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Mechanical Regulation of Cardiac Aging in Model Systems
Aging and Autophagy in the Heart
Macromolecular Degradation Systems and Cardiovascular Aging
Mitochondrial Metabolism in Aging Heart

Dietary Interventions, Cardiovascular Aging, and Disease: Animal Models and Human Studies
Pharmacological Strategies to Retard Cardiovascular Aging

Guest Editors: Guido Kroemer and Lorenzo Galluzzi

Dietary Interventions, Cardiovascular Aging, and Disease Animal Models and Human Studies

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Abstract: Recent studies indicate that dietary interventions have the potential to prevent and even treat cardiovascular disease, which is the leading cause of death. Many of these studies have focused on various animal models that are able to recreate one or more conditions or elevate risk factors that characterize the disease. Here, we highlight macronutrient-focused interventions in both mammalian model organisms and humans with emphasis on some of the most relevant and well-established diets known to be associated with cardiovascular disease prevention and treatment. We also discuss more recent dietary interventions in rodents, monkeys, and humans, which affect atherosclerosis and cardiovascular diseases with focus on those that also delay aging. (*Circ Res.* 2016;118:1612-1625. DOI: 10.1161/CIRCRESAHA.116.307473.)

Key Words: CR ■ DR ■ FMD ■ health span ■ protein restriction

In recent years, a range of dietary interventions have been proposed as alternative to drugs for the prevention and treatment of cardiovascular and other age-related diseases. This is in part because of research demonstrating the beneficial effects of specific dietary interventions in reducing risk factors associated with cardiovascular diseases (CVD), as well as mortality from CVD in both nonhuman and human primates. Although the dietary guidelines put forth for CVD prevention take into consideration some of the research on dietary restriction (DR), they are mostly focused on epidemiological and some clinical studies related to diet composition (The 2015 Dietary Guidelines for Americans; US Department of Agriculture, Center for Nutrition Policy and Promotion. Available from: <http://www.cnpp.usda.gov/DietaryGuidelines> (cited 2/23/2016)). In 2013, CVD, the leading cause of death in the world, accounted for 30.8% of all deaths in the United States. A major risk factor for fatal cardiovascular events is atherosclerosis, which is characterized by the accumulation of

fatty materials, including cholesterol, in the arteries leading to thickened walls.¹ In this review, we discuss the effect of macronutrient modulations on CVDs in both animal models and humans. We do not cover other important studies that evaluate various types of dietary and pharmacological interventions that are not centered on macromolecules or health-span. A comprehensive overview of interventions with the potential to affect human aging is discussed elsewhere.²

Definitions

1. Caloric restriction (CR) in most cases refers to a 20% to 40% reduction in total calorie intake but normal levels of micronutrients.
2. DR is sometimes used interchangeably with CR. However, in this review, DR is used to refer to restriction of particular macronutrient, such as proteins, carbs, or fats with or without caloric restriction. This type of intervention may also involve CR.

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Nonstandard Abbreviations and Acronyms

Apo	apolipoprotein
CETP	cholesteryl ester transfer protein
CHD	coronary heart disease
CHF	congestive heart failure
CR	caloric restriction
CVD	cardiovascular disease
DR	dietary restriction
HDL	high-density lipoprotein
HFD	high-fat diet
IGF-1	insulin-like growth factor 1
LDL	low-density lipoprotein
LDLR	low-density lipoprotein receptor
NIA	National Institute of Aging
SHR	spontaneously hypertensive rat
SHROB	spontaneously hypertensive obese
WNPRC	Wisconsin National Primate Research Center

- Fasting refers to a complete absence of food intake. Fasting can be administered for various time frames and can be repeated many times. The term short-term starvation is used to describe *in vitro* studies on fasting and also to describe a 48- to 72-hour fasting periods in mice. Prolonged fasting refers to fasting periods exceeding 2 days in mice and 3 days in humans. Intermittent fasting or alternate-day fasting in most cases refer to the long-term use of feeding every other day in mice or humans a standard level of food with no or minimal food being administered on the fasting day.
- Fasting mimetics are pharmacological agents that, when administered, trigger some of the effects of fasting.
- Fasting mimicking diet are formulations compositions of macronutrients and micronutrients specially formulated to trigger responses, assessed by the measurements of several markers, including glucose and insulin-like growth factor 1 (IGF-1), while minimizing CR.

Murine Models for CVDs

Rats and mice are valuable organisms to understand the mechanisms of CVDs and identify potential therapies. However, murine models have limitations, including a small heart size, fast heart rate, and absence of pericardial fat.^{3,4} In addition, rodents are not particularly representative of human heart physiology, mostly because the major myosin heavy chain in rodents is α -myosin heavy chain, whereas larger mammals including humans express β -myosin heavy chain. Moreover, the difference in lipid metabolism between humans and rodents largely affects the formation of atherosclerotic plaques and the onset of CVD. Because of the relatively high natural resistance of mice and rats to atherosclerosis compared with humans, the knockout and transgenic models with accelerated CVD phenotypes are often used.⁵ It is important to be aware of these limitations when assessing the literature discussed in the following sections.

Diet-Induced Murine Models

High-fat diet (HFD) interventions leading to metabolic syndrome have gained center stage as murine CVD and diabetes mellitus models. Both male and female mice on a HFD with 60% calories from fat have increased body weight, increased white adipocyte tissue, increased fasting glucose levels, cardiac dysfunction, and more pronounced insulin resistance in male mice.^{6–9} The HFD-fed mice also display higher levels of serum total cholesterol, triglycerides, and low-density lipoprotein (LDL) when compared with standard chow-control mice.^{7–9} Resveratrol, a natural activator of SIRT1 found in red grapes and green tea,¹⁰ mimics some of the effects of CR and has been shown to reverse the detrimental effects of the HFD in mice.⁹ Resveratrol also increases insulin sensitivity and lowers fasting glucose levels in HFD-treated mice.⁹

Cardiovascular homeostasis is maintained by a cascade of biochemical reactions known as the renin–angiotensin system.^{11,12} Increased intake of high-fat foods directly affects this homeostasis and contributes to vascular diseases by promoting major changes in arterial tissue, especially through the activation of renin–angiotensin system and the induction of oxidative stress and proinflammatory factors.¹³ The HFD promotes increased disorganization of collagen fiber in the vascular wall of the aorta and induces aortic stiffening partly by elevating oxidative stress damage and activating renin–angiotensin system in vascular tissue.¹³

A variation of the HFD, called the Paigen diet, can also induce CVDs-associated damage in murine models. This diet was developed in 1985 by combining the atherogenic Thomas–Hartroft diet,¹⁴ which contains 30% cocoa butter (primarily saturated fats) with Purina breeder chow, which contains 10% fat. The resulting diet contains 1.25% cholesterol, 0.5% cholic acids, and 15% of fat, mainly oleic, palmitoleic, palmitic, and stearic fatty acids with a ratio of polyunsaturated to saturated fats of 0.69. The Paigen diet overcomes the high mortality observed with the Thomas–Hartroft diet while maintaining the atherogenic properties.¹⁴ The lesions observed in mice directly correlate with the percent saturated fatty acids and circulating cholesterol.^{15–17} Verges et al demonstrated that when each of the 3 components thought to affect plasma cholesterol levels were selectively eliminated, fat had no influence, whereas cholate and cholesterol had small and large effects, respectively.¹⁸ Additionally, in the same study, dietary cholesterol was involved in acute hepatic inflammation and influenced genes associated with hepatic fibrosis. The cholate accentuated diet-induced hypercholesterolemia by inhibiting cholesterol's conversion to bile acid.¹⁸ Despite the variation in the degree of penetrance and effects of the Paigen diet between different mouse strains, and on the levels of high-density lipoprotein (HDL) in each mouse strain and rats,¹⁹ the diet-dependent lesions resemble the ones observed in humans.²⁰

In a study by Tanner et al evaluating cardiovascular response to fasting in mice on chow versus HFD diet, in the fed state, HFD mice had elevated heart rates and mean arterial blood pressure; however, after 16 hours of fasting, these parameters decreased in both groups.²¹ As expected, HFD mice had higher fasting triglycerides; larger body weight, gonadal fat pad mass, and total fat mass; and elevated serum leptin,

regardless of feeding condition. The study highlighted that the metabolic and cardiovascular changes that are brought on by fasting occur sooner in obese mice than in lean ones, as measured by heat production and oxygen consumption. In addition, fasting also affected the locomotion of HFD animals because the animals were markedly less active during the fasting period cycle than during their fed state. HFD mice also consumed less water and produced less urine over the 24-hour periods of feeding and fasting when compared with control animals.²¹

Over 40 years ago, Small et al demonstrated that excessive amounts of cholesterol in cells can destroy membrane functions or result in atherosclerotic damage to blood vessels.²² Hypercholesterolemia observed during aging can be explained by the change in homeostasis between the many signaling and metabolic constituents of the cholesterol signaling pathway that includes transcriptional regulation of 3-hydroxy-3-methylglutaryl coenzyme A reductase and LDL receptor (LDLR), which, in turn, are dependent on sterol regulatory element-binding proteins.²³ Caloric restriction, which generally refers to a chronic 20% to 40% reduction in calorie intake compared with ad libitum consumption, is well established to reduce cholesterol levels and alleviate its detrimental effects.²⁴ Martini et al investigated whether the beneficial effects of CR are because of its impact on the cholesterol biosynthesis and signaling pathway, specifically 3-hydroxy-3-methylglutaryl coenzyme A reductase and LDLR.²⁵ The authors demonstrated that in rats, CR reduces the aging-related effects of factors involved in the synthesis and degradation rate of 3-hydroxy-3-methylglutaryl coenzyme A reductase. They also demonstrated that this effect depends on both the correct membrane LDLR localization and on the proper restored 3-hydroxy-3-methylglutaryl coenzyme A reductase activity (Figure).²⁵

Obesity Knockout and Transgenic Models

Several genetic rodent models of obesity and insulin resistance have become available to test the effect of dietary interventions on the onset and development of CVDs. Here we consider and describe those that allow the evaluation of some of the most crucial markers leading to CVD, such as hormonal signaling, fatty acid metabolism/transport, and inflammation. We also report the results of studies aimed at addressing the effect of CR on CVD onset in some of these models. Among others, the models described include the *db/db* and *ob/ob* mouse strains, as well as rats carrying the 2 mutant genes *fat (fa)* and *corpulent (cp)*.

Leptin is a circulating hormone produced by adipose cells that acts by binding to the leptin receptor in the hypothalamus to inhibit hunger and stimulate satiety. In both murine and rat *db* and *ob* mutant strains, the primary defect has been found to reside in the leptin system. In particular, the *ob/ob* mouse produces a defective leptin protein that does not bind to the leptin receptor (or ObR), and the *db* mouse mutation leads to a defective ObR.^{26–28} Mice homozygous for the fat cell-specific hormone receptor leptin (*Lepr^{db}* or *db/db*) have a defective receptor unable to bind leptin and develop significant obesity at around 3 to 4 weeks of age. The *db/db* mouse

model, discovered by JAX labs,²⁹ was first shown by Chen et al to contain a G→T point mutation, which results in a donor splice site that converts the 106 nt region to a novel exon retained in the OB-R transcript.³⁰ *db/db* mice are a genetic model of noninsulin-dependent diabetes mellitus that display many of the characteristics of human disease, including hyperglycemia, insulin resistance, and obesity.^{31,32} Importantly, as in human noninsulin-dependent diabetes mellitus, *db/db* mice have a marked decrease in skeletal muscle glucose utilization because of a defect in glucose transport that is not accompanied by significant alterations in GLUT4 expression,³³ which makes them an ideal model for the study of this disease. As observed in other animal models, *db/db* mice do not develop atherosclerosis or ischemia as expected. They, instead, develop symptoms associated with diabetic condition, such as abnormal cardiac metabolism and function, glomerular sclerosis, impaired vascular function, and retinal damage. As shown by Sleeman et al, some of the markers of metabolic function, such as serum nonesterified fatty acids and circulating triglyceride levels, can be reduced by food restriction.³⁴ Because the increased levels of nonesterified fatty acids have also been associated with higher risk of developing myocardial infarction and stroke,³⁵ CR is a potentially beneficial intervention to reduce some CVD-associated markers in a system with unbalanced leptin signaling, such as in obese subjects.³⁶

Mice homozygous for the obese spontaneous mutation, *Lep^{ob}*, commonly referred to as *ob* or *ob/ob*, eat excessively and present with obesity, hyperglycemia, glucose intolerance, elevated plasma insulin, subfertility, impaired wound healing, and an increase in hormone production from both pituitary and adrenal glands.³⁷ The *ob/ob* arose from a random mutation in the C57BL/6J colony at JAX labs.³⁸ The obesity is characterized by an increase in both the number and size of adipocytes. Although increased food consumption contributes to this obesity, homozygote mice gain excess weight and deposit excess fat even when the intake is restricted to that leading to normal weight maintenance in wild-type mice. Similarly to what is observed in *db/db* mice, this strain is defective in leptin signaling because of the production of a mutant form of leptin that does not bind to the ObR.³⁷ This mouse model has been used mostly to test the effect of leptin on glucose and insulin metabolism, rather than as a CVD model. Both *ob/ob* and *db/db* mice manifest myocardial hypertrophy, insulin resistance, and lipid accumulation.

Both *ob/ob* and *db/db* mice subjected to CR demonstrate that a reduced caloric regimen is sufficient to normalize body weight and glucose homeostasis, induce a 3-fold increase in circulating leptin levels, and reverse myocardial hypertrophy.^{39,40} However, CR alone is not sufficient to completely reverse the leptin deficiency in the transgenic animals and also results in increased circulating free fatty acids and triglycerides, possibly because of increased adiposity at least in caloric-restricted *ob/ob* mice.⁴⁰ Despite the significant increase in leptin levels during CR, Sloan et al associate the protection from myocardial hypertrophy to obesity prevention rather than to hormonal changes.⁴⁰ Furthermore, changes in cardiac metabolism that characterize obesity and diabetes mellitus

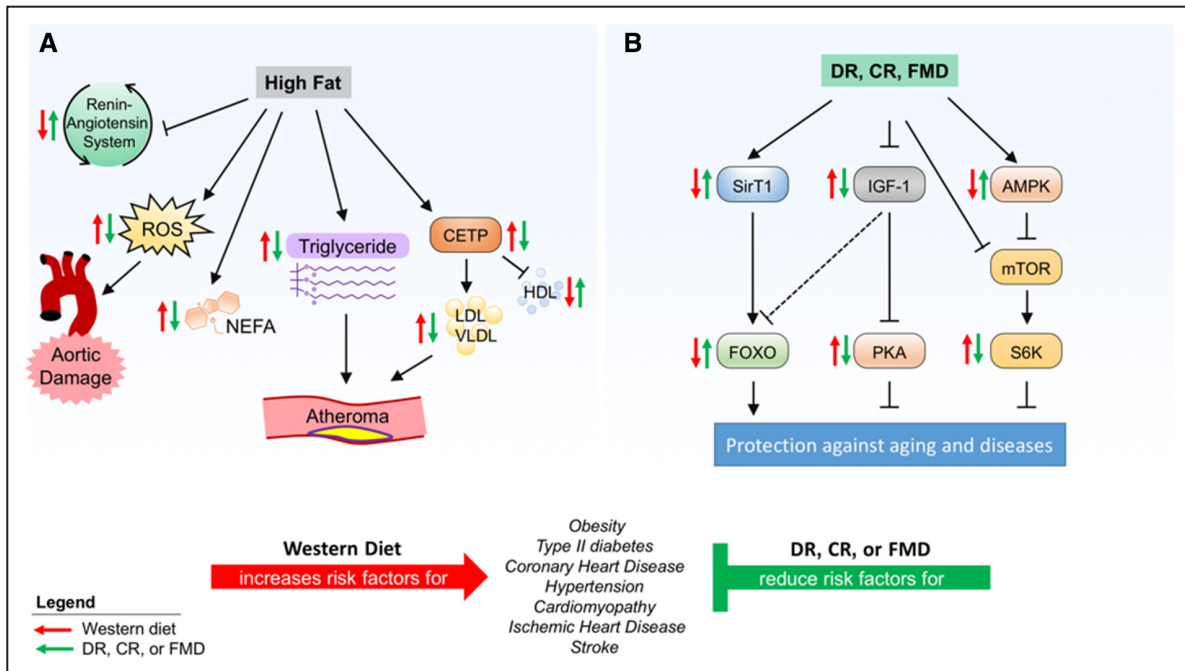


Figure. Effects of diets on cardiac health and aging. **A**, An overview of some of the major signaling pathways that are affected by high-fat diets and dietary restrictions discussed in this review. **B**, Molecular signaling pathways affected by dietary interventions and their effect on cardiovascular health and aging. AMPK indicates AMP-activated protein kinase; CETP, cholesteryl ester transfer protein; CR, caloric restriction; DR, dietary restriction; FMD, fasting mimicking diet; FOXO, forkhead box O transcription factors; HDL, high-density lipoprotein; IGF-1, insulin-like growth factor 1; LDL, low-density lipoprotein; mTOR, mammalian target of rapamycin; NEFA, nonesterified fatty acids; PKA, protein kinase A; S6K, S6 kinase; ROS, reactive oxygen species; and VLDL, very low-density lipoprotein.

persist in leptin-deficient mice but can be prevented by CR.^{39,41} This phenomenon is mediated by increased free fatty acid delivery to the heart.

Rat Knockout and Transgenic Models

Koletsky developed the spontaneously hypertensive obese (SHROB) rat model in 1969 by crossing a hypertensive female rat with a normotensive male Sprague–Dawley rat. The SHROB obese genotype (indicated as *fa^k* or fatty) entails a recessive mutation of the leptin receptor gene, which results in a premature stop codon that impacts the extracellular domain of the leptin receptor.⁴² Lean littermates of the SHROB rats carrying one copy or no *fa^k* allele are spontaneously hypertensive, but show only mild insulin resistance compared with normotensive rats.⁴² The *fa^k/fa^k* SHROB rat model is incapable of central and peripheral responses to leptin and is therefore leptin-resistant, showing circulating leptin levels that are nearly 170-fold higher than those of their lean littermates.^{42–44} SHROB rats are hyperlipidemic, hyperinsulinemic (fasting insulin levels are 20-fold higher than their littermates), and normoglycemic, in addition to having an abnormal response to a glucose load compared with their lean counterparts.⁴⁴ They also have significantly higher fasting plasma glucagon concentrations and higher insulin/glucagon molar ratios than lean spontaneously hypertensive rat (SHR) littermates.^{45,46} As a result, this strain shows an obese, spontaneously hypertensive phenotype useful for investigating clinical signs of metabolic syndrome X.⁴⁴

Because of the spontaneous development of hypertension, this rat model is best suited for the study of CVD onset and for

the development of preventative actions and lifestyle changes, such as dietary habits. However, Ernsberger et al have reported that although a low calorie diet can reduce blood pressure in this model (as has also been observed in humans), the extreme change of nutrient supply during refeeding is not beneficial for blood pressure and can lead to mild hypertension.⁴⁷

Wild-type rats are naturally not prone to developing atherosclerosis, although different kinds of stress can lead to myocardial lesions. As described earlier, a strain carrying a mutant corpulent (or *cp*) gene, which arose spontaneously in the Koletsky's hypertensive rat strain, has been shown to cause obesity, hyperlipidemia, and a fulminant atherosclerosis.⁴⁸ The Koletsky rat was introgressed by Kahle et al to produce 2 fully backcrossed and stable inbred lines, the LA/N and SHR/N strains.⁴⁹ Partially backcrossed strains (based on a reduced number of backcrosses) were also produced and are known as Jcr.La-*cp* and SHHF/Mcc-*cp* strains. Although genetically identical, the different strains retain different degrees of genetic contribution from the parental strain and show major metabolic differences.⁵⁰

Bosse et al demonstrated that a low-carb HFD can reduce blood pressure in 6-week-old SHRs without affecting insulin resistance when compared with Wistar–Kyoto rats (normotensive control).⁵¹ Cardiac hypertrophy in SHR is regulated by liver kinase B1 (LKB1, also known as Serine/threonine kinase 11 [STK11]) and S6 kinase (S6K).^{52,53} In SHRs, phosphorylation of cardiac LKB1 is reduced in comparison to that in Wistar–Kyoto animals. Interestingly, phosphorylation of S6K was increased only in the low-carb high-fat diet–treated SHR group and not in the Wistar–Kyoto rat or the control diet SHR

groups.⁵¹ The control diet used in this study consisted of 10% fat, 70% carbohydrate, and 20% protein, whereas the low-carbohydrate/HFD consisted of 20% carbohydrate, 60% fat, and 20% protein.

When homozygous for the corpulent *cp* gene (*cp/cp*), the JCR:LA-*cp* phenotype consumes excessive food, resulting in obesity, hyperlipidemia, and insulin resistance. Russel et al have previously described the presence of atherosclerotic disease in nontreated corpulent male rats of the JCR:LA strain together with myocardial, ischemic lesions that include cell loss and old organized scars.⁵⁴ The corpulent rats have a marked very low-density lipoprotein hyperlipidemia, resulting in greatly raised triglycerides and moderately raised plasma cholesterol concentrations.⁵⁵ These rats exhibit insulin resistance, impaired glucose tolerance, and myocardial lesions that are milder in the females than in the males.⁵⁰ The JCR:LA-*cp* rat is a valuable model for the study of atherogenesis and myocardial damage because it develops the disease state by excessive consumption of a normal, low-fat diet and mimics the at-risk human population in many regards.⁵⁵ As reported by Russel and Proctor, intermittent cycles of CR/refeeding reduced plasma levels of leptin and the inflammatory cytokine interleukin-1 β and improved macrovascular dysfunction in JCR:LA-*cp* rats.⁵⁶

Obesity in the *SHHF/Mcc-cp* rat model is caused by expression of the autosomal recessive *cp* trait. The original SHR/N-*cp* strain animals were developed by backcrossing rats heterozygous for the *cp* gene to SHR/N. This model exhibits hypertension (100%), obesity (25%), and congestive heart failure (CHF; 100%). Both lean and obese SHHF rats develop clinical signs associated with CHF, including generalized and subcutaneous edema, hydrothorax, ascites, dyspnea, cyanosis, enlarged hearts, left atrial thrombosis, and hyperemia of the lungs, liver, and kidneys.^{57,58} These rats are hypertensive, but blood pressure returns to the normal range with the onset of severe CHF. Increased levels of plasma atrial natriuretic factor are positively correlated with the severity of CHF in these rats,⁵⁹ an observation also reported in other animal models of CHF and in humans.^{60,61} Levels of plasma aldosterone, renin, and norepinephrine follow similar patterns to those observed in humans with CHF. Although multiple studies have demonstrated the beneficial effects of physical activity, CR, and body weight reduction on survival,^{62,63} there is a lack of information regarding the effects of fasting in this model. The outcome of such a study would be of great clinical impact considering that coronary heart disease (CHD), hypertension and, in the long term, the use of certain chemotherapy drugs can lead to the development of CHF.⁶⁴

Apolipoprotein-Associated Models

Apolipoproteins are a family of proteins that bind to lipids to form lipoproteins and transport them through the lymphatic and circulatory systems. A major difference between rodents and humans affecting the cardiovascular system is in the metabolism of fatty acids: rodents use HDL to transport the hydrophobic fatty acids through the circulatory system, whereas humans rely on LDL and very low-density lipoprotein.

The level of cholesteryl ester transfer protein (CETP) correlates inversely with increased HDL levels and is considered

to be antiatherogenic. Human subjects with CETP deficiency and an HDL cholesterol level >60 mg/dL have a reduced risk of CHD.⁶⁵ In recent years, there has been an increasing effort to not only reduce the circulating levels of LDL but to also increase the levels of HDL for the prevention of CVD.⁶⁶ For this reason, there is much interest in factors that determine levels of CETP activity. Wang et al reported that cycles of prolonged CR can reduce plasma levels of CETP and increase HDL in obese subjects affected by type II diabetes mellitus.⁶⁷

Mice and rats are naturally deficient in CETP activity.⁶⁸ In the absence of CETP, rats and mice are relatively resistant to diet-induced atherosclerosis.^{69,70} When fed a diet high in fat and cholesterol, mice carrying the human CETP gene show a significant increase in circulating and liver CETP mRNA and, as a result, also show reduced levels of plasma HDL. Transgenic mice expressing both human apolipoprotein (apo) B and human CETP display a 3-fold higher serum CETP activity than humans and also have apoB levels comparable to those of healthy subjects. The distribution of total cholesterol within the HDL, LDL, and very low-density lipoprotein fractions in the apoB/CETP animals also closely represents the cholesterol plasma of normolipidemic humans.⁷¹ For this reason, CETP transgenic mice have been an invaluable model for the study of lipid metabolism, for atherosclerosis, and for the identification and evaluation of nutrients and compounds that increase HDL cholesterol levels in a model that closely resembles part of the human physiology.

Increased plasma levels of LDLs are associated with increased incidence of CVD and stroke.⁶⁶ Although atherosclerosis was previously thought to be mainly a degenerative disease, it is now well established that chronic inflammation, particularly when extended to the vessel wall, has a focal role in its onset.⁷² For this reason, interleukin-1 β and other proinflammatory cytokines secreted by plaque-infiltrating macrophages have been the center of several studies aimed at determining the role of inflammation in the pathogenesis of atherosclerosis. With 6 times the total plasma cholesterol concentration of its littermate (C57BL/6 strain), the apoE is one of the most widely used mouse model in the study of inflammation and CVD. Prolonged fasting has been shown to generally decrease inflammation, reduce interleukin-1 β ,⁴⁷ and increase apoE⁶⁷ levels, suggesting that it has the potential to reduce inflammation-associated CVD. However, whether intermittent/periodic fasting or chronic caloric restriction can prevent atherosclerosis development in this model remains to be answered. Cardiovascular lesions in this model are naturally limited to the aorta, but when mice are fed a high-cholesterol diet, atherosclerosis can develop to an extent comparable to that observed in humans.⁷³ However, when exposed to a high-cholesterol diet, mice lacking the apoE gene develop intimal lesions rather than lesions of the arterial system as observed in humans.⁷⁴

Although both CR and control *apoE*^{-/-} mice develop early-stage atherosclerosis, as determined by the presence of foam cells and free lipids in the aortic wall, mice undergoing 60% CR have 33% lower atherosclerotic lesions than those observed in ad libitum-fed animals.⁷⁵ CR also attenuates the formation of atherosclerotic plaques and results in lower levels of reactive oxygen species and lipid hydroperoxide (a marker for oxidative damage) in the aorta.⁷⁵

The endoplasmic reticulum is a major site for the production of many membrane and soluble proteins, and it is strictly regulated to guarantee the correct folding and assembly of proteins. When the endoplasmic reticulum releases defective proteins, it can cause malfunction or loss of protein function. This is the case observed in patients with familial hypercholesterolemia carrying single or multiple mutations in their LDL receptor (LDLR), which leads to misfolding of the receptor. The LDLR^{-/-} mouse is a model of familial hypercholesterolemia, which is characterized in humans by the decreased ability to clear cholesterol-rich LDL particles from circulation, resulting in elevated blood cholesterol levels and leading to early onset of atherosclerosis as well as an increased risk of cardiac disease. Similarly to what is observed in familial hypercholesterolemia in humans, LDLR^{-/-} transgenic mice show aortic atherosclerosis that is also extended to other vessels. This model is highly suitable for testing the role of LDLR in the effects of dietary interventions on CVD onset because hypercholesterolemia is the most recurring marker associated with cardiac failure and its levels increase with aging.

In addition to insulin, glucose, and IGF-1 levels, fasting, CR, and DR all probably function in overlapping ways by regulating proteins in complex pathways involved in both growth and aging, including SirT1, PKA, AMP-activated protein kinase, forkhead transcription factors, mammalian target of rapamycin, and even changing the ratio of NAD⁺ to NADH.^{2,76-78}

Transgenic and knockout mouse model studies have linked SirT1, a multifaceted histone deacetylase, to heart protection from oxidative and hypertrophic stresses.⁷⁹ Although SirT1 overexpressing mice display many of the phenotypes observed in CR mice,^{78,80} tissue-specific models show varying results depending on the fold increase in expression.⁸⁰ Tissue-specific α -myosin heavy chain transgenic mice with a roughly 5-fold increase in the expression of SirT1 are protected against cardiac stress and apoptosis induced by paraquat.⁸⁰ Furthermore, age-dependent cardiac dysfunction was delayed in these animals.⁸⁰ However, further increase of SirT1 expression (13-fold) had an inverse effect, leading to oxidative stress, apoptosis, and cardiomyopathy.^{76,80} IGF-1 has been shown to counteract some of the activities of SirT1,⁸¹ which is not surprising because both act on targets, including forkhead box O transcription factors, in cardiomyocytes.

Vinciguerra et al developed a cardiac-specific locally acting IGF-1 propeptide-expressing mouse model, which also conditionally expressed Sirt1.⁷⁸ They demonstrated that IGF-1 propeptide induces the activity of SirT1 and infers protection against oxidative stress in the heart muscle.⁷⁸ Furthermore, depletion of SirT1 in these mice eliminates the protective effects of the IGF-1 propeptide.⁷⁸

Rodgers et al have shown that fasting can activate LDLR regulation in a SIRT1-dependent manner.⁸² SIRT1 is an important factor in systemic and hepatic glucose, lipid, and cholesterol homeostasis. Under low nutrient conditions, upregulation of SIRT1 promotes hepatic glucose production.⁸³ Rodgers et al also demonstrated that, in hepatic SIRT1 knockdown mice, glucose tolerance and insulin sensitivity increased, whereas hepatic glucose production was lowered. In addition, free fatty acid accumulation and serum cholesterol was lowered in this model.⁸² Cholesterol levels are closely regulated by the

efflux-mediating cell membrane ATP-binding cassette transporters A BCA1 and SR-B1, both of which are regulated by SIRT1.⁸² In addition, cholesterol transport and metabolism is modulated by peroxisome proliferator-activated receptor gamma coactivator 1-beta (PGC-1 β), expression, which is lowered under fasting conditions in the absence of SIRT1.^{82,84} These results further suggest the importance of testing dietary interventions in this animal model to address the potential role of LDLR in mediating the effect of fasting and CR in the delay of CVD onset (Table 1).

Caloric Restriction and Nonhuman Primate Studies

The potential to model human diseases is one of the most important considerations when choosing an animal model. However, translation into clinical research must also be balanced with the time, cost, ethics, and feasibility of the animal project. Thus, nonhuman primates are exceptionally well-suited to provide insight into some of the questions regarding the biology of human aging and disease. Among the nonhuman primate models, the rhesus monkey, with 93% sequence identity to humans, is a popular species for biomedical research (Rhesus Genome Consortium; <https://www.hgsc.bcm.edu/rhesus-monkey-genome-project>). The similarities between *Rhesus macaque* and *Homo sapiens* extend beyond genetics to both biology and physiology with a maximum lifespan of 40 years compared with 120 years human maximum lifespan. Moreover, aging *Rhesus macaque* develops many of the same phenotypes observed in aging humans, including graying and thinning of hair, sarcopenia, and increased incidence of diabetes mellitus, cancer, and other aging-related diseases.^{85,86}

To date, only 2 major projects have been designed to evaluate the long-term effects of moderate CR on general physiology, health span, and lifespan in nonhuman primates. However, these studies have yielded complex, if not contradictory, results. In an ongoing study by the National Institute of Aging (NIA), where rhesus monkeys were placed on a 30% caloric restriction diet, no significant impact on lifespan has been observed despite beneficial effects on health span.⁸⁷ In contrast, the 2-decade longitudinal adult-onset study by Wisconsin National Primate Research Center (WNPRC) showed a 26% mortality in the CR group in comparison to 63% mortality in the control diet group.^{88,89} Although the studies have differing results for mortality outcomes, both groups demonstrate the health span extension benefits of CR.⁸⁷⁻⁹⁰

Although the WNPRC reported lifespan extension as a significant finding, the NIA study found no apparent difference in the cause of death between the control and CR groups. Both groups showed a similar distribution of CVD, amyloidosis, neoplasia, and general health deterioration. In the report, the WNPRC classified 16 out of the 38 control animals and none of the CR animals as diabetic or prediabetic, demonstrating prevention of diabetes mellitus and maintenance of glucose homeostasis by CR.⁸⁸ Similarly, the instances of CVD were reduced by 50% in the animals subjected to CR. However, the most frequent cardiac pathologies observed during necropsy were valvular endocardiosis, cardiomyopathy, and myocardial fibrosis.⁸⁸

In the NIA study, necropsy indicated that cardiovascular abnormalities were the cause of death for 3 control and 4 CR

Table 1. Rodent Models Used in CVD Studies That Utilizes Dietary Interventions

Animal Model	Type of Dietary Intervention	Cardiovascular Parameters Analyzed
<i>ob/ob</i> mice; <i>db/db</i> mice	50% calorie restriction	Reversal of myocardial hypertrophy and lipid accumulation ^{34,36,39–41}
SHROB or <i>fa^k</i> (fatty) rat	83% calorie restriction	Reduction of high blood pressure ⁴⁷
Spontaneously hypertensive rats (SHR)	Low carbohydrate (20%)/high-fat (60%) diet	Reduction of high blood pressure ⁵¹
<i>SHHF/Mcc-cp</i> rats	No dietary intervention	Hypertension (100%), obesity (25%), and CHF (100%). Enlarged heart, left atrial thrombosis ⁵⁷
JC:LA-cp rats	Two-day caloric restriction/refeeding cycles	Reduction of circulating leptin and IL-1 β and improved macrovascular dysfunction ⁵⁶
apoB/CETP mice	No dietary intervention	Reduced levels of plasma HDL. LDL, HDL, VLDL, and total cholesterol levels representative of normolipidemic humans ⁷¹
LDLR ^{-/-}	No dietary intervention	Aortic atherosclerosis also extending to other vessels ⁸²
apoE mice	60% caloric restriction	Reduced atherosclerotic lesions and inflammation ⁷⁵
Wild-type mouse fed high-fat diet	16-hour fasting	Reduction of heart rate and mean arterial blood pressure, delay of elevated triglycerides and serum leptin onset, and accumulation of gonadal/total fat mass ²¹
Wild-type mouse fed Paigen diet	1.25% cholesterol, 0.5% cholic acid, 15% fat (polyunsaturated/saturated=0.69)	Low HDL, high plasma cholesterol and saturated fatty acids, fatty streaks, and atherosclerosis ^{14–17}

apoB indicates apolipoprotein B; CETP, cholesteryl ester transfer protein; CHF, congestive heart failure; HDL, high-density lipoprotein; LDL, low-density lipoprotein; IL, interleukin; and VLDL, very low-density lipoprotein.

monkeys. Among those, myocardial fibrosis and degeneration were reported as significant pathologies, but not the primary cause of death. Other cardiac instances reported included heart failure because of right ventricular necrosis in a 40-year-old CR monkey, 2 cases of pathological changes in the heart tissue, and 1 CR monkey with a change associated with a previous myocardial infarct.⁸⁷

Obvious differences are apparent when comparing the experimental design of the NIA and WNPRC studies. Perhaps, the most important difference is the composition of the diet. The WNPRC developed a semipurified, nutritionally fortified diet specifically for the study to be able to accurately control for nutrient intake. Lactalbumin was used as the sole source of protein, providing 15% of total calorie intake. Additionally, the diet was composed of 10% fat (primarily from corn oil), 5% cellulose, and 28.5% sucrose. This diet is similar to the chow diet the animals were on before entering the study, with the exception that it contained twice as much fat and less fiber.^{91,92} In contrast, the NIA diet, which was in the form of a biscuit, was specifically formulated with natural ingredients using the standard high-fiber diet used for monkeys at the National Institutes of Health.⁹³ For this diet, protein was derived from wheat, corn, soybean, fish, and alfalfa meal, providing 17.3% of total calorie intake with the addition of 5% fat, 5% crude fiber, and only 3.9% sucrose (plus standard vitamins and minerals). Nutrient concentration was estimated based on National Research Council data published in 1978. To safeguard for deficiencies, CR animals received a 40% increase in vitamin and mineral supplements. In addition to the biscuits, all animals received a daily allowance of low-calorie treats and weekly ration of fresh fruits.

In the NIA study, animals were fed twice a day, and food allotment was calculated per animal based on age and body weight as per the National Research Council protocol (1978), with all unconsumed food removed daily.

In addition to differences in CR diet composition, the implementation of the diet also differed. Although both groups gradually reduced food intake over a few months for the CR group, the control WNPRC monkeys were fed ad libitum to more closely represent human feeding habits, but the NIA monkeys were portion controlled, as performed in classical CR experiments. This difference resulted in marked variance in body weight between age- and sex-matched animals in both studies, with NIA animals weighing less than WNPRC animals. Interestingly, when both studies were compared with The Internet Primate Ageing Database (iPAD; <http://ipad.primat.wisc.edu/>), the WNPRC animals weighed higher than average, whereas the NIA animals weighed lower.

In addition, a point of divergence between the 2 studies was also medical management, which can potentially have an effect on determining the end point of lifespan. The NIA's policy was to withhold treatment for cases of long-term chronic disease, meaning that animals were only kept alive so long as they were free of pain. In contrast, the WNPRC pursued drug interventions for treatment of chronic diseases.^{86,87}

In summary, what may have accounted for the more convincing effects of CR in the Wisconsin study is the use of a less healthy diet for the controls, resulting in a relative more pronounced change in the health-associated components of the diet for the monkeys placed on CR compared to the CR group monkeys at the NIA.

Other Nonhuman Primate Studies

Several smaller studies have evaluated the short-term beneficial effects of CR in promoting health span in nonhuman primates. The NIA conducted a squirrel monkey study using

Saimiri sciureus and *Saimiri boliviensis* in parallel with the Rhesus monkey study. However, after 5 years, they did not observe any reliable difference between the CR and the control groups.⁹⁴ One possible explanation was that the CR group only received a 21% to 23% reduced calorie intake compared with the control group. Bodkin et al evaluated mortality in a cohort of 117 monkeys for roughly 25 years and observed 2.6-fold increase in risk of death in the 109 (88 male and 21 female) ad libitum-fed animals in contrast to the 8 (all male) dietary restricted animals.⁹⁵ This study also found that ad libitum animals had increased levels of circulating insulin compared with the DR group. In contrast to the NIA and the WNPRC, the DR animals were fed the same food (Purina Mills) as the ad libitum group. However, the amount of food was tittered for each animal based on total body weight and maintained at 10 kg±1.⁹⁵

A study from Wake Forest University School of Medicine examined the relation between incidences of atherosclerosis and increased insulin sensitivity in calorically restricted *Macaca fascicularis*.⁹⁶ As expected, the 4-year study of 32 randomized subjects revealed increased sensitivity to insulin in the CR group and reduced body fat. No significant difference was observed in either the lipid profile or extent of atherosclerosis between the 2 groups, although it should be noted that the study was designed to maintain similar plasma lipid levels between groups by adding crystalline cholesterol to the CR diet. This supplementation might have been sufficient to reduce the benefits of CR on atherosclerosis by affecting insulin sensitivity and visceral fat mass.⁹⁶ In fact, not taking into account effects on mortality, all nonhuman primate dietary intervention trials to date have demonstrated that CR significantly improves cardiovascular risk factors by reducing body weight, high blood pressure, visceral fat, and insulin sensitivity.

Future Consideration for Nonhuman Primate Studies

Investigations of nonhuman primate model systems have shed much light on the potency of CR as it relates to humans. However, there are still certain aspects of nonhuman primate studies that mandate further evaluations. First is the concern regarding the ability to fight off infections and other acute challenges, which may depend both on animal fitness and on the robustness of the immune system. Second, because most studies are conducted under controlled environments, it is important to establish how CR would affect challenges commonly encountered in wild environments. Examples of such a condition could include exercise, extreme temperatures, and infectious diseases.

Human Clinical Trials, Epidemiological Studies, Diet, and CVD

Independent cohort studies in the last decade have attempted to examine the effects of various dietary habits on health and the incidence of aging-related diseases, such as cardiovascular dysfunctions, diabetes mellitus, and cancer. Among the many dietary interventions, Mediterranean diets have received much attention since they have been associated with lower risk of chronic disease including CVD.^{97–99} The Mediterranean diet

(or diets) encompass the historical dietary habits of cultures that border the Mediterranean Sea. Although there is no universally defined Mediterranean diet, the American Heart Association highlights the following characteristics: olive oil is the central source of fat; high consumption of fruits, vegetables, and nuts; low consumption of animal products, with fish being a major source of protein; and low to moderate consumption of wine. Although most people in the Mediterranean areas only partially comply to these guidelines, there are a sufficient number of people to determining the association between the Mediterranean diet and health.

Sofi et al defined a scoring system with absolute cutoff values for all food components of Mediterranean diets that range from 0 (minimal adherence) to 18 (maximal adherence). Their metadata analysis study used data obtained from 4.1 million subjects and found that (using their Mediterranean diet score system) an increase of only 2 points in adherence to the Mediterranean diet correlated with a 10% reduction in CVD risk.¹⁰⁰ A year after the study was published, a cohort of over 19 000 mixed sex participants of the Health Alcohol and Psychosocial in Eastern Europe (HAPIEE) study from the Czech Republic, Poland, and Russian Federation was analyzed to evaluate the correlation between the Mediterranean diet score as defined by Sofi et al and total, CVD, CHD, and stroke mortality.¹⁰¹ In the 7-year study, 1314 participants died and, although the adherence to a Mediterranean diet was only 25%, the authors reported that an increase in Mediterranean diet score was inversely associated with death from CVD (hazard ratio of 90% and confidence interval of 0.81–0.99).¹⁰¹ Of the 19 263 participants, the study identified 438 cases of CVD, 226 cases of CHD, and 109 cases of stroke. Furthermore, this study demonstrated that the absolute cutoff Mediterranean diet score system was suitable for diet adherence evaluation.¹⁰¹

Estruch et al studied 7447 at-risk men and women to further evaluate the effect of an energy-unrestricted Mediterranean diet supplemented with 1 L of extra-virgin olive oil per week or a Mediterranean diet supplemented with 30 g of mixed nuts per day in comparison to a control diet with reduced dietary fat.^{102,103} The participants were between the ages of 58 and 80 and were not diagnosed with CVD at the time of enrollment, but were considered at risk for being diagnosed with type II diabetes mellitus or had at least 3 of the following risk factors: smoking, hypertension, elevated LDL, low HDL, obesity, or a family history of premature CHD.¹⁰² The primary end point of this study was the rate of major cardiovascular events for which they reported to be 3.8% in the Mediterranean diet group supplemented with extravirgin olive oil, 3.4% in the Mediterranean diet group supplemented with mixed nuts, and 4.4% in the control group. Their results also showed a significant reduction in the rate of CHD in the group with supplemented mixed nuts or olive oil,¹⁰² corroborating observations in past studies.^{104–106} In a 6-year follow-up analysis of the same study, the authors reported that intake of mono- and polyunsaturated fats correlated with reduced CVD risks, whereas intake of saturated and trans fats increased the incidence of CVDs.¹⁰³ Interestingly, saturated fats from fish and vegetables had an inverse association with CVD and death.¹⁰³ Moreover, substitution of 5% of energy from saturated fats with mono- or poly-unsaturated fats showed a 37% and 33% lower risk of

CVDs. Similarly a replacement of 1% energy from trans fats with mono-unsaturated fats reduced risk of CVD by 8%.¹⁰³ Other studies have also highlighted the inverse observation of olive oil intake and prevalence of CHD.^{104,105,107,108}

In addition to the benefits attributed to Mediterranean diet, a comparative review of all major US and Swedish cohort studies has indicated a positive correlation between a low-carbohydrate and high-protein diet and the prevalence of chronic aging-associated metabolic syndromes and cancer.

In 2 major US follow-up studies, the Nurses' Health Study and the Health Professionals' Follow-up Study, a simple scoring system allowed investigators to evaluate the impact of each macronutrient group on human health and mortality.¹⁰⁹

From the 21 233 documented cumulative deaths among 85 168 women and 44 548 men in the Nurses' Health Study and the Health Professionals' Follow-up Study cohorts, respectively, CVD was the cause for 5204 deaths.¹⁰⁹ This study highlighted that in both men and women, a low-carbohydrate diet, with a high intake of animal-based foods, was directly correlated with increased all-cause mortality.¹⁰⁹ Although the focus of the study was on the negative effects of a low carbohydrate content, the diet also contained a high level of protein, which Levine et al proposed may be responsible for part of the association between the low-carbohydrate diet and mortality. In a separate study, initial evaluation of total protein intake in the Health Professionals' Follow-up Study cohort did not show any statistically significant associations between the 1057 incidents of stroke and the 2959 incidents of ischemic heart disease with respect to total protein intake. However, the same study showed an inverse correlation between diet and stroke or ischemic heart disease for higher plant protein intake and a positive correlation for higher animal protein intake.^{110,111}

In a multivariable analysis of the Nurses' Health Study cohort, Bernstein et al (2010) corroborated the findings from the Health Professionals' Follow-up Study cohort that an increased intake of red meat and high fat correlated with elevated risk of ischemic heart disease among women, whereas lower risk was associated with intake of nuts and beans. This study evaluated 2210 cases of nonfatal infarctions and 952 deaths from CHD.¹¹²

Likewise, a positive correlation was observed between red and processed meat intake and risk of diabetes mellitus in a multiethnic study of 29 759 white, 35 244 Japanese-American, and 10 509 Native Hawaiian men and women in Hawaii, aged 45 to 75 years.¹¹³

Similar observations were made in the Swedish women cohort of 43 396 by Lagiou et al (2012). They reported a 5% increase in incidences of CVD with a 5 g increase in protein intake or 20 g reduction in carbohydrate intake.¹¹⁴ Although at first, the association between decreased carbohydrate intake and CVD might seem unprecedented, the investigators found out that most participants often substituted carbohydrates with animal protein, resulting in a total increase in protein intake.¹¹⁵ However, when evaluating a northern Swedish population, no general association between low carbohydrate or high protein score and mortality was found.¹¹⁵ It is possible that this observation is because majority of the participants had a diet with protein intake amounts comparable to the Swedish national food recommendations, while simultaneously reducing

carbohydrate intake and exceeding fat intake recommendation. It should also be highlighted that studies of Swedish cohorts have an advantage over studies of American cohorts in that the nationwide data linkage in Sweden allows for virtually complete follow-up and objective ascertainment of cardiovascular outcomes.

The studies mentioned earlier use a combined age group analysis that does not allow for distinction of age-related protein intake needs and, consequently, may distort the effects that protein intake has. Although increased protein intake has been shown to inversely affect cardiac health and other chronic diseases in young and middle-aged adults, few studies have specifically looked at dietary consequences in adults versus older adults. This is especially important considering that the levels of IGF-1, a central factor in aging-related diseases whose levels are mostly regulated by proteins, are known to decrease during aging. In addition, several other physiological changes are observed in aging individuals above the age of 65, such as sarcopenia and decreased motility, may be negatively affected by low or very low protein intake.

In the last 20 years, the optimal required protein intake for the elderly population has been the subject of investigation of many groups. Many have tried to calculate a recommended dose based on nitrogen homeostasis. However, the results have been mixed, with some reports suggesting to increase the current 0.8 g/kg per day of quality protein Recommended Dietary Allowance for the elderly, whereas others have suggested no change is needed.¹¹⁶

A study by Levine et al demonstrated that although low protein diets reduce the risk for cancer and overall mortality in the population aged ≤ 65 , these beneficial effects are not observed in individuals over the age of 65, which instead display an increased risk for mortality while on a low protein diet.¹¹⁷ This increased death risk in the ≤ 65 population may be evidence of both a potential higher requirement for protein intake in the elderly, but also the presence of frail and sick individuals among those reporting low protein intake over the age of 65. For example, to prevent muscle loss in the elderly, the PROT-AGE study group (an international study group to review dietary protein needs with aging, appointed by the European Union Geriatric Medicine Society [EUGMS], in cooperation with other scientific organizations the International Association of Gerontology and Geriatrics, the International Academy on Nutrition and Aging, and the Australian and New Zealand Society for Geriatric Medicine) recommends a daily protein intake of 1.0 to 1.2 g/kg a day, while also advocating that the elderly should consume 25 to 30 g of protein per meal containing 2.5 to 2.8 g of leucine.^{118,119} Metabolic studies have corroborated these finding by highlighting that intake of quality protein is required for maintenance of muscle mass and function in the elderly. Amino acid composition, digestibility, absorption, and regulatory roles of specific amino acids in cellular processes are used to evaluate the quality of protein source.¹¹⁸

Interestingly, muscle protein synthesis is stimulated by increased protein consumption of ≤ 30 g per serving, but no added benefits are observed with higher protein intake.¹²⁰ Additionally, muscle protein synthesis is attenuated in the elderly by limitation of essential amino acids, such as

leucine, and when protein is consumed with glucose.^{116,120,121} Thus, it is likely that consuming 1 g of protein/kg of body weight per day with at least 25 to 30 g in one meal is sufficient to prevent malnourishment in the great majority of subjects, while also minimizing the risk of cancer and overall mortality. The combination of this level of proteins and certain types of exercise is necessary to optimize muscle protein synthesis and growth.

Consuming a diet with a high percentage of calories coming from added sugars has been linked to increased risk of obesity, type II diabetes mellitus,^{122–125} dyslipidemia,¹²⁶ and hypertension,^{127–131} all risk factors for CVD. To add to this, there has been a link established between increased sugar consumption and upregulation of de novo lipogenesis in the liver, increased hepatic triglyceride synthesis, and consequently increased triglyceride levels.¹³² The increase in circulating lipids contributes to the development of atherosclerosis and subsequently CVD. Prolonged consumption of added sugars is linked to increased LDL levels and decreased HDL levels, highlighting the effects of dietary sugar on risk factors for CVD, specifically dyslipidemia.^{132–134} More recent findings have shown that there may be a link between excess intake of added sugars and increased prevalence of inflammatory markers in the blood, contributing to atherosclerosis in concert with the induced dyslipidemia.^{130,131,135} Epidemiological studies suggest that although consumption of added sugars may be trending downward, most Americans obtain a significant portion of their calories from these sources, resulting in an increased incidence of CVDs.¹³⁶ Findings from The Third National Health and Nutrition Examination Survey (NHANES III) study indicate that the percentage of calories coming from added sugars had decreased from 15.7% in 1994 to 14.9% in 2010. However, 71.4% of adults have diets consisting of at least 10% added sugars. When comparing populations, which consume varying amounts of sugar, the difference in risk for CVD is striking. Compared with people who consume 8.0% of daily calories from added sugars, those who consumed 17% to 21% had a 38% higher risk of CVD, with those in the highest quintile of consumption (above 21% calories from added sugars) displaying a more than double that relative risk. This association between sugar and risk factors for CVD has also been found in adolescents.¹³⁶

Dietary Interventions to Treat Coronary Heart Disease

A project led by Dr Roy Walford for the inhabitants at Biosphere 2 was the first known study on the effects of CR on human health markers.¹³⁷ During the first 6 months, the biospherians encountered scarcity of food because of problems in generating sufficient crops, which led them to participate in a CR diet providing an average of 1780 calories/d in contrast to the 2500 calories/d that they consumed before entering the biosphere. After 6 months on CR, Walford and colleagues reported a drastic reduction in cholesterol, blood pressure, fasting blood glucose, and body weight.¹³⁷ The Biospherians diet was mostly vegetarian consisting of fruits, cereal grains, split peas, beans, peanuts, greens, potatoes, other vegetables, and small quantities of goat milk and yogurt (≈ 84 g/d), goat meat, pork, fish, and eggs (≈ 2 – 6 g/d).¹³⁷

Since the Biosphere 2 study, long-term DR in human volunteers has confirmed the remarkable effects of CR on CHD risk factors, including a reduction of total cholesterol, LDL cholesterol, and triglycerides, with an increase in HDL cholesterol, reduced fasting glucose levels, and insulin resistance index and, furthermore, CR caused a reduction in both systolic and diastolic blood pressure and reduced anti-inflammatory markers, such as C-reactive protein and tumor necrosis factor.^{137–139}

However, chronic dietary intervention may not be necessary to obtain beneficial effects on aging and disease risk factors. In a study by Brandhorst et al, the use of 3 cycles of a 5 days per month periodic fasting mimicking diet was able to reduce risk factors for aging and age-related disease without major adverse effects in both mice and in a pilot clinical trial.¹³⁹ In mice, this periodic intervention also caused an 11% increase in the mean lifespan. In both mice and humans, the fasting mimicking diet was designed to mimic the effects of fasting, including reducing IGF-1 and glucose levels, while increasing insulin-like growth factor binding protein-1 (IGFBP1) levels and ketone bodies. In the human trial, the experimental group displayed lower CVD and inflammatory marker C reactive protein (CRP) after 3 cycles of the diet. Other effects of FMD included reduced body weight and body fat without loss of lean mass in the experimental group.¹³⁹

Disease treatment is much more common, because preventive measures are often difficult to implement in the general

Table 2. Macronutrient Composition of Western Diet Compared With Other Dietary Interventions

Macronutrient	Western Diet	Caloric Restriction*	Mediterranean Diet	Fasting Mimicking Diet	Protein Restriction
Protein (animal)	High	Low/Normal	Normal	None	Low
Protein (plant)	Low	Low/Normal	Moderate	Low	Low
Saturated fats	High	Low/Normal	Low	Very low	Normal
Unsaturated fats	Low	Low/Normal	High	High	Normal
Sugars	High	Low/Normal	Moderate	Very low	Normal
Complex Carbohydrates	Low	Low/Normal	High	High	Normal

CR indicates caloric restriction.

*See CR in definition section above.

population. Consequently, many groups have been investigating the use of dietary intervention to treat CVD and other age-related diseases. A randomized controlled trial indicated that a comprehensive lifestyle change consisting of a low-fat vegetarian diet, mild to moderate exercise, stress management, and group support improved coronary atherosclerosis after just 1 year.¹⁴⁰ Although it did not impose CRs, this diet is highly restricted and contains no animal product, no caffeine, 10% calories from fat obtained from grains, vegetables, fruit, beans, legumes or soy foods, and only 12 g of sugar. The authors demonstrated that among the 28 patients placed on the diet, 82% showed regression of atherosclerosis, whereas the health of subjects in the control group continued to decline.¹⁴⁰ Furthermore, in the 5-year follow-up study, the investigators used positron emission tomography and demonstrated that, with intense risk factor modification, the size and severity of myocardial perfusion abnormalities had decreased in the experimental group in contrast to the control group, both at rest and under drug-induced cardiac stress.¹⁴¹ Notably, the efficacy of this diet must be confirmed in much larger randomized and controlled clinical trials, which also focus on compliance in the general population. The allowance of specific high-fat healthy foods could not only improve compliance, but also enhance efficacy, especially considering the demonstrated beneficial effects of olive oil and nuts, and the many studies suggesting that the Mediterranean diet is protective against CVDs (Table 2).^{103–105,107,108,142,143}

In summary, both calorie-restricted and normocaloric diets have the potential to prevent and treat CVDs. Further research in this field is needed to minimize caloric restriction and the potential side effects associated with it, while maximizing effects on compliance, aging, metabolic syndrome, and CVDs.

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Dr Longo has equity interest in L-Nutra, a company that develops medical food. The other authors report no conflicts.

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