Mechanisms Underlying Caloric Restriction and Lifespan Regulation: Implications for Vascular Aging

Zoltan Ungvari, Cristina Parrado-Fernandez, Anna Csiszar, Rafael de Cabo

Abstract—This review focuses on the emerging evidence that attenuation of the production of reactive oxygen species and inhibition of inflammatory pathways play a central role in the antiaging cardiovascular effects of caloric restriction. Particular emphasis is placed on the potential role of the plasma membrane redox system in caloric restriction–induced pathways responsible for sensing oxidative stress and increasing cellular oxidative stress resistance. We propose that caloric restriction increases bioavailability of NO, decreases vascular reactive oxygen species generation, activates the Nrf2/antioxidant response element pathway, inducing reactive oxygen species detoxification systems, exerts antiinflammatory effects, and, thereby, suppresses initiation/progression of vascular disease that accompany aging. (Circ Res. 2008;102:519-528.)

Key Words: aging ■ antioxidants ■ caloric restriction ■ nuclear receptors ■ redox

Almost a century ago, Moreschi and Rous published separately their observations on the impact of underfeeding laboratory animals on transplanted and induced tumors.1,2 Two decades later, McCay et al first observed lifespan extension in laboratory rats maintained on a caloric restriction (CR) diet.3 Since then, CR has been studied intensively, with consistent results showing its beneficial effects on longevity, age-associated diseases, attenuation of functional declines, and carcinogenesis across a broad variety of species and diet formulations.4–5 Despite these observations, the precise mechanism(s) underlying the effects of CR protection and lifespan extension remains unknown. It is safe to say that CR reduces metabolic rate and oxidative damage improves markers of diabetes such as insulin sensitivity. CR decreases the incidence of cardiovascular disease and has been shown to alter neuroendocrine and sympathetic nervous system in laboratory animals, and some of these are replicating now in ongoing human studies. In particular, the National Institute on Aging, through its program, CALERIE (Comprehensive Assessment of Long-Term Effects of Reducing Intake of Energy, initiated in 2002) endeavors to fund clinical trials that address the feasibility of using CR as a therapeutic tool as well as its effects and mechanisms in disease prevention. CALERIE studies examine the delay of aging-related comorbidities, particularly those associated with metabolic rate and biomarkers of aging, studying those that predict...
oxidative stress as an early stage in its development, as confirmed by a decrease in antioxidant defenses and an increase in oxidative damage.20,21 Aging is also associated with changes in levels of antioxidant capacity and oxidative damage, ostensibly leading to mitochondrial impairment. These changes have been coupled to increased oxidative damage to DNA, lipids,26,27 and proteins.23,28–30 Accumulation of mitochondrial DNA mutations, commonly identified in age-related diseases, induce impairments of mitochondrial complexes,31–33 including mitochondrial complex III activity in the aged heart.34 Impaired mitochondrial function causes a shortage of ATP supply, resulting in induction of further problems in biochemical pathways.31 The free radical theory of aging35,36 has generated considerable interest regarding the search for possible biochemical bases of aging processes. Many past studies have shown that CR decreases production of ROS production thus minimizing oxidative damage.77,38 These studies have lead collectively to the hypothesis that CR by reducing oxidative stress extends the lifespan. The mitochondrial79 and plasma40 membranes are sites of active and abundant ROS production and thus are at high risk of ROS damage. Therefore, it follows that a central mechanism for the actions of CR may involve membrane alterations that either reduce ROS production or resist oxidative damage.

It has been proposed that lifespan is inversely related to the degree of membrane phospholipid unsaturation41,42 and that elucidation of this relationship can provide insight on the mechanism for lifespan extension with CR.43 Modulation of membrane susceptibility to peroxidation, however, may be too simplistic to explain aging processes because this hypothesis, for the most part, does not consider other membrane-associated processes. Such processes include changes in cellular signaling, leakage of protons (and other ions),44 production of ROS,39 induction of apoptosis,45 and maintenance of antioxidant systems.46–49 Membrane-induced alterations in any of these processes could have major consequences that influence oxidative stress and lifespan.

CR Increases Coenzyme Q–Dependent Reductases in Plasma Membranes

Coenzyme (Co)Q contributes to stabilize plasma membrane, regenerates antioxidants such as ascorbate and α-tocopherol, and regulates the extracellularly induced ceramide-dependent apoptosis pathway.49,50 NAD(P)H-dependent reductases act at the plasma membrane to regenerate CoQH₂, contributing to maintain its antioxidant properties. As a whole, both CoQ and its reductases (Figure 1) constitute a transplasma membrane antioxidant redox system responsible of the above described functions.51–53 The aforementioned antioxidants are maintained in their reduced forms at the plasma membrane by different CoQ-dependent reductases, NADH-dependent cytochrome b₅ reductase53 and NAD(P)H:quinone-oxidoreductase-1 (NQO1).55 Different dietary modifications can modulate these enzyme activities to protect the plasma membrane.49,57,58 Our previous work has shown that these 2 enzyme activities are increased in plasma membranes from rat and mouse tissues under long-term CR compared with ad libitum conditions.46–48 Increases in the activities of these enzymes are attributable to enhanced concentration of these proteins at the plasma membrane.49,57,58 Both enzyme activities are known to be present in the cardiovascular system,59–62 and we posit that they are regulated by CR in a similar manner. Data from our laboratories and others provide support that the plasma membrane redox system is, at least in part, responsible of the maintenance of the antioxidant capacity during oxidative stress challenges induced by the diet and aging. The upregulation of the plasma membrane redox system that occurs

Figure 1. A diagram of the plasma membrane redox system. The redox cycle is shown in blue. CoQ indicates oxidized form of coenzyme Q; CoQ·¹, semiquinone radical; CoQH₂, reduced form of coenzyme Q; NQO1, NADH-quinone oxidoreductase. Modified from Hyun et al.46
during CR decreases the levels of oxidative stress in aged membranes. CR modifies composition of fatty acid in the plasma membrane, resulting in decreased oxidative damage including lipid peroxidation. More importantly, plasma membrane redox activities and also the content of CoQ, which decline with age, are enhanced by CR, providing protection to phospholipids and preventing the lipid peroxidation reaction progression.46–48,64

The plasma membrane also contributes to the regulation of the cellular redox homeostasis through the maintenance of NAD(P)H/NAD(P) ratio. This function is driven in cooperation with mitochondria, an interaction particularly observed in ρ0 cells.48,68,69 The ratio of pyridine nucleotides is an important regulator of yeast lifespan, as well as the establishment of respiration.70 The ratio of NAD+/NADH is also an important regulator of the deacetylase activity of Sir2, an enzyme involved in the regulation of lifespan in yeast. We and others have shown that expression of mammalian Sir2 (SIRT1) is induced under CR in laboratory animals and humans, as well as in cells in culture that are treated with serum from CR animals.11,47,71–73 As we have indicated above, CR increases the activity of NAD(P)H-dependent reductases in the plasma membrane and CoQ, which likely contributes to the regulation of the NAD(P)H/NAD(P) ratio. Because NADH and NADPH are substrates for NAD(P)H oxidases, the availability of these electron donors also influences the generation of ROS by these enzymes.38 There is increasing evidence for age-related upregulation of NAD(P)H oxidases in the cardiovascular system; however, neither the role of CR-induced alterations in NAD(P)H/NAD(P) ratio in modulation of NAD(P)H oxidase activity nor the role of the plasma membrane redox system in this process is well understood. Plasma membrane-associated redox system and mitochondria are the major source of ROS in cells, which are generated mainly when CoQ-dependent electron transport is disrupted.77,78 Aging is associated with increased rates of stress-induced apoptosis in multiple organs,77 including an increased rate of endothelial apoptosis.75,78 CR promotes the activation of stress response genes and attenuates stress-induced apoptosis by inducing SIRT1.72–74 Ceramide is a major signal molecule that mediates stress responses and induces apoptosis through the activation of caspases.81 We have previously shown that CoQ within plasma membranes prevent the cytosolic accumulation of ceramide by inhibiting the neutral sphingomyelinase present in membranes.50,82 It is conceivable that changes in CoQ concentration observed in liver plasma membrane induced by CR (see above) modulates the activity of neutral sphingomyelinase. We have studied this activity in plasma membrane–enriched fractions of rat liver and brain and observed that the activity of neutral sphingomyelinase decreases significantly after long-term CR.46–48

CR Induces SIRT1 Protein Levels In Vivo and In Vitro

SIRT1 is distributed in all mammalian tissues studied and modulates cellular and tissue homeostasis, interacting with metabolic and stress response proteins and factors. Mounting evidence suggests that SIRT1 regulates energy metabolism, endocrine signaling, and some stress responses.83 SIRT1 is also inducible by a broad variety of signals, in response to CR79 or fasting,84 suggesting a broad role in mammalian physiology. It is becoming clear that sirtuins are regulated by stress and nutritional status in yeast, worms, flies, and mammals.70,79,86,87 Endocrine and energy metabolism pathways coordinate organismal development and physiology and are intrinsic to pathologies such as cancer, neurodegeneration, and diabetes. These systems respond to a variety of external signals, as diverse as environment, stress, and nutrients. Sir2 regulates, in opposite ways, both replicative and chronological lifespan in yeast. Extra copies of sirtuin genes extend the lifespans of multicellular organisms such as worms, flies, and fish.86,90,91 In principle, understanding how these pathways respond to environmental and nutritional factors could enable us to develop successful therapies. SIRT1 regulates several transcription factors that regulate stress responses, energy metabolism, and endocrine signaling, including peroxisome proliferator-activated receptor (PPARγ), PPARγ coactivator 1 (PGC1)-α, forhead box transcription factors (FOXOs), liver X receptor (LXR), and p53.92–98 There is mounting data supporting that SIRT1 regulates energy metabolism, endocrine signaling, and some stress responses.83,99 The biological effects identified for sirtuins have fueled speculation that sirtuins modulate processes that affect longevity, age-related disease, diabetes, and tumorigenesis.100 CR animals and humans have significantly higher levels of SIRT1 protein in most tissues, including brain, kidney, muscle, visceral fat pads, and liver.11,79,101 Upregulation of SIRT1 by CR is also observed in cultured cell models that recapitulate the key in vivo proliferative and phenotypic features of CR.72 Increasing the resistance of cells to apoptosis is beneficial if a cell is not critically damaged and is difficult to replace. However, this situation is clearly not always desirable if, for example, a cell is mutated or otherwise irreparably damaged. Under conditions of severe stress or proapoptotic signals such as tumor necrosis factor-α (TNFα), SIRT1 can switch into a proapoptotic mode.79 A recent study by Alt and colleagues102,103 found that mouse embryonic cells lacking the SIRT1 gene continue to divide long after they should have senesced because of chronic cell stress, indicating that SIRT1 is able to suppress the proliferation of damaged cells. SIRT1 regulates several transcription factors that regulate stress responses, energy metabolism, and endocrine signaling, including PPARγ, PPARγ coactivator 1α, forhead box transcription factors,92–96 forkhead box transcription factors,92–96 liver X receptor,104 and p53. There is mounting data supporting that SIRT1 regulates energy metabolism, endocrine signaling, and some stress responses.83,99 Recent reports associate SIRT1 with the regulation of apoptosis, senescence, and proliferation.79,105–107

Vasoprotective Effects of CR

CR was shown to attenuate atherogenesis in rodents. The cardiovascular effects of CR observed so far are consistent with the view that CR may confer vasoprotection in humans, although the effects of CR on progression of atherosclerosis and plaque composition in elderly humans or aged primates are still not well documented. In general, CR may affect vascular health both by improving systemic risk factors for coronary artery disease (e.g., plasma lipid and glucose
levels, blood pressure) and by modulating cellular functions and gene expression in endothelial and smooth muscle cells that create a microenvironment in the vascular wall, which does not favor atherogenesis (eg, attenuation of ROS production, antiinflammatory effects).

CR Improves Cardiovascular Risk Factor Profile
Most present knowledge on the effects of CR on cardiovascular risk factors in humans emanates from studies in which obese individuals were treated with some form of relatively short-term dietary restriction to lose weight. High-calorie diets and the resulting obesity are major risk factors for hypertension and coronary artery disease. In addition, weight loss has been associated with significant improvement in the cardiovascular risk factor profile in these individuals (including a decreased weight, body mass index, waist circumference, hip circumference, waist-to-hip ratio, total body fat, total cholesterol, serum triglyceride).110,111 CR exerts beneficial effects on risk factors of atherosclerosis in nonobese individuals as well. This effect has also been shown both in studies on the 8 individuals (including Dr Roy Walford, an early proponent of CR) sealed inside Biosphere 2 for 2 years and on 18 individuals who had been on voluntary diets and the resulting obesity are major risk factors for hypertension and coronary artery disease. Accordingly, CR elicited significant improvement of both agonist- and flow-induced, NO-mediated dilation of resistance arteries from the skeletal muscle of aged F344 rats (Figure 2A and 2B), suggesting that CR increases bioavailability of NO. Available data also suggest that weight reduction with very-low-calorie diets increases flow-mediated vasodilation in obese individuals.121,122 It is yet to be determined whether CR can also improve endothelial function in nonobese aged monkeys109 and elderly humans independent of weight reduction.

The mechanisms by which CR increases bioavailability of NO improving endothelial function in aged rodents likely include upregulation of eNOS (Figure 2C and 2D). Although the upstream mediator(s) of the vascular effects of CR are not well understood, there are data suggesting that CR may regulate both eNOS activity and expression via activation of SIRT1. An interesting study recently reported that SIRT1 and eNOS colocalize in endothelial cells and that SIRT1 deacetylates eNOS, stimulating eNOS activity and increasing endothelial nitric oxide.123 Moreover, CR in mice leads to deacetylation of eNOS, whereas SIRT1 overexpression or SIRT1 activators were shown to induce eNOS expression in endothelial cells.124 Further studies are definitely needed to elucidate whether SIRT1 activation results in increased NO bioavailability improving endothelial function in aged CR individuals.

CR Increases Bioavailability of NO and Improves Endothelial Function
The direct effects of CR on vascular function and phenotype in aging are not well characterized. It is generally accepted that tonic release of NO from the endothelium exerts vasculoprotective and cardioprotective effects, such as maintenance of normal coronary blood flow, inhibition of platelet aggregation and inflammatory cell adhesion to endothelial cells, and disruption of proinflammatory cytokine-induced signaling pathways. Abundant experimental and clinical data show that aging impairs endothelial NO production (recently reviewed elsewhere119), which has been suggested to play a role in atherogenesis. The severe impairment of NO bioavailability in aging also limits cardiac blood supply and alters myocardial O2 consumption and cardiac contractility.120 Our recent data suggest that lifelong CR in rats prevents aging-induced endothelial dysfunction. Accordingly, CR elicited significant improvement of both agonist- and flow-induced, NO-mediated dilation of resistance arteries from the skeletal muscle of aged F344 rats (Figure 2A and 2B), suggesting that CR increases bioavailability of NO. Available data also suggest that weight reduction with very-low-calorie diets improves flow-mediated vasodilation in obese individuals.121,122 It is yet to be determined whether CR can also improve endothelial function in nonobese aged monkeys109 and elderly humans independent of weight reduction.

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CR May Attenuate Vascular Inflammation in Aging

Atherosclerotic vascular disease is now recognized as a chronic inflammatory disease. There is abundant evidence showing that aging is associated with vascular inflammation-promoting atherogenesis (reviewed recently elsewhere). For example, aging promotes endothelial activation, increasing the expression of adhesion molecules, and enhancing leukocyte adhesion to the endothelial cells. Previous studies by our and other laboratories have shown that endothelial activation in aging is mediated, at least in part, by oxidative stress–induced increased nuclear factor-κB activation. In this regard, it is important that CR seems to attenuate vascular nuclear factor-κB induction and endothelial activation in aged rats. CR also protected against the age-associated increase of c-Jun N-terminal kinase and p38 activities in aged rat aortas. Moreover, CR similarly reversed the age-related increase of activator protein-1 DNA binding activity. In aging, a proinflammatory shift develops in the vascular cytokine expression profile (including upregulation of TNFα, interleukin [IL]-1β, and IL-6). Aging is also associated with increased plasma levels of inflammatory mediators (eg, TNFα, IL-6, and C-reactive protein), both in humans and rodents. In studies of CR in rats and mice, it was found that CR results in marked decreases in these inflammatory markers. The observation that CR in humans also seem to decrease serum C-reactive protein and TNFα provides preliminary evidence that CR may also reduce vascular inflammation in humans.

CR Attenuates Oxidative Stress in the Vasculature

Advanced age is associated with endothelial oxidative stress, which leads to functional inactivation of NO by high concentrations of O$_2^-$, resulting in an enhanced ONOO$^-$ formation. The role of increased oxidative and nitrosative stress in eliciting endothelial dysfunction and activation of proatherogenic inflammatory processes in aging has been reviewed recently. In 1996, Sohal and Weindrusch proposed that the antiaging action of CR stems from the attenuation of the age-associated increase in oxidative stress. Indeed, it has been amply demonstrated that CR decreases the age-associated accumulation of oxidatively damaged lipids, proteins, and nucleic acids in multiple organ systems, including the liver and skeletal muscle. Our findings suggest that CR in aged rats significantly decreases vascular O$_2^-$ production (Figure 2E). These data are in line with the findings that endothelial cells obtained from CR mice exhibit decreased O$_2^-$ and H$_2$O$_2$ production as compared with those obtained from mice fed ad libitum. CR also significantly attenuates oxidative DNA damage and normalizes the tissue content of lipid peroxidation–derived aldehydes (HNE, MDA) in aortas of aged rats. There are studies extant suggesting that reduction of oxidative stress in the arterial wall may contribute to the antiatherogenic effect of CR in apolipoprotein E–null (apoE$^{-/-}$) mice. In parenchymal tissues of experimental animals, CR modulates the expression of various antioxidant enzymes; however, at present, it is unclear whether this is the case in the vasculature as well. Previous studies have identified vascular NAD(P)H oxidases as an important source of ROS production in small coronary arteries, aorta, and carotid arteries of aged rodents. In addition, aging also increases mitochondrial ROS generation in the endothelial cells. Future studies should elucidate how CR affects NAD(P)H oxidase activity/expression and mitochondrion–derived ROS generation in the aged blood vessels.

There are data in the literature attributing some of the effects of CR to a decreased insulin-like signaling. Studies in Caenorhabditis elegans provided the first evidence that reduced insulin-like signaling may actually promote longevity in lower organisms. By now, it is well established that insulin-like signals promote the phosphorylation and deactivation of DAF-16, a forkhead transcription factor that is a key regulator of oxidative stress resistance and metabolism in C elegans (reviewed elsewhere). There is also evidence that loss of insulin-like growth factor (IGF)-like signaling contributes to longevity response to CR in Drosophila. The first evidence to support a role of insulin-like signals in regulation of mammalian longevity came from the observation that mice with hereditary dwarfism (Ames dwarf) have low circulating IGF-I and extended longevity and exhibit many symptoms of delayed aging. However, the link between IGF signaling and vascular oxidative stress is likely complex. In Ames dwarf aortas, endothelial ROS generation are more than in vessels of wild type mice (Z.U., unpublished data). Moreover, in cultured coronary arterial endothelial cells, treatment with IGF significantly reduces cellular O$_2^-$ and H$_2$O$_2$ production and ROS generation by mitochondria and upregulates expression of antioxidant enzymes and eNOS (Z.U., unpublished data, 2008). These in vitro findings accord with the observations that in humans, growth hormone and IGF-I deficiency is associated with premature atherosclerosis and elevated cardiovascular disease mortality. Recent evidence suggests that cardiovascular disease risk also may be elevated among apparently healthy individuals who have serum IGF-1 levels in the low normal range. There is also increasing evidence that IGF-1 may exert vasculoprotective effects in aging. By now, it has been firmly established that IGF-1 protects cardiomyocytes from apoptotic cell death. Cardiac stem cells and early committed cells were also demonstrated to express IGF-1 receptors and secrete IGF-1, and IGF-1 was shown to promote cardiac stem cell survival and proliferation. The findings that cardiac overexpression of IGF-1 significantly improved cardiomyocyte contractile function in old mice support the view that IGF-1 signaling plays a protective role in the cardiovascular system and that loss of IGF-1 contributes to cardiac aging. Thus, low IGF-1 levels are less likely to be the cause of reduced ROS production and increased bioavailability of NO in the vasculature in CR.

Nrf2: A Novel Pathway for Vasoprotection

Nrf2 (NF-E2–related factor 2) is a transcription factor that binds to the antioxidant response element (ARE) of target genes and increases the transcription of a variety of antioxidant proteins. Kelch-like ECH–associated protein–1 (Keap1) normally sequesters Nrf2 in the cytoplasm, but on oxidation of cysteine...
residues, Nrf2 dissociates from Keap1, translocates to the nucleus, and binds to ARE sequences, leading to transcriptional activation of phase II detoxifying genes (such as glutathione S-transferase and NQO1) and antioxidant enzymes (such as glutathione reductase, glutathione peroxidase, and catalase). In parenchymal tissues of the aged rat, there is a significant decline in transcriptional activity of Nrf2, which causes age-related loss of glutathione synthesis, likely promoting cellular oxidative stress. In a series of current studies, we are testing the hypothesis of whether Nrf2 induction plays a role in attenuation of cellular oxidative stress in aged tissues. In this context, our recent studies demonstrated that induction of Nrf2 is responsible for the anticarcinogenic effects of CR but is dispensable for increased insulin sensitivity. Accordingly, Nrf2-deficient mice developed tumors more readily in response to carcinogen exposure than did wild-type mice, and CR was ineffective in suppressing tumors in the Nrf2-deficient mice. The aforementioned Nrf2-dependent ROS detoxification systems are expressed in endothelial cells, and previous studies have provided solid evidence that the ARE-mediated genes are regulated by antiprotective laminar flow through a Nrf2-dependent mechanism. Also, induction of Nrf2 in cultured endothelial cells results in a marked increase in ARE-driven transcriptional activity and protected the cells from H2O2-mediated cytotoxicity. Nrf2 also suppresses TNFα-induced endothelial activation and inhibits monocyte adherence to the endothelial cells. Although presently it is unknown how aging affects Nrf2 transcriptional activity in the vascular endothelial and smooth muscle cell, we have strong evidence for an age-dependent decline in glutathione synthesis in aged rat aortas, which is prevented by CR (A.C., Z.U., and J. Pinto, unpublished data, 2008). Further studies are evidently needed to test the hypothesis that the Nrf2/ARE pathway is induced in aged arteries, which acts as an endogenous atheroprotective system for antioxidant protection and suppression of redox-sensitive vascular inflammation.

Conclusions and Perspectives
Oxidative stress plays an important role in the pathogenesis of coronary artery disease by mediating expression of inflammatory genes and eliciting oxidative modification of lipoprotein particles. CR seems to attenuate both vascular oxidative stress and exert antiinflammatory effects in aged animals. We posit that CR activates the Nrf2/ARE pathway, which may serve as an endogenous antioxidant system within the vasculature, increasing cellular oxidative stress tolerance. CR also increases bioavailability of antiatherogenic NO and augments endothelial function. In addition, CR exerts beneficial effects on a range of systemic cardiovascular risk factors. There is a great deal of effort to dissect the pathways that invoke CR benefits to develop pharmacological agents that would act as CR mimetics. Several of the currently proposed CR mimetics are phytochemicals (resveratrol, quercetin, and curcumin) that act, at least in part, through the activation of Nrf2 pathway. Importantly, newly identified CR mimetics, such as resveratrol, exert cardiovascular effects that are remarkably similar to those of CR. Accordingly, resveratrol increases vascular oxidative stress resistance, upregulates eNOS, inhibits endothelial activation and vascular inflammatory gene expression, and activates both SIRT1 and the Nrf2/ARE pathways, providing a pharmacological alternative for CR for the prevention of coronary artery disease in the elderly.

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Disclosures
None.

References


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57. Mataix J, Manas M, Quiles J, Battino M, Cassinello M, Lopez-Frias M, Ungvari et al Caloric Restriction and Vascular Aging 525


63. Deleted in proof.


69. Gomez-Diaz C, Villalba JM, Perez-Vicente R, Crane FL, Navas P. Ascorbate stimulation is stabilized in rho(0)HL-60 cells by CoQ10 increase at the plasma membrane. Biochem Biophys Res Commun. 1997;234:79–81.


84. Deng XQ, Chen LL, Li N. The expression of SIRT1 in nonalcoholic fatty liver disease induced by high-fat diet in rats. Liver Int. 2007;27:708–715.


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