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.4–6 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, Aging, and the Plasma Membrane

Mitochondria are the main source of ATP production. During mitochondrial oxidative phosphorylation, reactive oxygen species (ROS) are produced. ROS are associated with damage to DNA, lipids, and proteins. The pathology of aging and age-related diseases involves oxidative stress as an early stage in its development, as confirmed by a decrease in antioxidant defenses and an increase in oxidative damage. 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, and proteins. Accumulation of mitochondrial DNA mutations, commonly identified in age-related diseases, induce impairments of mitochondrial complexes, including mitochondrial complex III activity in the aged heart. Impaired mitochondrial function causes a shortage of ATP supply, resulting in induction of further problems in biochemical pathways.

The free radical theory of aging 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. These studies have lead collectively to the hypothesis that CR by reducing oxidative stress extends the lifespan. The mitochondrial and plasma 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 unsaturation and that elucidation of this relationship can provide insight on the mechanism for lifespan extension with CR. 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), production of ROS, induction of apoptosis, and maintenance of antioxidant systems. 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 In Vivo and In Vitro

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. 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.

The aforementioned antioxidants are maintained in their reduced forms at the plasma membrane by different CoQ-dependent reductases, NADH-dependent cytochrome b₅ reductase and NAD(P)H:quinone-oxidoreductase-1 (NQO1). Different dietary modifications can modulate these enzyme activities to protect the plasma membrane. 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. The upregulation of the plasma membrane redox system that occurs...
during CR decreases the levels of oxidative stress in aged membranes.46–48,64 CR modifies composition of fatty acid in the plasma membrane, resulting in decreased oxidative damage including lipid peroxidation.65,66 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)H ratio.67 This function is driven in cooperation with mitochondria, an interaction particularly observed in ρ0 cells.48,68,69 The ratio of pyridine nucleotides is 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 replicative88 and chronological89 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 (PPAR)γ, PPARγ coactivator 1 (PGC1)-α, forkhead 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,97 PPARγ coactivator 1α,98 forkhead box transcription factors,92–96 liver X receptor,104 and p53.105 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.108 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 primates109 are still not well documented. In general, CR may affect vascular health both by improving systemic risk factors for coronary artery disease (eg, 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 (Figure 2A and 2B), suggesting 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.

The mechanisms by which CR increases bioavailability of NO 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 May Attenuate Vascular Inflammation in Aging

Atherosclerotic vascular disease is now recognized as a chronic inflammatory disease.125 There is abundant evidence showing that aging is associated with vascular inflammation-promoting atherogenesis (reviewed recently elsewhere119,126,127). For example, aging promotes endothelial activation, increasing the expression of adhesion molecules,75,124,128,129 and enhancing leukocyte adhesion to the endothelial cells.124,129,130 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 kB activation.124,129 In this regard, it is important that CR seems to attenuate vascular nuclear factor kB induction and endothelial activation in aged rats.128,129 CR also protected against the age-associated increase of c-Jun N-terminal kinase and p38 activities in aged rat aortas.131 Moreover, CR similarly reversed the age-related increase of activator protein-1 DNA binding activity.131 In aging, a proinflammatory shift develops in the vascular cytokine expression profile (including upregulation of TNFα, interleukin [IL]-1β, and IL-6).74,78,132 Aging is also associated with increased plasma levels of inflammatory mediators (eg, TNFα, IL-6, and C-reactive protein), both in humans and rodents.7,133,134 In studies of CR in rats and mice, it was found that CR results in marked decreases in these inflammatory markers.135,136 The observation that CR in humans also seem to decrease serum C-reactive protein and TNFα137 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 $\cdot O_2^-$, resulting in an enhanced ONOO⁻ formation.74,75,120,138,139 The role of increased oxidative and nitrosative stress in eliciting endothelial dysfunction and activation of proatherogenic inflammatory processes in aging has been reviewed recently.119,126 In 1996, Sohal and Weindruch138 proposed that the antiaging action of CR stems from the attenuation of the age-associated increase in oxidative stress.140 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.141,142 Our findings suggest that CR in aged rats significantly decreases vascular $\cdot O_2^-$ production (Figure 2E). These data are in line with the findings that endothelial cells obtained from CR mice exhibit decreased $\cdot O_2^-$ and $H_2O_2$ production as compared with those obtained from mice fed ad libitum.130 CR also significantly attenuates oxidative DNA damage143 and normalizes the tissue content of lipid peroxidation–derived aldehydes (HNE, MDA) in aortas of aged rats.131 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.108 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.74,75,119 In addition, aging also increases mitochondrial ROS generation in the endothelial cells.124 Future studies should elucidate how CR affects NAD(P)H oxidase activity/expression and mitochondrial-derived ROS generation145,146 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 elsewhere147). There is also evidence that loss of insulin-like growth factor (IGF)-like signaling contributes to longevity response to CR in Drosophila.148 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-1 and extended longevity and exhibit many symptoms of delayed aging.149 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, 2008). Moreover, in cultured coronary arterial endothelial cells, treatment with IGF significantly reduces cellular O$_2^-$ and $H_2O_2$ 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.150 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.151 There is also increasing evidence that IGF-1 may exert vasculoprotective effects in aging.152,153 By now, it has been firmly established that IGF-1 protects myocardocytes from apoptotic cell death.154–156 Cardiac stem cells and early committed cells were also demonstrated to express IGF-1 and IGF-I provided the first evidence 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 nu-
cleus, 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 atheroprotective laminar
flow through a Nrf2-dependent mechanism. Also, in-
duction 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
adhesiveness 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 athero-
protective 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 inflam-
matory genes and eliciting oxidative modification of lipopro-
tein particles. CR seems to attenuate both vascular oxidative
stress and exert antiinflammatory effects in aged animals. We
post that CR activates the Nrf2/ARE pathway, which may
serve as an endogenous antioxidant system within the vascu-
lature, 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 mi-
metics, such as resveratrol, exert cardiovascular effects that
are remarkably similar to those of CR. Accordingly, resvera-
trol increases vascular oxidative stress resistance, upregu-
lates 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.

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