in flavonoids is associated with a decreased cardiometabolic and other chronic disease risk (27,28). This is particularly true for cocoa products, which contain high amounts of Epi. Notably, Kuna Indians who regularly consume a cacao-based beverage are protected against age-dependent reductions in vascular and renal function (12,21) and demonstrate extremely low mortality indices for chronic diseases (29). In support of these observations, clinical studies have demonstrated reductions in blood and organ OS levels with cocoa and/or dark chocolate consumption (30).

The brain is a highly metabolically active organ and as such very susceptible to damage from excess ROS (31). Multiple studies suggest that age-associated, chronic neurodegenerative diseases like Alzheimer's and Parkinson's are partly due to long-standing OS (32,33). Here, we demonstrate that 2 weeks of Epi treatment of S mice reduces brain OS levels as determined by protein-free carbonyl content while partly restoring the GSH/GSSG levels towards those noted for Y animals. The deacetylase SIRT3 primarily located in mitochondria appears to play an important role on the regulation of ROS buffering systems. A major target of SIRT3 deacetylation is SOD2 (13). Epi treatment increased SIRT3, SOD2, and catalase protein levels in aged brain suggesting a possible means to partly mitigate excess ROS. Significant increases were also observed for GPx and TRX. There is precedent for the beneficial effects of flavonoids on OS. In a recent study 14-month-old female mice were given green tea catechins in

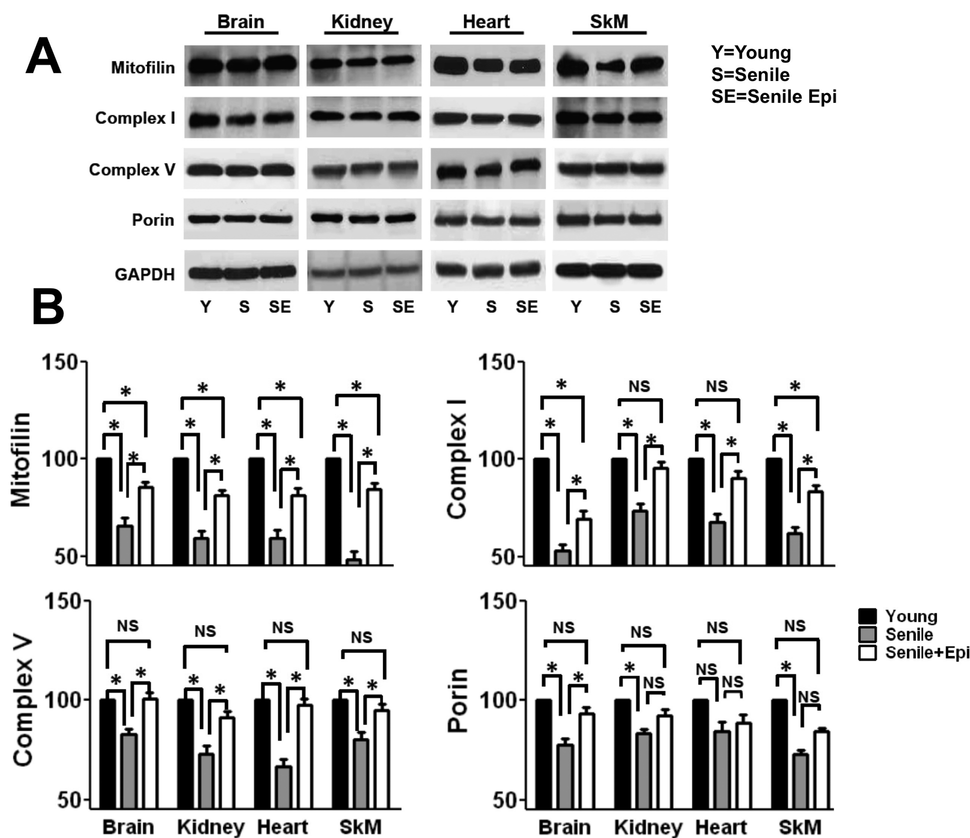

Figure 4. Mitochondrial protein levels in young (Y), senile (S), and S mice treated with (–)-epicatechin (Epi). (**A**) Representative Western blot images observed in mitofilin, complexes I and V, and porin protein levels. (**B**) Changes observed in proteins of interest in S and Epi-treated S mice compared with Y mice (set as 100%). Protein levels were normalized using glyceraldehyde-3-phosphate dehydrogenase values (n = 5/group, *p < .05).

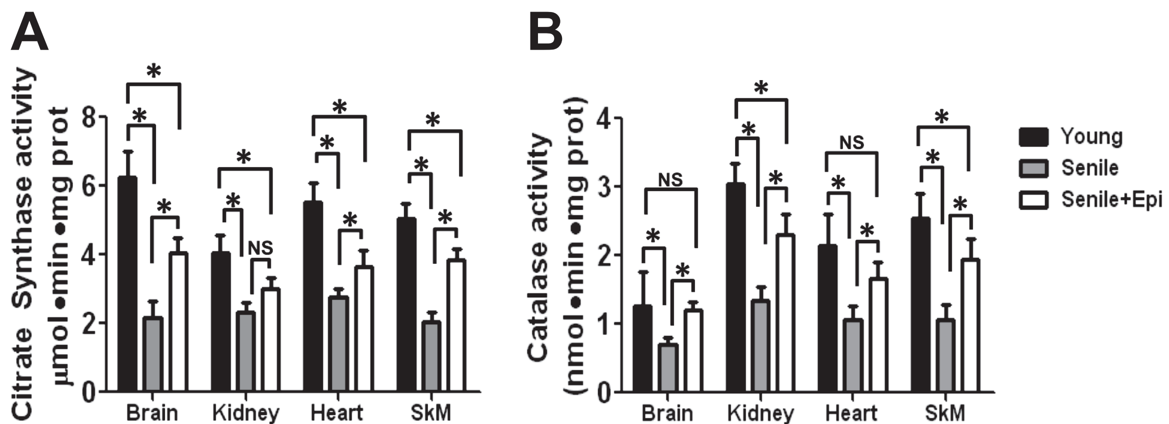

Figure 5. Citrate synthase (CS) activity and catalase activity in young, senile, and senile mice treated with (–)-epicatechin (Epi). (**A**) Changes observed in CS. (**B**) Changes observed in catalase activity. Enzyme activity was normalized by protein in the samples. (n = 5/group, *p< .05).

drinking water for 6 months. Tea catechin supplementation prevented decreases in total SOD and GPx activities in serum as well as reduced protein carbonyl content in the hippocampus (34). These results support the concept that long-term supplementation with catechins may indeed ameliorate aging-induced increases in brain OS levels.

Age-associated loss of kidney function has been recognized for decades. With aging, many subjects exhibit progressive decreases in glomerular filtration rate and renal blood flow (35). Most of these changes are often related to and may be caused by OS (36). In the kidney, Epi treatment of S mice resulted in a complete recovery of GSH/GSSG levels and an amelioration of protein carbonyls whose levels were comparable with those of Y mice. With the exception of SOD2, levels all other regulators were significantly improved. These results are similar to those reported by Tanabe and colleagues (37), whereby Epi treatment prevented cisplatin-induced increases in kidney OS.

Cardiac aging in mice closely recapitulates the human condition, which includes chamber hypertrophy, fibrosis, and diastolic

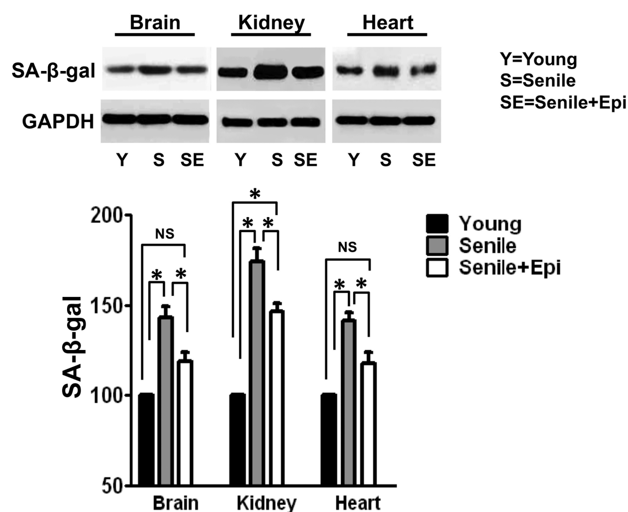

Figure 6. Senescence-associated β-galactosidase (SA-β-gal) protein levels in young (Y), senile (S), and S mice treated with (–)-epicatechin (Epi). (**A**) Representative Western blot images observed in SA-β-gal protein levels. (**B**) Changes observed of SA-β-gal protein levels in S and Epi-treated S mice compared with Y mice (set as 100%). Protein levels were normalized using glyceraldehyde-3-phosphate dehydrogenase values. (n = 5/group, $*p < .05$).

dysfunction, whereby OS appears to play a central role in mediating these changes (38). In myocardium, Epi treatment restored GSH/GSSG levels and reduced protein carbonyls and with the exception of TRX was accompanied by significant increases in ROS regulator protein levels. The protective role played by these OS regulator systems in the heart was documented in cardiomyocyte SOD2-deficient mice, which develop over time heart failure and die at about 6 months of age (39). In these mice, the administration of apple polyphenols rich in procyanidins and catechins increased survival by approximately 30% and mitigated the severity of heart failure (40).

One of the most significant effects of aging is loss of SkM mass and function, known as sarcopenia, a process that appears partly dependent on OS (41). In agreement with other reports (42,43), we documented increases in SkM OS and reductions in ROS regulatory systems in S mice. The administration of Epi reversed these changes towards those of Y mice. In agreement with these changes, using a high-fat diet mouse model of obesity and insulin resistance, we previously reported increases in SkM protein carbonylation and nitrotyrosilation, as well as a decrease in SOD2 and catalase activity. Two weeks of Epi treatment reversed such changes towards those of normal mouse muscle (44). Thus, Epi appears capable of protecting SkM from OS under a variety of "pathological" conditions.

During the process of aging, the abundance, morphology, and functional properties of mitochondria decay leading to mitochondrial dysfunction (15). Despite age-associated mitochondrial dysfunction being extensively investigated, the precise mechanisms remain to be elucidated. However, these mechanisms may include decreased mitochondrial DNA repair, defective mitochondrial removal, and decreased MB (14). Accumulating evidence suggest that SIRT1 and PGC1α play a key role as upstream activators of MB. SIRT1 regulates PGC1α through a functional protein–protein interaction (45), and once PGC1α is activated, it works in concert with several transcription factors to activate the expression of a broad set of mitochondrial genes (46). These transcriptions factors include NRF1 and NRF2 whose activation increases the expression of many mitochondrial proteins, including oxidative phosphorylation complexes and TFAM (47). Reduced levels of PGC1α have been found in aged mice (42), and loss of PGC1α has been suggested to be an important contributor to mitochondrial dysfunction in aging-associated diseases (48). Therefore, we examined the effects of Epi treatment on protein levels of SIRT1 and PGC1α in various organs from S mice.

In agreement with previous reports (42,49,50) we document reduced SIRT1, PGC1α, NRF1, and TFAM protein levels in brain, kidney, heart, and SkM from S mice compared with Y mice. Several studies have evidenced the importance of the SIRT1-PGC-1α-NRF1-TFAM pathway in the process of MB and aging. In a recent study using brain-specific SIRT1-overexpressing transgenic mice, it was demonstrated that SIRT1 extends life span and delays aging in mice (51). The polyphenol resveratrol has also been shown to exert protective effects in aged animals by augmenting SIRT1 activity. However, higher doses than those used for Epi were administered (52). In S mice, we document reduced mitochondrial protein content in all the organs examined suggesting lower mitochondrial mass compared with Y cohorts. Interestingly, 2 weeks of Epi treatment reverted this reduction towards those levels noted for Y animals. Previously, we have shown that 15 days of treatment with Epi resulted in the increase in complexes I, II, III, and V, mitofilin, porin, and TFAM protein levels as well as mitochondrial volume and cristae abundance in hind limb and cardiac muscles from middle-aged mice (12-month-old) (23). Therefore, Epi at low dose appears capable of preserving mitochondrial structure and/or function from the detrimental effects of aging.

In addition to OS, many mechanisms have been proposed to contribute to the process of aging (53). Cellular senescence has been hypothesized as contributing to age-associated tissue dysfunction, reduced regenerative capacity, and disease (53). Senescent cells have been shown to increase with aging in multiple organs and tissues (54,55). This is further reinforced by tracking the levels and/or activity of the pH-dependent SA-β-gal, which is widely used as a biomarker of cellular senescence (55). In agreement with other reports, we document increased SA-β-gal protein levels in S mice. A recent study also noted higher expression of SA-β-gal in hippocampal pyramidal cells (CA3 region) from 24-month-old rats compared with Y rats (56). These neurons, in particular, have been shown to be most vulnerable to OS (57) and are functionally related to age-related memory loss and cognition disorders (58). In another study, SA-β-gal gradually increased in renal tubules as mice aged (59), as we previously reported for SkM (60); Epi treatment was able to revert the increased SA-β-gal protein levels in the organs examined.

Taken together, findings from this study demonstrate that Epi shifts mitochondrial-related biology of S mice towards that of Y animals. Favorable changes are observed on antioxidant defense systems, MB and/or structure and activity, as well as in SA-β-gal levels. In addition, Epi effects were not universal in magnitude in all organs analyzed; some variability may be related to differential effects ascribed to tissue-specific gene expression and/or length of treatment necessary to evoke changes in the endpoints examined. This study also demonstrates that the naturally occurring flavonoid Epi provided at low dose has the potential to reduce many of the negative consequences of aging in multiple organs and/or tissues, with the prospect of yielding an overall improvement in health. These results warrant the implementation of rigorous clinical studies.

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Conflict of Interest

Dr. Villarreal is a co-founder of Cardero Therapeutics and Dr. Ceballos is a stockholder of the company.

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