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Review

Caloric restriction: powerful protection for the aging heart and vasculature

Edward P. Weiss[1,2] and Luigi Fontana[2,3]

[1]*Department of Nutrition and Dietetics, Saint Louis University, Saint Louis, Missouri;* [2]*Division of Geriatrics and Nutritional Sciences, Department of Medicine, Washington University School of Medicine, Saint Louis, Missouri; and* [3]*Division of Nutrition and Aging, Istituto Superiore di Sanitá, Rome, Italy*

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Weiss EP, Fontana L. Caloric restriction: powerful protection for the aging heart and vasculature. *Am J Physiol Heart Circ Physiol* 301: H1205–H1219, 2011. First published August 12, 2011; doi:10.1152/ajpheart.00685.2011.—Cardiovascular disease (CVD) is the leading cause of morbidity and mortality in the United States. Research has shown that the majority of the cardiometabolic alterations associated with an increased risk of CVD (e.g., insulin resistance/type 2 diabetes, abdominal obesity, dyslipidemia, hypertension, and inflammation) can be prevented, and even reversed, with the implementation of healthier diets and regular exercise. Data from animal and human studies indicate that more drastic interventions, i.e., calorie restriction with adequate nutrition (CR), may have additional beneficial effects on several metabolic and molecular factors that are modulating cardiovascular aging itself (e.g., cardiac and arterial stiffness and heart rate variability). The purpose of this article is to review the current knowledge on the effects of CR on the aging of the cardiovascular system and CVD risk in rodents, monkeys, and humans. Taken together, research shows that CR has numerous beneficial effects on the aging cardiovascular system, some of which are likely related to reductions in inflammation and oxidative stress. In the vasculature, CR appears to protect against endothelial dysfunction and arterial stiffness and attenuates atherogenesis by improving several cardiometabolic risk factors. In the heart, CR attenuates age-related changes in the myocardium (i.e., CR protects against fibrosis, reduces cardiomyocyte apoptosis, prevents myosin isoform shifts, etc.) and preserves or improves left ventricular diastolic function. These effects, in combination with other benefits of CR, such as protection against obesity, diabetes, hypertension, and cancer, suggest that CR may have a major beneficial effect on health span, life span, and quality of life in humans.

cardiovascular disease; inflammation; oxidative stress; arterial stiffness; cardiovascular aging

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In the last century both life expectancy at birth and life expectancy beyond the age of 65 have markedly increased (146). The percentage of the total U.S. population over the age of 65 was 4% in 1900, has reached 13% in 2010, and is expected to increase to ~20% in 2030, amplifying concerns about health care spending. In fact, ~80% of older adults (≥65 yr) have at least one chronic disease, and 50% have at least two chronic diseases, which often lead to disability (109).

Coronary heart disease, heart failure, stroke, and diabetes are the leading causes of morbidity and mortality, accounting for ~37% of total deaths in the United States (109). The incidence and prevalence of these diseases rise with advancing age because of the increased exposure time of the heart and arteries to harmful cardiometabolic risk factors (e.g., smoking, dyslipidemia, elevated blood pressure, insulin resistance, abdominal obesity, inflammation, and oxidative stress). Accordingly, participants of the Framingham Heart Study with low cardiometabolic risk (i.e., total cholesterol < 180 mg/dl, HDL-cholesterol > 40 mg/dl in men and > 50 mg/dl in women, blood pressure < 120/80 mmHg, fasting glycemia ≤ 125 mg/dl, body mass index < 25 kg/m^2, and no smoking) at age 50 had substantially lower lifetime risk of developing cardiovascular disease (CVD) than participants with two or more major risk factors (5.2 vs. 68.9% in men; and 8.2 vs. 50.2% in women) (127). However, according to the most recent scientific literature, some of the criteria used in this study to define the low CVD risk profile are indeed nonoptimal. Optimal fasting glucose concentration should be below 86 mg/dl and LDL-cholesterol levels below 70 mg/dl, optimal blood pressure values should be below 115/75 mmHg, and optimal waist circumference should be ≤94 cm for men and ≤88 cm for women (3, 33, 41, 80, 145), suggesting that it is possible to achieve an even lower lifetime risk of developing coronary heart disease, heart failure, and stroke.

Nonetheless, aging has profound effects on the heart and arterial system, independently of the presence of clinical and subclinical CVD (114). There is a progressive deterioration in

Address for reprint requests and other correspondence: E. P. Weiss, Dept. of Nutrition and Diabetics, Doisy College of Health Sciences, St. Louis Univ., St. Louis, MO, 63104 (e-mail: eweiss4@slu.edu).

cardiovascular function and structure with advancing age, including a reduction in maximal heart rate (111), decreased heart rate variability (6, 60, 161), increased arterial stiffness (9, 10, 112, 134, 181, 192), diminished left ventricular systolic reverse capacity (112, 150), diastolic dysfunction (103, 112, 140), and impaired endothelial function (74). These anatomical and physiological changes that occur with normal aging and reduced physiological reserves of most body systems are not synonymous with disease but with an increased vulnerability to challenges which may decrease the ability of the organism to survive stressful conditions.

Several studies have now demonstrated that intrinsic cardiovascular aging can be affected by changes in food intake or mutations in single genes (61, 65, 80, 131, 200). Studies conducted on laboratory rodents have shown that calorie restriction without malnutrition (CR) promotes longevity and ameliorates the age-associated impairment of left ventricular diastolic function and arterial elasticity and improves heart rate variability (24, 130, 164, 179). The purpose of this article is to review the current knowledge on the effects of CR on the aging of the cardiovascular system and CVD risk in rodents, monkeys, and humans.

Calorie Restriction Defined

"Calorie restriction" refers to a state in which energy intake in animals or humans is minimized to low-normal levels while adequate intakes of protein and micronutrients are maintained at sufficient levels to avoid malnutrition. CR typically consists of an energy intake that is 30–50% below that which is required to maintain normal body weight and adiposity and thus results in a very lean phenotype. In the strict use of the term, CR is not an intervention in which excessive energy intake is reduced. In animal studies, CR is typically introduced before physical maturation. Although it results in growth retardation, weight loss does not occur, which makes it clear that the health and longevity effects of CR in these studies are not attributable to weight loss. However, in human intervention studies, CR is introduced during adulthood and invariably results in weight loss, making it difficult to differentiate between the effects of weight loss and CR, per se. These and other aspects of animal and human CR study designs are presented in Table 1. The primary reason for studying CR in animals is to learn about human aging. In recent years, major advances in understanding the effects of CR on human aging and disease have been made by performing studies on human CR. However, despite this progress, none of the studies on humans can provide clear evidence of the effects of lifelong CR on life span or even major age-related diseases because of study design limitations.

The term "calorie restriction" has also been used less specifically to describe any reduction in energy intake, regardless of baseline energy intake, and is sometimes used to describe diet-induced weight loss in obesity. In this context, although calorie restriction results in weight loss, it is being used to alter the severity of a pathological/abnormal condition. This contrasts with the more strict use of the term, as described above, in which calorie restriction is intended to change a "normal" physiological state into "supernormal" state (i.e., a state in which physiological function is far better than that which would be necessary to be considered normal). For example, while a reduction in energy intake in overweight individuals has been shown to reduce serum C-reactive protein concentration from an abnormal level of 2.3 mg/l to a more clinically normal 1.6 mg/l (141), average serum C-reactive protein con-

Table 1. *Comparison of animal versus human CR study design features*

	Animal Studies	Human Studies
Energy intake	30-50% less than the intake of free-fed or modestly restricted animals (199)	Energy intake sufficiently low to achieve a lean phenotype or BMI of 18.5-21.0 kg/m^2 (64, 197) or energy intake that is sufficiently low to induce weight/fat loss in subjects with high-normal body weight or very mild overweight (i.e., BMI, 23.5-27.0 kg/m^2) (48, 86, 153)
Micronutrient and protein intake	Adequate to meet recommended intakes in some but not all studies (31)	Often stated that intakes are adequate to meet recommended intakes (64); however, micronutrient data have not been reported.
Initiation of CR	Typically after weaning but well before physical maturation (199)	Initiated in physically mature adults. Inevitably, this results in weight loss (48, 86, 153), making it difficult to differentiate between the effects of CR, per se, and weight loss.
Duration of CR	Generally lifelong after weaning (199)	6-12 mo in intervention trials (48, 86, 153); 2-30 yr in observational studies (64, 197)
Effects on growth	Growth retardation (131)	No growth retardation (initiated during adulthood)
Effects on body weight and fat mass	Due to growth retardation, body weight increases less than in control animals during early years; CR does not result in weight loss (199)	In response to the introduction of CR, body weight and fat mass decrease until energy balance is reestablished (48, 86, 153, 197). In cross-sectional research, body weight and fat mass are stable in CR practitioners (64).
Outcomes	Mean and maximal life span, disease prevention, and outcomes related to mechanism for life-span increases (65).	Risk factors for age-related diseases, biomarkers thought to reflect biological aging, and biomarkers shown in animal studies to change with CR (63, 67, 86, 89, 204).

CR, calorie restriction; BMI, body mass index.

centration in men and women practicing long-term CR is 0.2 mg/l (64), suggesting that CR results in a supernormal state of inflammatory control. This review article focuses specifically on the more strict use of the term "calorie restriction" as a means for optimizing the health and function of the cardiovascular function in the absence of overt preexisting disease. Studies in which dietary energy intake is reduced to correct obesity and related conditions will not be reviewed, as they are not within the scope of this paper.

Extensive research over the past seven decades has demonstrated that in animal species ranging from worms to rodents, 30–50% restriction of energy intake without introducing protein or micronutrient malnutrition (i.e., CR) increases life span by 30–50% (65). Part of this effect is mediated by preventing or postponing death because of chronic diseases such as cancer (up to 62% reduction in cancer incidence), obesity, type 2 diabetes, and autoimmune, cardiovascular, kidney, and neurodegenerative diseases (61, 131, 200). Furthermore, postmortem pathological studies have demonstrated that 30% of the rodents undergoing CR die in very old age without any evidence of lethal pathology (166), suggesting that it is possible to live a long life without overt disease. However, in addition to disease prevention, CR also slows the lifelong deterioration of structure and function in organs and tissues that occurs even in the absence of disease (61, 65, 131, 200). As a result, CR results in a more youthful biological phenotype and increases maximal life span (defined as the average age of the oldest 10% of animals in a cohort).

Ongoing studies are evaluating the effect of lifelong CR on aging and life span in nonhuman primates (i.e., Rhesus monkeys). Early evidence indicates that long-term CR results in many of the same adaptive responses that occur in rodents undergoing CR, such as increased insulin sensitivity, improved lipid profile, reduced blood pressure, decreased inflammatory and oxidative stress markers, lower serum triiodothyronine concentration and body temperature, and prevention of the age-associated decline in serum concentrations of dehydroepiandrosterone sulfate and melatonin (5, 61). The monkeys undergoing CR also appear to be partially protected against immune senescence, sarcopenia, and brain atrophy in subcortical regions that control motor and executive function (35, 36, 136). While it is premature to determine whether life span is increased by CR in these higher mammals, preliminary evidence based on small sample sizes suggests that CR may protect against cancer, CVD, and type 2 diabetes in monkeys (35). More definitive data on the effects of CR on life span in monkeys will likely be available in 10–15 years.

Data from studies on humans are far less definitive with respect to the effects of CR on life span than those from animal studies. The effect of CR on human longevity was evaluated in a retrospective analysis of data from men and women living in Okinawa Japan during the 1940s–1960s. The results indicate that these individuals were moderately calorie restricted (~1,785 kcal per day) with a lean phenotype (body mass index, ~21 kg/m^2). When compared with residents of greater Japan and the United States, the Okinawans had markedly lower mortality from coronary heart disease and cancer and one of the highest numbers of centenarians in the world (~50 per 100,000 inhabitants) or about four to five times the average for most developed countries (93, 208). Studies have also shown that in individuals undergoing long-term self-imposed CR (i.e., ~8 yr) and those undergoing 6–12 months of CR in randomized trials, CR results in adaptations that would be expected to reduce chronic disease risk, including greater insulin sensitivity, lower body fat levels, a better plasma lipid profile, lower blood pressure, less oxidative stress/damage, and lower levels of chronic systemic inflammation (61, 62, 64, 86, 89, 138). Furthermore, these studies also demonstrate that CR in humans results in lower leptin, sex hormones, and triiodothyronine hormone levels, lower core body temperature, and a lower metabolic rate (23, 63, 86, 169, 204), all of which are hallmark adaptations seen in response to CR in animals. However, a notable difference exists between human and animal studies of CR. In rodents, CR results in large reductions in insulin-like growth factor-1 (171); this adaptation may mediate the cancer-protective and life span-extending effects of CR (65). In contrast, in humans, CR does not lower insulin-like growth factor-1 levels unless protein intake is also restricted (67).

Cardiovascular System Aging and Calorie Restriction

The cardiovascular system undergoes extensive structural and functional changes during the adult life span. Some of these changes are accelerated and exacerbated by the presence of harmful environmental and lifestyle factors and may eventually result in overt disease. For example, years of exposure to cardiometabolic risk factors cause atherogenesis and may culminate in the rupture of an advanced plaque, ultimately resulting in ischemic heart disease and possibly heart failure. However, other age-related changes in the cardiovascular system may not necessarily be precursors to disease. For example, maximal heart rate decreases very predictably with increasing age (182, 203), yet this change is not a disease itself and is not known to be involved in disease pathogenesis. In this context, studies on the effects of CR on cardiovascular aging are especially interesting. Insights into the disease-protective effects of CR can be gained by evaluating the effects of CR on CVD risk factors and disease processes. However, because CR also appears to slow basic biological aging, independent of disease processes, studies on the effects of CR on disease-independent aspects of cardiovascular aging are important for learning about aging, per se.

Metabolic and Molecular Cardiovascular Targets of Calorie Restriction

Vascular oxidative stress. Vascular aging is associated with increases in the production of reactive oxygen species (ROS) from vascular tissue mitochondria and decreases in endogenous antioxidant system activity (1, 44, 54, 92, 188, 193). In the healthy vasculature, oxidative stress activates the transcription factor NF-E2-related factor 2 (Nrf2), which moves into the nucleus, binds antioxidant-response elements, and induces proteins involved in protection against oxidative and free radical stress, including glutathione-*S*-transferases, NADPH:quinone oxidoreductase 1, and heme oxygenase 1 (91, 106, 144). With increasing age, the oxidative stress-induced activation of Nrf2 and the expression of its target genes is attenuated or becomes entirely absent (187, 190), thereby downregulating antioxidant system activity. This, together with increases in the production of ROS (92, 188, 193), results in increases in oxidative stress and damage that are common in old age (12, 187) (Fig. 1).

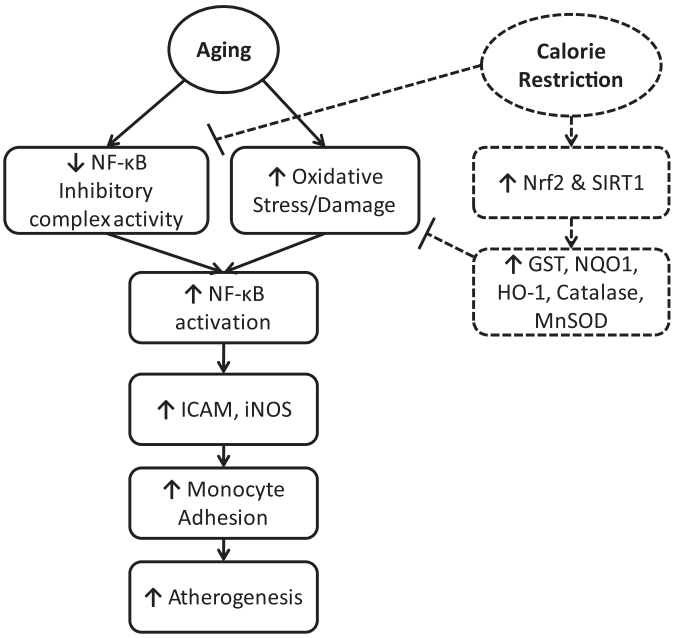

Fig. 1. The effects of aging and calorie restriction (CR) on oxidative stress and inflammation in the vasculature and their relationship to atherogenesis. Increasing age is associated with increased production of reactive oxygen species, which results in oxidative stress and damage. Aging is also associated with a reduction in the inhibitory complex that prevents the transcription factor NF-κB from entering the nucleus where it can induce inflammatory genes. Together these factors result in increased expression of adhesion molecules (ICAM-1) and the proinflammatory enzyme inducible nitric oxide synthase (iNOS), thereby promoting monocytes adhesion to the endothelium and intimal infiltration and consequently initiating atherogenesis. CR attenuates the age-related loss of NF-κB inhibition, thereby keeping NF-κB in the inactive state. Furthermore, by activating transcription factor NF-E2-related factor 2 (Nrf2) and sirtuin 1 (SIRT1), CR induces proteins involved in protection against oxidative and free radical stress including glutathione-*S*-transferases (GST), NADPH:quinone oxidoreductase 1(NQO1), and heme oxygenase 1 (HO-1), manganese superoxide dismutase (MnSOD), and catalase. Together, these effects of CR prevent the NF-κB-mediated vascular inflammatory response and attenuate the age-related increase in atherogenesis.

It has been theorized that CR slows the aging process, at least partly by attenuating the age-related increases in oxidative stress and the accumulation of oxidative damage (170). In support of this theory, at least in the context of the vasculature, CR has been shown to attenuate the production of ROS and oxidative damage (43, 80, 189) and increases levels of the endogenous antioxidants glutathione and ascorbate in the aorta (43). As has been found in other cells/tissues, the histone deacetylase and gene repressor sirtuin 1 (SIRT1) may be involved in the antioxidant effects of CR, as serum from CR animals induced SIRT1 in cultured endothelial cells and SIRT1 knockdown attenuated the CR-mediated reduction in ROS production (43). CR also induces endogenous antioxidant proteins by activating Nrf2 (149) (Fig. 1). Studies on humans also support the notion that CR attenuates oxidative stress. Twelve months of CR in humans have been shown to increase the activity of the antioxidant glutathione peroxidase in plasma (137), decrease plasma protein carbonyl levels as a marker of oxidative damage (137), and lower levels of oxidative damage to DNA and RNA in white blood cells and urine (86, 89).

Vascular inflammation. In addition to the direct effect of oxidative stress on the age-related deterioration of vascular structure and function, oxidative stress also increases vascular inflammation including "endothelial activation" [i.e., increased expression of leukocyte recognition and adhesion molecules, a phenotypic change from antithrombotic to prothrombotic, and increased expression of cytokines and growth factors (17)]. As inflammation has been implicated as a major contributor to atherogenesis (125), this may contribute to the development of atherosclerosis with increasing age. At least part of the mechanism by which oxidative stress promotes inflammation appears to involve the activation of the redox-sensitive transcription factor NF-κB (54, 177, 188), which has been implicated as an important contributor to atherogenesis (83) (Fig. 1). Oxidative stress-induced activation of NF-κB has been shown to increase vascular expression of adhesion molecules and inducible nitric oxide (NO) synthase and to increase monocytes adhesion to endothelial cells (188), all of which promote atherogenesis.

CR has several effects on vascular inflammation (Fig. 1). CR attenuates the age-related increase in expression of vascular adhesion molecules, an effect that coincided with reductions in ROS production (218). This effect of CR appears to be mediated by the prevention of the age-related reductions in NF-κB inhibitory complexes, thereby preventing NF-κB from entering the nucleus to promote inflammatory gene transcription. CR has also been shown to attenuate age-related increases in prostanoids in both serum (prostaglandin E_2) and in the aorta (prostaglandin E_2 and thromboxanes A_2); this effect appears to be mediated by an attenuation of the age-related increases in cyclooxygenase 2 and cytosolic phospholipase A_2, both of which are involved in prostanoid synthesis. Although the mechanism for cyclooxygenase 2 and cytosolic phospholipase A_2 downregulation by CR is not clear, it is possible that NF-κB is involved because the genes for these proteins have NF-κB binding sites in their promoter regions (128, 211). Aging is also associated with increases in the proinflammatory cytokines in vascular tissue and serum from rodents (TNF-α, IL-1β, and IL-6) (45, 46) and in serum from humans (TNF-α, TNF receptor-1, and IL-6) (148, 175). CR has been shown to decrease levels of several inflammatory cytokines in rodents (80, 95, 172) and with seven to eight years of calorie restriction in humans (64) but not with shorter-term CR (i.e., 6–12 mo) in humans (118, 202).

Effects of Calorie Restriction on Vascular Health and Function

Endothelial function. Another means by which oxidative stress contributes to age-related vascular dysfunction is by altering the bioavailability of endothelium-derived free radical, NO. In the presence of oxidative stress, NO bonds with ROS, thereby eliminating its bioactivity. Furthermore, age-related reductions in endothelial production of NO also likely contribute to lower NO bioavailability, as a result of several age-related changes including reductions in the expression of endothelial NO synthase (eNOS) (44, 180), decreases in tetrahydrobiopterin (eNOS cofactor) levels (168), and decreases in intracellular L-arginine (eNOS substrate for NO production) availability (16). Among other compounds produced by the endothelium, NO serves an important role in the maintenance of vascular health and in preventing atherosclerosis, since it inhibits platelet and leukocyte adherence to the endothelium

(108), prevents platelet aggregation (174), and suppresses vascular smooth muscle cell proliferation (73). Endothelium-derived NO causes vascular smooth muscle relaxation and is the most potent endogenous vasodilator known (39). NO-mediated vasodilation is commonly measured as an index of NO bioavailability (152). In both rodents and humans, impaired NO-mediated vasodilation (i.e., endothelial dysfunction) develops with increasing age in both conduit and resistance vessels (15, 29, 97, 178). Not only does this blunt the minute-by-minute adjustments in blood vessel diameter that are important for regulating blood flow to target organs such as the heart (1, 191) and skeletal muscle (151, 185), but it is also associated with a greater likelihood of developing coronary artery disease (165, 213).

CR preserves endothelial function with advancing age through two known mechanisms. As reviewed earlier in this article, CR attenuates age-related increases in oxidative stress. This attenuates the degree to which NO is inactivated by ROS, improves NO bioavailability, and thus preserves endothelial function (43, 157, 214). It does not appear that these effects are mediated by alterations in the sensitivity of vascular smooth muscle to NO, as neither aging or CR alter the vasodilatory response to the endothelium-independent NO donor, *S*-nitroso-*N*-acetyl-penicillamine (43). CR also appears to prevent age-related losses in endothelial function by attenuating the age-related decrease in eNOS expression (157, 189) and activity (214). Although the means by which CR increases eNOS activity are not clear, SIRT1, which is induced by CR, has been shown to associate with eNOS in the cytosol of vascular endothelial cells, deacetylate eNOS, and increase eNOS activity (133). Furthermore, CR results in elevated levels of circulating adiponectin in both humans and rodents (62, 202, 217), which may activate eNOS in vascular tissue through a pathway involving the phosphorylation/activation of AMP-activated protein kinase (53). CR has also been shown to increase Akt phosphorylation in heart tissue (75), which may contribute to eNOS activation (71); however, it is not known whether CR alters Akt activity in the vasculature. Whether CR increases eNOS activity by altering levels of the eNOS cofactor tetrahydrobiopterin or the eNOS substrate L-arginine is not known.

Arterial stiffness. Aging is associated with increases in the stiffness of the large elastic arteries (102, 192). This hardening of the arteries occurs throughout adulthood (184) and in the absence of atherosclerosis (8, 192), thereby making it an attractive biomarker for biological aging. However, the process is also accelerated in the presence of diseases including hypertension, diabetes, and kidney dysfunction (162), and greater stiffness is associated with future development of coronary artery disease, and all cause mortality (194, 210). Age-related increases in arterial stiffness are primarily caused by structural alterations in the media including increases in collagen content (173), decreases in elastin (34), decreases in elastin cross linkage (which are responsible for elasticity) (198), accumulation of advanced glycation end products (163), smooth muscle atrophy (184), and calcification, especially in the elastin-rich regions (56). Age-related alterations also occur in the intima; however, these changes are more closely associated with atherogenesis, as covered later in this review.

CR has been shown to attenuate age-related increases in arterial stiffness, at least in animal models. In older rats, CR resulted in less arterial stiffness compared with free-fed rats, as evidenced by greater fractional expansion of the aorta during the cardiac cycle (i.e., arterial distensibility) and slower pulse wave velocity (2). These effects of CR are accompanied by less fibrous matrix (i.e., collagen) accumulation, more elastin, and better preservation of vascular smooth muscle (2, 68). Furthermore, CR appears to prevent age-related vascular accumulation of the proteoglycan decorin (68), which has been shown to promote vascular fibrosis and mineralization through the activation of TGF-β (158, 212). Less arterial stiffness has also been shown in arteries isolated from spontaneously hypertensive rats exposed to short-term CR (i.e., 5 wk) (53). To our knowledge, studies have not been published on the effects of CR on arterial stiffness in humans.

The mechanisms by which CR attenuates age-related increases in arterial stiffness may involve reductions in oxidative stress and systemic inflammation and alterations in NO bioavailability. Age-related increases in oxidative stress promote the production and accumulation of lipid peroxidation products including 4-hydroxynonenal (HNE) in the rodent aorta (24). HNE is known to promote fibrosis by inducing the fibrinogenic cytokine TGF-β (122), at least partly through the activation of activator protein-1, which is a transcription factor for the TGF-β gene (21). CR in rodents has been shown to attenuate the age-related increases in aortic HNE, TGF-β, and fibrosis (24). The reductions in oxidative stress that result from CR, as well as increased eNOS levels, contribute to greater NO bioavailability in old age, which can also contribute to CR-mediated reductions in arterial stiffness (207). With respect to chronic inflammation, both cross-sectional (94, 196) and intervention studies (195) have demonstrated that systemic inflammation increases arterial stiffness. Furthermore, inflammation-reducing medications, including TNF-α antagonists and statins, have been shown to decrease arterial stiffness (72, 142). As outlined earlier in this article, CR has a potent anti-inflammatory effect in the vasculature, and this may be partly responsible for the protection against the development of arterial stiffness with increasing age.

Atherosclerosis. The development of atherosclerosis is a complex process and is not fully understood. Because atherogenesis can occur for many decades before atheromas develop and because many of the physiological conditions that promote atherogenesis (i.e., oxidative stress, chronic systemic inflammation, type 2 diabetes, endothelial dysfunction, high blood pressure, and dyslipidemia) develop in older age (18, 29, 70, 77, 82, 139, 186), advanced atherosclerotic lesions and their clinical sequelae (such as coronary artery disease and cerebrovascular disease) are far more common in older age than in young adulthood (114).

It is beyond the scope of this review to provide a detailed description of the mechanisms involved in atherogenesis; comprehensive reviews have been published elsewhere (84, 125). However, in brief, and as described in the review articles (84, 125), atherogenesis is a three-stage process. The earliest stage involves "injury" to the endothelium, resulting from oxidative stress and other factors. Blood constituents such as oxidized LDL leak through the injured endothelium and trigger an inflammatory response, in which macrophages phagocytose the invading agents to form foam cells. During the second stage, a fibrous cap consisting of smooth muscle, collagen, and calcium forms on the developing plaque to provide structural stability. Within the plaque, a core containing free cholesterol/lipid and

necrotic tissue forms and becomes enriched with procoagulants. At the most advance stage, focal degradation of collagen and thinning of the fibrous cap make the plaque prone to rupture. Plaque rupture results in rapid activation of coagulation, arterial lumen obstruction, and ischemia to downstream tissues.

CR may protect against atherogenesis; however, data are limited. A major limitation to using rodents for studies on the effect of CR on atherogenesis is that rodents are generally not prone to atherosclerosis (primary cause of death is generally cancer). However, one study circumvented this problem by using a genetically engineered mouse model that is prone to atherosclerosis (i.e., the homozygous *apolipoprotein E* knockout) (80). In this study, mice undergoing 60% CR and those in a free-feeding control group all had early stage atherosclerosis, as evidenced by the presence of foam cells and free lipids in the wall of the aorta. However, the atherosclerotic lesions were 33% smaller in the CR group. Furthermore, CR appears to have blunted the development of more advanced atherosclerotic plaques, as evidenced by half as many mice with evidence of fibrous caps and plaque calcification and two-thirds fewer plaques with an "acellular" necrotic lipid core. These intriguing data were accompanied by lower levels of lipid hydroperoxide (oxidative damage marker) in the aortia and lower levels of the ROS production (i.e., superoxide and hydrogen peroxide) (80).

Preliminary data have been published on the effects of CR on atherosclerosis in nonhuman primates. In a preliminary report from a 20-year longitudinal study of Rhesus macaques (CR, $n = 38$; and control, $n = 38$) in which longevity will eventually be the primary outcome, 13% ($n = 5$) of the CR-group monkeys have died from age-related causes, which is significantly fewer ($P = 0.03$) than that in the control group (37%, $n = 14$), indicating a significant longevity-enhancing effect of CR. Although only half as many monkeys in the CR group died of CVD (CR group, $n = 2$; and control group, $n = 4$), the number of cardiovascular deaths is far too small to make any conclusions. Furthermore, most of the CVD deaths were attributed to valvular endocardiosis, cardiomyopathy, and myocardial fibrosis, and thus these data do not provide insights regarding the effects of CR on atherogenesis. Another study of nonhuman primates used cynomolgus monkeys (26, 28), which are reported to be a good model for human atherosclerosis (201). The feeding intervention was four years in duration, was initiated in adulthood, and provided equal amounts of dietary cholesterol in the CR and control groups so that the effect of CR, per se, could be evaluated. Results showed no difference between the CR and control groups in terms of intimal thickness, as an index of atherosclerosis extent, in the coronary and abdominal arteries (26, 28). However, it has been argued that intimal thickening is not necessarily indicative of atherosclerosis, per se. Rather, a thicker intima simply reflects an aging artery that is more prone to atherogenesis (114). In this context, the data showing that CR in monkeys does not affect intimal thickness could be interpreted to indicate that four years of CR in adult monkeys is not sufficient for altering primary vascular aging, without drawing conclusions about the effects of CR on atherosclerosis.

The only data on the effect of CR on atherosclerosis in humans are based on the observational data from Japanese Okinawans. Men and women living in Okinawa during the 1940s–1960s were estimated to have been calorie restricted without evidence of malnutrition (208). Age-adjusted mortality from coronary heart disease in these individuals was 49% lower than that of other Japanese men and women and 87% lower than individuals residing in the United States (208). While this study provides evidence that moderate CR provides powerful protection against coronary artery disease, the information should be interpreted cautiously, as numerous other aspects of the Okinawans' diet (or lifestyle in general) could have contributed to protection against atherosclerosis. For example, they had high intakes of antioxidants from vegetables and fruits and their diet was rich in isoflavones from soy and other legumes; furthermore, they rarely consumed processed foods such as refined sugar (209).

Risk factors for atherosclerosis. In light of the difficulties in evaluating the effect of CR on atherogenesis, several studies have evaluated the effect of CR on risk factors for atherosclerosis. Not surprisingly, CR results in lower body weight and lower levels of both total and central/visceral fat mass in studies on numerous species including monkeys (27, 28, 37) and humans (49, 64, 153, 155). In humans, these changes corresponded with reductions in adipocyte size and with lower hepatic lipid content; however, intramyocellular lipid was not altered by CR (117).

One of the hallmark characteristics of CR in rodents and monkeys is lower plasma insulin levels and improved glucoregulation (14, 27, 79, 96, 101, 115, 132). Furthermore, 20 years of CR in monkeys has been shown to completely prevent glucoregulatory impairment, whereas in the control animals, 13% have developed diabetes and an additional 29% have developed prediabetes (35). While improvements in glycemic control have been hypothesized to contribute to the antiaging effect of CR (30), they would also be expected to protect against atherosclerosis and CVD, as hyperglycemia, insulin resistance, and diabetes are associated with atherosclerosis and CVD risk (13, 69, 76, 90, 215). Three randomized trials on men and women have demonstrated clear improvements in glucoregulation in response to CR. Two of these trials showed that CR decreases fasting insulin with no change in fasting glucose, indicating improved insulin action (48, 117); one of these trials also showed a strong tendency for CR-induced improvements in insulin action as measured by using an intravenous glucose tolerance test (117). Our trial showed that 20% CR for 12 months reduced fasting insulin (−37%) and oral glucose tolerance test insulin (area under curve, −24%); this, paired with a tendency for lower glycemia, resulted in a 26% improvement in insulin action (202). Additional insights about the effects of CR in humans comes from the Biosphere-2 experiment, in which eight men and women were sealed into a closed 3.14-acre dome for two years for the purposes studying ecological factors that influence the earth's biosphere (4). During the two-year experiment, food production was inadequate to meet the needs of the crew, and as a result, they were calorie restricted but consumed a micronutrient-rich diet. Relative to preentry levels, fasting glucose decreased by 21% and fasting insulin decreased by 42% (197), suggesting a profound improvement in glucoregulation. While data from the randomized trials, the Biosphere-2 experiment, and the studies on nonhuman primates show clear and powerful effects of CR to improve glucoregulation, the results from our observational study on adults undergoing 3–20 years of strict self-imposed

CR are not as clear. When compared with nonobese control subjects consuming a typical western diet, the CR practitioners had 13% lower fasting glucose and 80% lower fasting insulin levels, suggesting that CR improved insulin action as shown in the other studies. However, 2 h glucose from an oral glucose tolerance test was 14% higher in the CR group, an effect that may have been attributable to somewhat lower postprandial insulin levels. Furthermore, 39% of the CR practitioners met the criterion for impaired glucose tolerance and another 21% had high-normal 2-h glucose values. An explanation for this unanticipated finding is not clear, and it is not known how this finding of low-fasting glycemia paired with high-postprandial glycemia would affect CVD risk. However, the relationship between aging/aging-related diseases and insulin resistance is confounded by associated factors such as excessive abdominal adiposity, decreased physical activity, hyperinsulinemia, dyslipidemia, inflammation, and other metabolic and hormonal components of the metabolic syndrome (14, 154). Interestingly, the CR individuals with high-postprandial glycemia values were extremely lean and had very low fasting plasma concentrations of glucose and insulin and an outstanding metabolic profile (very low triglyceride, high HDL-cholesterol, high adiponectin, and extremely low C-reactive protein concentrations) (62).

Studies on the effects of CR on blood lipids and lipoproteins in primates have yielded mixed results. Studies of adult cynomolgus monkeys exposed to four years of CR on a diet that was enriched with crystalline cholesterol (i.e., dietary cholesterol was equal in the CR and control groups) generally revealed negative findings. CR did not affect serum concentrations of total, HDL-, LDL-, or very low-density lipoprotein-cholesterol, triglycerides, or intimal thickness (28). Furthermore, despite strong evidence that CR reduces oxidative stress in the vasculature (reviewed above), LDL oxidation was not affected by CR (25). These findings suggest that CR, in the absence of a reduction in dietary cholesterol, does not alter lipid profile. In studies that did not enrich the diet with cholesterol and which also used a different species of monkey (i.e., rhesus), CR resulted in a very large 50% reduction in plasma triglycerides but did not alter total, HDL-, or LDL-cholesterol (55). Although LDL oxidation was not altered by CR (25), LDL particles from CR animals were found to have less proteoglycan binding (55), which would be expected to result in lower levels of LDL in the arterial wall, thereby making the LDL particles less atherogenic (22, 126). This finding is supported by research on rodents showing that CR attenuated the age-related increase in elastin-associated LDL in the aortic wall (68). Plasma levels of lipoprotein (a) were lower in the CR monkeys, which is another adaptation that would be expected to reduce atherosclerotic disease risk (47, 216). Furthermore, although HDL-cholesterol was not altered by CR, the HDL_{2b} subfraction was higher in CR monkeys. HDL_{2b} is the HDL subfraction that is most closely associated protection against atherosclerosis (20, 98) and plasma concentrations were also found to be 38% higher in human centenarians compared with normolipidemic healthy weight controls (11).

In CR studies on humans, the effects of CR on lipoproteins are profound (Table 2). In the randomized intervention trials, total and LDL-cholesterol decreased by 5–15% (48, 66, 121), whereas in observational trials on more strict CR in humans, total, and LDL-cholesterol levels were ~30% lower than in control subjects with total cholesterol values of 130–160 mg/dl in the CR group (64, 197). Although one randomized trial and one observational trial reported increases in HDL-cholesterol (48, 64), others reported no change in HDL-cholesterol (66, 121) and one reported a large reduction (197). Large reductions in plasma triglyceride concentrations were seen in all studies with CR resulting in an average triglyceride concentration of ~85 mg/dl (48, 64, 66, 121, 197).

Blood pressure was not affected by four years of CR in adult cynomolgus monkeys (28). However, although there was no evidence of an age-related increase in blood pressure in rhesus monkeys, CR resulted in ~10 mmHg lower systolic and diastolic blood pressures (116). Randomized controlled trials on human CR have also produced equivocal results for blood pressure (Table 2). One reported no effect of 6 months of CR on blood pressure. While the other trial also reported no effect of CR on blood pressure (66), a subsequent analysis which excluded subjects who were not compliant with the CR intervention revealed a modest reduction in blood pressure (i.e., 10 mmHg systolic, and 6 mmHg diastolic) in response to 12 months of CR (156) (Table 2). Observational trials on longer-term strict CR showed substantial blood pressure lowering effects of 20–25% with CR, resulting in systolic pressures of ~95 mmHg and diastolic pressures of ~60 mmHg (64, 197).

Effects of Calorie Restriction on Cardiac Function

Increasing age is associated with a thickening of the myocardium and an increase in left ventricular mass/hypertrophy,

Table 2. *Result from human trials on the effects of CR on plasma lipid concentrations, BP, and CRP*

	TC	LDL-C	HDL-C	TG	Systolic BP	Diastolic BP	CRP
Randomized trials with 6-12-mo interventions							
Tufts University (48)	↓5	↓7	↑13	↓16	NR	NR	NR
PBRC (121)	NR	↓6	(↑9)	↓21	(↓2)	(↓2)	(↓29)
Washington University (66, 156)	↓10	↓14	(↑6)	↓23	↓8	↓9	(↓30)
Observational trials of long-term strict CR							
Biosphere 2 (197)	↓30	↓33	↓27	↓22	↓25	↓22	NR
CRON (64)	↓23	↓32	↑24	↓67	↓23	↓23	↓81
Mean of all trials	↓17	↓18	↑5	↓30	↓15	↓14	↓47

Values are percent changes from baseline. Values in parentheses were not significantly different from the control group in the Pennington Biomedical Research Center (PBRC), Washington University, and Washington University study on self-imposed calorie restriction with optimal nutrition (CRON) studies or were not significantly different from zero in the Tufts University and Biosphere-2 studies (these studies did not include a control group). BP, blood pressure; CRP, C-reactive protein; TC, total cholesterol; C, cholesterol; TG, tryglycerides; NR, not reported.

even after accounting for age-related increases in blood pressure, obesity, and vascular and valvular heart disease (124). Furthermore, aging is associated with myocardial fibrosis (40), increases in the number of collagen cross-linkages (19), and increases in the size and decreases in the number of cardiomyocytes (7, 147). These alterations result in an increase in stiffness, a reduction elastic recoil, a reduction in the passive suction-mediated early diastolic filling, and a greater reliance on the atrium for diastolic filling (32, 103, 140). There is also an age-related shift in myosin isoform in cardiac muscle from the fast V1 (α-myosin heavy chain) isoform to the slow V3 (β-myosin heavy chain) isoform (38, 129, 160), which may contribute to the slower contraction and relaxation of cardiac muscle seen in older hearts (110). Furthermore, age-related increases in arterial stiffness, as reviewed earlier in this article, result in a greater afterload on the heart (111). Although systolic function (based on ejection fraction) is generally preserved with increasing age in individuals without CVD (59), the greater myocardial work required to overcome the increased afterload contributes to further cardiac hypertrophy (32).

Studies on rodents have demonstrated that CR prevents part of the age-related deterioration in diastolic filling as evidenced by a greater ratio of Doppler-measured earlier to late diastolic filling velocity (i.e., E-wave-to-A-wave ratio) (179). Similar effects of CR have been demonstrated in nonobese spontaneously hypertensive rats (53) and in Dahl salt-sensitive rats (164), even in the presence of a high-salt diet (164). These effects may be partly mediated by reductions in blood pressure but were also accompanied by reductions in inflammatory cytokines (164) and improvements in vascular compliance and function (53). Histological studies have shown that CR also decreases cardiomyopathy, cardiac fibrosis, myocardial degeneration (100). Recent research has identified numerous other factors that are associated with CR-mediated preservation of diastolic function in rats, including reductions in cardiomyocyte size, less accumulation of β-galactosidase and lipofuscin (senescence markers), less myocyte apoptosis, less deterioration of intracellular calcium handling with faster diastolic relaxation, and increases in autophagy, which were associated with suppression of the mammalian target of rapamycin pathway (167) [inhibition of mammilian target of rapamycin with rapamycin has been shown to reverse cardiac hypertrophy (135) and may contribute to lifespan extension in mice (85)]. Insights into the mechanisms for the cardiac adaptive responses to CR also come from microarray gene expression studies on mouse hearts (52). Long-term CR, and to some extent, short-term CR alters the gene expression profile in a manner that is consistent with protection against age-related increases in myocardial remodeling and fibrosis and against age-related decreases in contractility and lipid metabolism. Additionally, and consistent with more youthful cardiac phenotype, immunohistochemical studies showed less perivascular collagen in the hearts of old CR mice and smaller myocyte size (52). Some of the beneficial effects of CR on the aged myocardium in rodents appear to be mediated by reductions in oxidative stress and damage, as evidenced by less dityrosine cross-linking of cardiac muscle proteins (120), better preservation of mitochondrial membrane fluidity (mitochondrial oxidative damage marker) and less lipid peroxidation (119), and less mitochondrial free radical production and less oxidative damage to mitochondrial DNA in the myocardium (78).

Studies on humans generally corroborate the findings from rodent studies. In middle- to older-aged individuals, we showed that diastolic function was better in subjects who practiced strict CR for 3–15 years than that in healthy age- and sex-matched control subjects, as evidenced by Doppler studies showing lower late diastolic (A wave) velocities, greater early filling fraction, a higher E-wave-to-A-wave ratio, and greater early diastolic relaxation (E′ wave from tissue Doppler imaging) (138) (Fig. 2). Insights into the physiological adaptations responsible for these improvements were obtained by using the parameterized diastolic filling (PDF) formalism (107), which indicated that CR subjects had less ventricular stiffness and less viscous loss of diastolic recoil (138), both of which would be consistent with less myocardial fibrosis. We also performed a randomized controlled trial to investigate the effects of one year of calorie restriction in middle-aged men and women with high-normal or slightly overweight body weight. In support of our cross-sectional study (138), results indicated that CR resulted in a lower isovolumic relaxation time and early diastolic filling with PDF-derived indexes, indicating less myocardial stiffness and less viscoelasticity (as an index of relaxation damping) (156) (Fig. 2). Furthermore, CR resulted in improvements in the PDF-based indexes of peak atrioventricular pres-

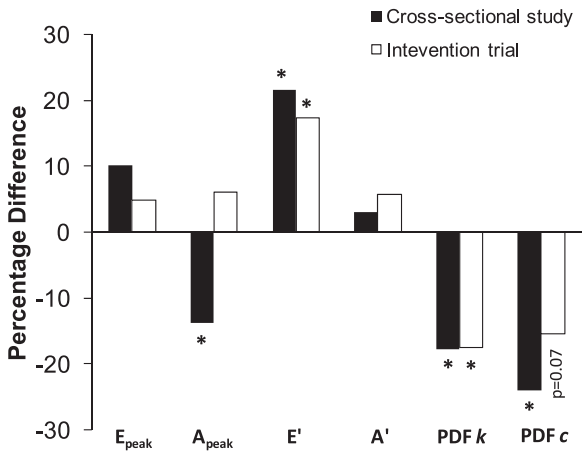

Fig. 2. Effects of CR on left ventricular diastolic function in humans. *$P < 0.05$ vs. zero. Collectively, these data indicate that CR improves left ventricular diastolic function in healthy humans, partly by decreasing ventricular stiffness and partly by decreasing the damping of elastic recoil of the ventricle during diastole. For the cross-sectional data, values are percent difference between individuals undergoing long-term (3–15 yr) of self-imposed strict CR compared with age- and sex-matched healthy control subjects who were consuming a typical U.S. diet (138). For example, the 10% value for E_{peak} in the figure (black bar) indicates that the CR group had a 10% greater E_{peak} value than the control group. For the intervention trial, values are percent difference between baseline and follow-up measures in men and women undergoing a yearlong CR intervention (156). For example, the 5% value for E_{peak} in the figure (white bar) indicates that E_{peak} increased by 5% during the CR intervention. E_{peak}, early suction-mediated diastolic ventricular filling as measured with pulsed-Doppler transmitral flow assessments; A_{peak}, late/active atrium-driven diastolic ventricular filling as measured with pulsed-Doppler transmitral flow assessments; E′, early diastolic function as measured by tissue Doppler imaging of the septal aspect of the mitral valve annulus; A′, late-diastolic function as measured by tissue Doppler imaging of the septal aspect of the mitral valve annulus; PDF *k*, global ventricular stiffness parameter based on the parameterized diastolic filling (PDF) formalism (107); PDF *c*, index of viscoelastic loss of ventricular recoil during diastole as determined by using the PDF formalism.

sure gradient and indexes of stored strain energy, both of which would contribute to enhanced diastolic function (156).

Surprisingly, some research on rodents suggests that CR augments some age-related changes in the heart. Both long- and short-term CR was shown to augment the age-related increase in contraction and relaxation times in isolated rodents hearts (105). This alteration appears to coincide with a CR-induced increase in the age-related shift in cardiac myosin isoform from the fast V1 isoform to slow V3 (81, 104, 143). In our CR studies on humans, we saw no evidence for prolonged ventricular relaxation based on isovolumic relaxation time, E-wave duration, or early diastolic transmitral flow (E wave) or early diastolic mitral annulus velocity (E′ wave) (138, 156).

Aging-related alterations in cardiac function are partly dependent on changes in autonomic function. These changes include decreases in the inotropic (50, 113) and chronotropic (176) responses to β-adrenergic stimulation, part of which are mediated by a reduction in myocardial β-adrenergic receptor density (206), reductions in maximal heart rate (182, 203), decreased heart rate variability (6, 60, 161), diminished left ventricular systolic reverse capacity (150), and a delayed cardiac response to carotid baroreceptor activation (58).

CR has been shown to alter several aspects of these age-related alterations in autonomic function. In rodents, CR has been shown to prevent the age-related decline in β-adrenergic receptor density, thereby preserving the inotropic and chronotropic responses to β-adrenergic (isoproterenol) stimulation (42, 105). Normal aging is associated with increases in circulating catecholamines (88), an effect that is associated with the induction of catecholamine biosynthetic enzymes in the adrenal medulla (99). CR has been shown to attenuate the expression of catecholamine biosynthetic enzymes (57) and reduce oxidative damage to the adrenal medulla (205). However, it is not clear whether long-term CR reduces circulating catecholamine levels (123). CR has also been shown to attenuate the age-related deterioration in the baroreceptor reflex in rodents (87, 183), suggesting an enhanced control of blood pressure through autonomic control of the heart and vasculature. Further evidence for a beneficial effect of CR on autonomic function comes from rodent studies showing a decrease in low-frequency diastolic blood pressure variability, suggesting lower sympathetic tone and greater high-frequency heart rate variability, implying greater parasympathetic tone (130). Studies using power spectral analysis of heart rate variability have also shown that six months of calorie restriction in nonobese humans results in decreases in sympathetic activity, increases in parasympathetic activity, and a decrease in the sympathetic/parasympathetic balance (51).

Summary and Future Research Directions

Calorie restriction has numerous beneficial effects on the aging cardiovascular system, many of which appear to be mediated, at least partly, by reductions in oxidative stress and inflammation in the vasculature and heart. With respect to vascular benefits, animal studies have demonstrated that CR attenuates the age-related decline in the antiadhesion and vasodilatory functions of the endothelium and blunts the increase in arterial stiffness that occurs with advancing age. Future studies are needed to determine whether CR in humans also has beneficial effects on endothelial function and arterial stiffness. Evidence from animal studies also suggests that CR protects against atherosclerosis; however, these findings are limited, largely because most animal species used in CR studies are not prone to atherosclerosis. While human CR studies involving direct measures of atherosclerosis are currently not practical or ethical for use in healthy human subjects, developing technologies for imaging of atherosclerosis [as reviewed elsewhere (159)] may make such studies feasible in the future. In the meantime, a large body of evidence, from both human and animal studies, indicates that CR has profound beneficial effects on risk factors for atherosclerosis. Furthermore, epidemiologic data from Japanese Okinawans suggest that CR protects against coronary heart disease.

CR also has beneficial effects on cardiac function. In animal models, CR has been shown to attenuate numerous age-related changes in the heart (including the development of hypertrophy and myocardial fibrosis), shifts in myosin isoform composition, histological changes, increases in cardiomyocyte apoptosis, and the deterioration of chronotropic and inotropic responses to adrenergic stimulation. Although such data are not available from human CR studies, echocardiographic studies have demonstrated that CR improves diastolic cardiac function in healthy nonobese humans and that these adaptations correspond with reductions in indexes of myocardial stiffness. Additional studies are needed, especially in humans, to elucidate other aspects of CR on the aging of the heart. For example, no studies have evaluated the effect of CR on the profound age-related loss of the ability of the heart to respond to acute physical stress/exercise (i.e., heart rate response and systolic reverse capacity) or on the shift in substrate metabolism that occurs in the aging heart. Additionally, more basic research is needed to elucidate the molecular adaptations that mediate the antiaging effects of CR on the cardiovascular system.

Conclusion

Research on animals indicates that CR has a powerful ability to prevent many of the age-related changes in the structure and function of the cardiovascular system. Furthermore, research on humans has demonstrated that CR, when introduced in mature adults, may reverse some of the age-related changes in the cardiovascular system, although much more research on humans is needed. Taken together, these findings suggest that CR protects against the progressive decline in cardiovascular function that occurs with increasing age. They also show that CR has a powerful effect to prevent diseases of the cardiovascular system. These effects on the cardiovascular system, in combination with other benefits of CR, such as the protection against the development of excess adiposity and insulin resistance/type 2 diabetes, hypertension, and protection against cancer, suggest that CR may have a major beneficial effect on health span, life span, and quality of life in humans.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

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