

Reviews: Current topics

Beneficial effects of intermittent fasting and caloric restriction on the cardiovascular and cerebrovascular systems

Mark P. Mattson*, Ruiqian Wan

Laboratory of Neurosciences, National Institute on Aging Intramural Research Program, Baltimore, MD 21224, USA

Abstract

Intermittent fasting (IF; reduced meal frequency) and caloric restriction (CR) extend lifespan and increase resistance to age-related diseases in rodents and monkeys and improve the health of overweight humans. Both IF and CR enhance cardiovascular and brain functions and improve several risk factors for coronary artery disease and stroke including a reduction in blood pressure and increased insulin sensitivity. Cardiovascular stress adaptation is improved and heart rate variability is increased in rodents maintained on an IF or a CR diet. Moreover, rodents maintained on an IF regimen exhibit increased resistance of heart and brain cells to ischemic injury in experimental models of myocardial infarction and stroke. The beneficial effects of IF and CR result from at least two mechanisms — reduced oxidative damage and increased cellular stress resistance. Recent findings suggest that some of the beneficial effects of IF on both the cardiovascular system and the brain are mediated by brain-derived neurotrophic factor signaling in the brain. Interestingly, cellular and molecular effects of IF and CR on the cardiovascular system and the brain are similar to those of regular physical exercise, suggesting shared mechanisms. A better understanding of the cellular and molecular mechanisms by which IF and CR affect the blood vessels and heart and brain cells will likely lead to novel preventative and therapeutic strategies for extending health span.

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Keywords: Heart rate variability; Ischemia; Insulin sensitivity; Oxidative stress; Stroke

1. Lifespan extension by caloric restriction and intermittent fasting

Experiments performed 70 years ago showed that caloric restriction (CR; reduction in energy intake with maintenance of nutrition) increases the lifespan of rodents [1], and this has been widely replicated and extended in studies that demonstrated increases both in the mean and in the maximum lifespan of rats and mice maintained on reduced calorie diets [2]. More recent studies suggest that reducing calorie intake can also increase the lifespan of monkeys [3,4]. The amount by which lifespan is extended increases progressively as calorie intake is reduced (until the point of starvation) as well as with the duration of the diet (the later the diet is begun, the less lifespan is increased). Interestingly, intermittent fasting (IF; diets with reduced meal frequencies such as every-other-day fasting) can also increase lifespan, even when there is little or no overall decrease in calorie intake [5,6]. Similar to CR, IF can

improve risk factors for diabetes and cardiovascular disease in rodents [6,7]. Diseases responsible for mortality in rodents such as cancers, diabetes and kidney disease are also delayed or prevented by CR and IF [8,9].

An increasing number of physiological effects of CR and IF that may contribute to their abilities to increase health span has been documented in studies on rodents, monkeys and humans [2,10]. Prominent among these are the following: increased insulin sensitivity that results in reduced plasma glucose and insulin concentrations and improved glucose tolerance [6,11]; reduced levels of oxidative stress as indicated by decreased oxidative damage to proteins, lipids and DNA [12]; increased resistance to various types of stress including heat, oxidative and metabolic stresses [10]; and enhanced immune function. The two leading hypotheses for the “anti-aging” effects of dietary restriction are the oxidative stress hypothesis and the stress resistance hypothesis [10,12]. Because CR and some IF regimens involve a reduction in energy intake, there are fewer free radicals produced in the mitochondria of cells and therefore less oxidative damage to the cells. When maintained on a CR or an IF diet, organisms ranging from yeast

* Corresponding author. Tel.: +1 410 558 8463; fax: +1 410 558 8465.
E-mail address: mattsonm@grc.nia.nih.gov (M.P. Mattson).

and worms to rats and mice exhibit increased resistance to many different types of stressors [13–15]. This resistance to stress is associated with increased resistance of cells in many different tissues to injury induced by oxidative, genotoxic and metabolic insults. The conservation of stress resistance responses to CR and IF across a range of species provides strong evidence that this mechanism contributes to the lifespan-extending action of dietary restriction.

2. Dietary restriction improves the function and resilience of the cardiovascular system and the brain

When rats are maintained on either a CR or an IF diet, their resting heart rate (HR) and blood pressure (BP) are decreased compared with control rats fed ad libitum [7,16]. Rats on an IF diet exhibit improved cardiovascular adaptation to stress, as indicated by attenuated BP and HR responses to immobilization stress and more rapid recovery of BP and HR to baseline levels following removal from the stress [7]. In a study of overweight human subjects, it was shown that a one-meal-per-day diet (with reduced calories) significantly improved left ventricular function and recovery of BP and HR following exercise [17]. CR improves flow-mediated vasodilation in obese humans, which is associated with an apparent increase in insulin sensitivity, suggesting a role for improved glucose metabolism in the beneficial effects of CR on endothelial function [18]. We have recently found that rats maintained on either a CR or an IF diet exhibit increased HR variability (Wan et al., Soc. Neurosci. Abstr. 2004). High HR variability is associated with improved cardiovascular function, whereas low HR variability is characteristic of people with poor cardiovascular function. For example, endurance athletes exhibit greater HR variability compared with untrained healthy individuals [19], whereas reduced HR variability is predictive of heart failure [20].

Several beneficial effects of dietary restriction regimens on the brain have been documented in studies on rats and mice. For example, an age-dependent decline in the performance of mice in a complex maze task was prevented by a CR diet (40% restriction) [21], mice maintained on CR beginning at 3 months of age performed better in a maze test of learning and memory at 15 months of age compared with controls [22] and rats maintained on CR beginning at 4 months of age exhibited significantly improved motor learning at 22 months of age compared with controls [23]. When exposed to the neurotoxin kainic acid, rats maintained on an IF diet exhibit increased resistance of neurons in the hippocampus (a brain region critical for learning and memory) to degeneration, which was associated with preserved learning and memory, compared with neurotoxin-treated rats that had been fed ad libitum [24]. Moreover, when mice are maintained on an IF regimen, the survival of newly generated neurons that arise from stem cells in the hippocampus is enhanced [25]. Collectively, the available data suggest that CR and IF diets enhance

synaptic plasticity, promote the survival of neurons and increase the number of neurons produced from stem cells.

3. Dietary restriction improves cardiovascular and cerebrovascular disease risk factors

Several prominent risk factors for cardiovascular disease and stroke have been shown to be modified by CR and IF. Individuals with impaired glucose regulation (insulin resistance), which is typically associated with elevated levels of plasma glucose and insulin, are at increased risk of cardiovascular disease and stroke [26,27]. Rodents and monkeys maintained on CR or IF exhibit enhanced insulin sensitivity [4,6], which would be expected to decrease their risk of diabetes and cardiovascular disease. High levels of low-density lipoprotein (LDL) cholesterol and low levels of high-density lipoprotein (HDL) cholesterol are associated with increased risk of cardiovascular disease and stroke [28]. Studies on rodents, monkeys and humans suggest that CR can decrease LDL cholesterol levels while increasing HDL cholesterol levels [4,29,30]. CR also reduces levels of oxidative stress in the cardiovascular system as indicated by decreased oxidative modifications of proteins and DNA and decreased levels of lipid peroxidation in the heart [31–33]. CR reduces inflammatory processes that likely contribute to atherosclerosis, as indicated by reduced levels of leukocytes and circulating levels of tumor necrosis factor and other inflammatory cytokines [34,35]. By suppressing atherosclerosis, CR may reduce the risk of cardiovascular disease and stroke.

Hypertension is a major risk factor for both coronary artery disease and stroke [36]. When rats are maintained on a CR or an IF diet, their resting BPs (both systolic and diastolic) are significantly decreased [7,37]. Resting HR is also decreased in rats on IF [7]. IF also improves cardiovascular stress adaptation. For example, when rats on an IF diet were subjected to immobilization stress, the magnitudes of stress-induced increases in BP and HR were less than those in rats on a control ad libitum diet (Fig. 1) [7]. BP and HR each returned to basal levels more rapidly following termination of stress in rats on the IF diet. Rats on the IF diet exhibited activation of the stress-responsive hypothalamic-pituitary-adrenal neuroendocrine system during immobilization stress as indicated by increases in plasma levels of adrenocorticotropin (ACTH) and corticosterone [7]. However, rats on the IF diet adapted more rapidly to repeated bouts of stress as indicated by reduced ACTH and corticosterone responses compared with those on the control diet. As described above, HR variability is increased in rats maintained on IF and CR diets, another physiological change that would be expected to reduce the risk of cardiovascular mortality.

At the National Institute on Aging, a study of the effects of 30% CR on the lifespan of rhesus monkeys was begun more than 17 years ago [4]. Data from that study suggest that CR reduces the incidence of several diseases and

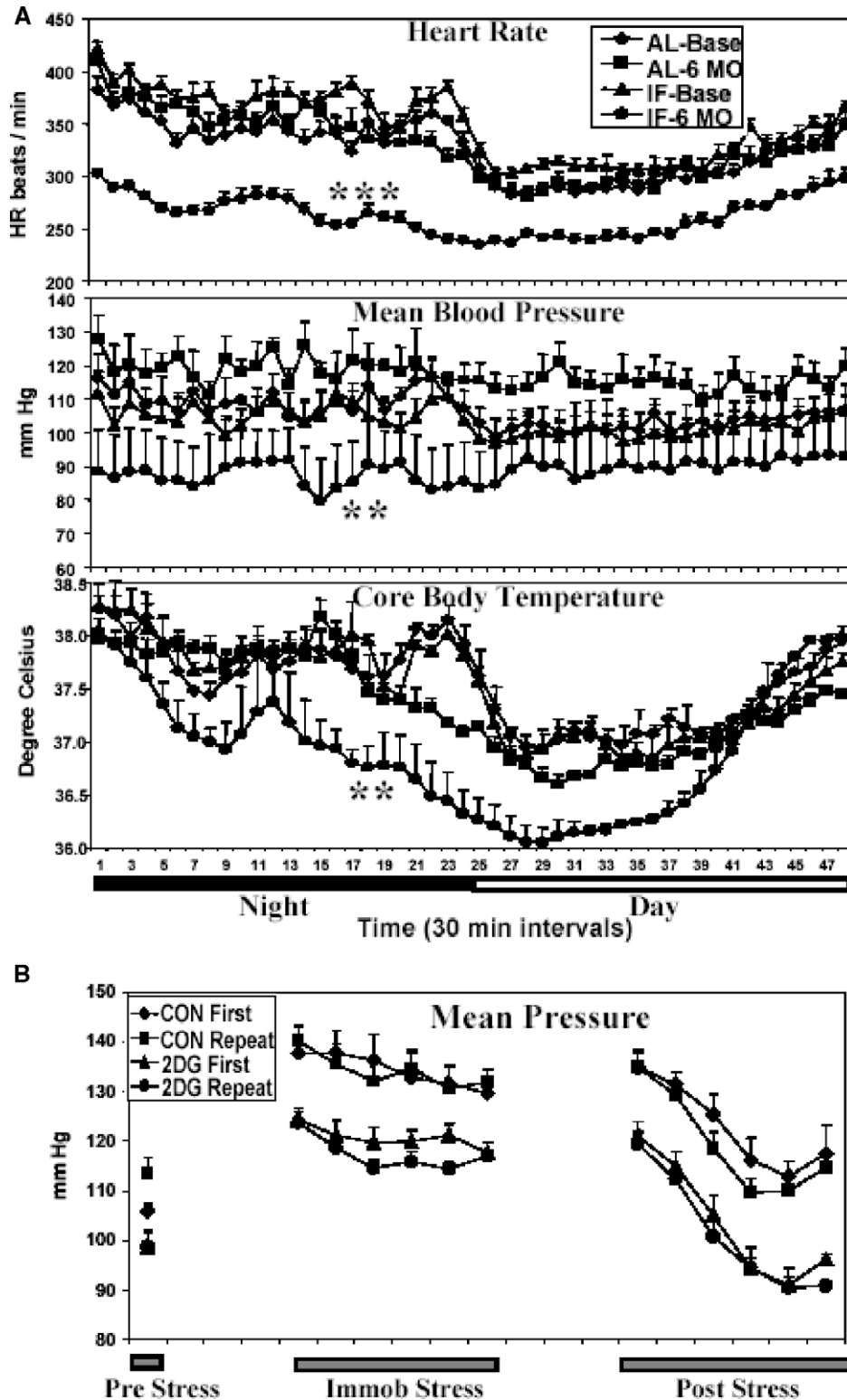


Fig. 1. IF and 2DG administration reduce BP in rats. Young adult rats were implanted with a probe/transmitter in the descending aorta to allow continuous recording of mean BP, HR and body temperature. (A) Rats were maintained on an ad libitum (AL) or an IF (qod fasting) diet. The traces show recordings during a 24-h period from rats in each group prior to diet initiation (base) and after 6 months on the diets. Rats in the IF group exhibited significant reductions in HR and BP compared with those in the AL group. Body temperature was significantly decreased in rats in the IF group on the fasting days. (Modified from Ref. [7].) (B) Rats were fed AL either a control diet or a diet supplemented with 2-deoxyglucose (2DG) (0.4% 2DG fed in a repeating pattern of 2 days of 2DG and 2-day control diet). After several months on the diets, the rats were subjected to either a single 1-h immobilization stress or five daily 1-h stress sessions. The records show mean BP before, during and after stress sessions. Note that BP responses to stress are significantly attenuated in rats on the 2DG diet. (Modified from Ref. [63].)

reduces mortality. With regard to the cardiovascular system, the monkeys on the CR diet exhibit lower body weights and decreased body fat, reduced BP, improved glucose regulation, reduced levels of triglycerides and an improved lipoprotein profile. Similar changes have been observed in human studies. For example, in a study of overweight women, 8 weeks of CR resulted in significant reductions in both systolic and diastolic BP, LDL cholesterol and triglycerides [38], changes that would be expected to decrease the risk of cardiovascular disease and stroke.

4. IF protects neurons and cardiac myocytes against ischemic injury

In a series of studies, we showed that IF imposes a mild stress on brain cells and that the brain cells respond to this mild stress by enhancing their ability to resist more severe stress (reviewed in Ref. [39]). For example, neurons in the brains of rats or mice on an IF regimen are more resistant to being killed by oxidative, metabolic and excitotoxic insults [24,40]. Associated with the neuroprotective effect of IF was increased levels of the stress resistance proteins and brain-derived neurotrophic factor (BDNF) [41,42]. Interestingly, administration of 2-deoxy-D-glucose (2DG; a non-metabolizable glucose analog) to rats, which mimics some aspects of CR, also increases the production of stress

resistance proteins in brain cells [43]. The latter findings suggested the possibility that IF would increase the resistance of neurons to a stroke because data from other studies have shown that protein chaperones and BDNF can reduce ischemic brain damage [43–46]. We therefore subjected rats that had been maintained for 3 months on either an IF or a control diet to transient middle cerebral artery occlusion, a model of ischemic stroke [41]. The amount of ischemia-induced damage to brain cells was significantly less in the rats on the IF diet compared with those on the control diet (Fig. 2). The amount of brain tissue damaged by the stroke was also decreased, and functional outcome improved in rats that had been administered 2DG (Fig. 2) [41]. Similar to the effects of IF, when rats were maintained for 3 weeks on a daily physical exercise regimen and then subjected to cerebral ischemia, the amount of damage to the brain was significantly less and functional outcome was improved compared with nonexercised control rats [47]. The latter study further showed that the exercise regimen increased BDNF levels in the brain and enhanced angiogenesis.

Although limited, the available data suggest that CR and IF can improve outcomes following myocardial infarction. Rats either maintained on a CR diet or subjected to exercise training for 10 weeks exhibited reduced mortality and improved outcomes following isoproterenol-induced myocardial infarction [48]. In another study, rats that had been

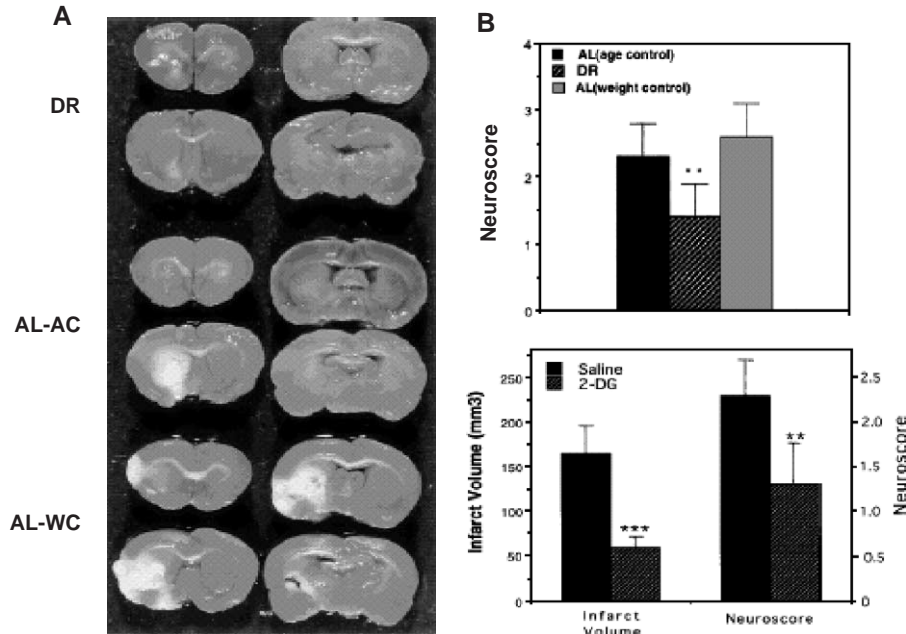


Fig. 2. IF and dietary supplementation with 2DG reduce stroke-induced brain damage and improve functional outcome in rats. (A) Rats were maintained for 3 months on an IF dietary restriction (DR) regimen. Because rats on the IF regimen maintain a lower body weight, two groups of AL-fed control rats were included in the study, an age-matched group (AL-AC) and a weight-matched group (AL-WC). Rats were subjected to middle cerebral artery occlusion–reperfusion and were euthanized 24 h later. Brains were sectioned in the coronal plane and stained with TTC dye. The amount of damage to cells in the cerebral cortex and striatum (white) was much greater in the two AL groups compared with the DR group. (B) Neurological function (neuroscore) following the stroke was significantly better in rats in the DR group compared with those in the two AL control groups. (C) Rats were treated with 2DG (seven daily injections of 100 mg/kg) or saline (control) and were then subjected to middle cerebral artery occlusion–reperfusion. Twenty-four hours later, neurological function was assessed (neuroscore) and the amount of brain damage was quantified (infarct volume). Neurological function was significantly better and brain damage was significantly less in the rats in the 2DG group compared with those in the saline control group. (Modified from Ref. [41].)

maintained on a CR or an ad libitum diet were subjected to occlusion of the left anterior descending coronary artery; rats on the CR diet exhibited decreased oxidative damage to heart cells and reduced inflammation in the ischemic zone [49]. Hearts from rats that had been maintained for 8 months on a CR diet exhibited improved recovery of function following ischemia/reperfusion as indicated by enhanced mitochondrial respiration [50]. The cardioprotective effect of ischemic preconditioning declines with increasing age; however, when rats are maintained on CR, ischemic preconditioning is preserved [51]. Recently, we have performed coronary artery occlusion (permanent ligation of the left anterior descending coronary artery) in rats that had been maintained for 3–4 months on either an IF or a control diet. The extent of damage to the heart as assessed by histological analysis in rats killed 24 h after coronary artery ligation and by echocardiographic analyses 1 week and 10 weeks after coronary artery ligation was significantly less in the rats on the IF diet compared with those on the control diet (Ahmet et al., AHA 2004 annual meeting). Thus, in several different laboratories, CR and IF diets have been shown to protect cardiac myocytes and neurons in the brain against ischemic injury. These findings suggest that CR and IF not only can reduce the risk of cardiovascular disease and stroke but can also improve outcome following a myocardial infarction or stroke.

5. Cellular and molecular mechanisms underlying effects of IF and CR on the cardiovascular system and the brain

In some strains of rats and mice, IF diets are also CR diets. For example, when Sprague–Dawley rats are maintained on a every-other-day fasting IF diet, they consume approximately 30% less food over time and maintain body weights 10–15% below that of rats fed ad libitum [7]. On the other hand, C57BL/6 mice maintained on the same IF regimen consume only 5% less food over time compared with mice fed ad libitum and maintain a body weight similar to that of mice fed ad libitum [6]. Although C57BL/6 mice on the IF diet were not calorie restricted, the diet resulted in beneficial physiological changes that were as great or greater than those in mice maintained on a 40% CR diet. For example, mice on the IF diet had fasting glucose and insulin levels as low or lower than those in mice on the 40% CR diet. In addition, the IF diet was more effective than the CR diet in protecting hippocampal neurons in the brain from being killed by a neurotoxin [6]. These findings are of considerable interest, with regard to understanding the cellular and molecular mechanisms underlying the beneficial effects of IF, because they suggest that health can be improved by reducing meal frequency without reducing calorie intake.

Two mechanisms that are believed to be responsible for the health span-extending effects of CR and IF are a decreased oxyradical production, thereby reducing the amount of oxidative damage to cells, and increased cellular stress resistance. Analyses of heart and brain tissues from

rodents maintained on CR have demonstrated reduced amounts of oxidative damage to proteins, lipids and DNA compared with rodents on an ad libitum diet [52–54]. Oxidative stress plays an important role in ischemic injury to cardiac myocytes and to neurons in the brain [55,56]. Although it remains to be established, it is likely that the ability of CR and IF to protect cells in the heart and the brain against ischemic injury results, in part, from reduced oxidative damage. An antioxidative effect of CR and IF would also be expected to suppress atherosclerosis, a process that involves oxidative damage to vascular endothelial cells and associated inflammation.

CR and IF can activate cellular stress response pathways in several different tissues of rodents including the heart and the brain where levels of heat-shock proteins and other protein chaperones are increased [42,57–59]. In particular, levels of the protein chaperones heat-shock protein 70 and glucose-regulated protein 78 were increased in brain cells of rats maintained on an IF regimen or administered with 2DG [41–43]. The increased production of BDNF in brain cells of rodents on IF also likely results from a cellular stress response because BDNF expression is known to be increased by several different types of mild stress [25,40]. IF may activate cellular stress resistance pathways more robustly than CR can because IF is considerably more effective than CR in inducing BDNF production in the brain (Duan and Mattson, unpublished data). The latter observation is consistent with data showing that IF is more effective than CR in protecting neurons against damage induced by a neurotoxin [6]. The specific mechanism(s) by which IF imposes a mild stress on neurons remains to be established. One possibility is that neurons are subjected to a metabolic stress as a result of the IF regimen. A second possibility is that neurons are more active during IF as a result of the animal feeling hungry and seeking food; when neurons are more active, they are subjected to energetic and oxidative stress that can stimulate the production of BDNF and other stress resistance mechanisms [60].

The antihypertensive effects of CR and IF may result, at least in part, from changes in autonomic regulation of vascular smooth muscle and heart cells. CR causes decreased activity of the sympathetic nervous system in both rodents and humans, and this may play an important role in the ability of CR to decrease BP [61]. It appears that reductions in norepinephrine and epinephrine levels play a role in mediating effects of CR on BP and HR. For example, it was recently reported that mice lacking dopamine beta-hydroxylase (an enzyme required for the production of norepinephrine and epinephrine) have reduced BP and HR and that the ability of CR to decrease BP and HR is abolished in these mice [62]. In our own studies, we established that IF reduces BP and HR in rats [7] and have provided evidence that these effects may result, at least in part, from increased parasympathetic tone (Wan et al., *Soc. Neurosci. Abstr.*, 2004). Effects of CR and IF on hypothalamic–pituitary neuroendocrine pathways may also influence

BP. Measurements of concentrations of ACTH and corticosterone under nonstress and stress conditions revealed differences in activity of the hypothalamic-pituitary-adrenal neuroendocrine system in rats on the IF diet compared with those on the control diet. Basal levels of ACTH and corticosterone were increased in rats on IF, but ACTH and corticosterone responses to repeated immobilization stress were significantly attenuated in rats on IF, suggesting improved neuroendocrine stress adaptation [7]. Mild energetic stress may play a role in the beneficial effects of IF on the cardiovascular system because rats fed a 2DG-supplemented diet exhibit changes in HR, BP and cardiovascular stress adaptation similar to rats on IF (Fig. 1) [63].

Recent findings suggest that the interplay between neurotrophic factor and neurotransmitter signaling in the brain can determine the risk of cardiovascular and cerebrovascular diseases (Fig. 3). We found that levels of BDNF are increased in several brain regions of rats and mice maintained on IF [40,64], the same diet that increases insulin sensitivity [6]. The increased amounts of BDNF in the brain may be responsible for the enhanced insulin sensitivity in animals on IF because infusion of BDNF into the ventricles of the brain of mice results in increased insulin peripheral insulin sensitivity [65]. Moreover, BDNF^{+/-} mice, which have reduced levels of BDNF in their brains, are hyperglycemic and their brain BDNF levels and blood glucose levels were normalized when they were maintained on IF [64]. Further evidence that BDNF signaling in the brain controls peripheral glucose metabolism comes from studies on the effects of antidepressant drugs that act by increasing serotonin signaling in the brain. For example, when the serotonin-selective reuptake inhibitor (SSRI) paroxetine was administered to hyperglycemic huntingtin mutant mice, BDNF levels in their brains were increased and their plasma glucose levels were normalized [66]. Several studies have shown that SSRI can increase insulin sensitivity in humans [67,68], although it remains to be established if BDNF mediates the effects of the SSRI. Collectively, the available data suggest that enhanced activity of serotonin and BDNF signaling pathways in the brain is involved in the improved glucose regulation induced by IF (Fig. 3). Such increased insulin sensitivity may contribute to the reduced risk of cardiovascular disease and stroke afforded by CR and IF.

Vascular endothelial cell growth factor (VEGF) is a potent inducer of angiogenesis that is expressed in the heart and the brain. VEGF can have beneficial effects on the cardiovascular system by enhancing coronary artery angiogenesis and by protecting cardiac myocytes against ischemic injury [69]. Similar actions of VEGF in the brain are suggested by studies showing that intracerebroventricular administration of VEGF can reduce brain damage, enhance neurogenesis and stimulate angiogenesis in a rat model of stroke [70]. In addition, VEGF and BDNF each alleviated neurological and vascular dysfunction caused by a high-fat diet in rats [71]. Moreover, VEGF can delay the senescence

of microvascular endothelial cells [72], suggesting that VEGF can counteract age-related vascular pathology. However, other findings suggest that VEGF can promote atherosclerosis [73]. VEGF and BDNF may act together to mediate beneficial effects of exercise on the heart and the brain. Thus, both VEGF and BDNF can stimulate angiogenesis and mediate, at least in part, exercise-induced angiogenesis [69,74–76]. Interestingly, an increase in circulating VEGF levels is critical for exercise-induced neurogenesis in the brain [77]. The latter findings suggest that BDNF and VEGF share biological activities. As is the case with IF-induced neurogenesis, BDNF plays an important role in exercise-induced neurogenesis in rodents [78]. Although the effects of CR and IF on VEGF signaling are unknown, the findings just described suggest a potential role for VEGF as mediator of some of the beneficial effects of dietary restriction in the brain and cardiovascular system.

Finally, data suggest that CR and IF can suppress inflammatory responses to damage to vascular endothelial cells, neurons and cardiac myocytes. For example, levels of proinflammatory cytokines that are believed to play important roles in the process of atherosclerosis are decreased in humans maintained on CR [79]. Inflammatory responses to ischemia/reperfusion injury in the heart were reduced in rats maintained on CR [80]. Microglial responses to excitotoxic brain injury were suppressed in mice maintained on IF compared with those fed ad libitum [81]. Inflammatory

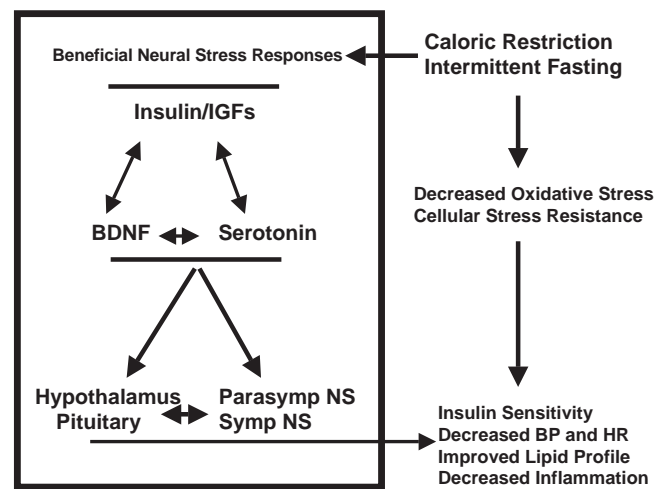


Fig. 3. Model of mechanisms by which DR may decrease the risk of cardiovascular disease and stroke. By inducing a mild cellular stress response in neurons, CR and IF may enhance signaling pathways in the brain involving BDNF, serotonin and insulin/insulin-like growth factors. The latter pathways increase the resistance of neurons to stress and promote adaptive plasticity in the brain (enhanced learning and memory and neurogenesis). The BDNF, serotonin and insulin/IGF signaling pathways in neurons may also exert beneficial effects on peripheral glucose regulation (increased insulin sensitivity) and cardiovascular risk factors (decreased BP and reduction in levels of atherogenic lipids, inflammatory cytokines and oxidative stress). In addition, CR and IF may exert more direct beneficial effects on the cells of the blood vessels and the heart including reduced oxidative stress and increased cellular stress resistance.

processes in blood vessels are increasingly recognized as a major factor involved in the processes of atherosclerosis and ischemic heart and brain injury. A better understanding of the mechanisms by which CR and IF influence cytokine production and activation of macrophages/microglia may provide novel approaches for therapeutic intervention in cardiovascular and cerebrovascular diseases.

6. What are the relative benefits of IF and CR in obese and nonobese individuals?

Most studies on the effects of CR and IF on laboratory animals and humans have involved overweight subjects as control subjects. There is no question that reducing food intake improves the health of overweight individuals: insulin sensitivity is increased, BP is decreased, cellular oxidative stress and inflammation are reduced and cellular stress resistance is increased. However, it remains to be determined if, and to what extent, CR and/or IF might benefit individuals whose body weights are within the “healthy” range. Studies in which calorie intake was progressively decreased in rats demonstrated a progressive increase in lifespan as calories were reduced [2]. If this is also true in humans, then even individuals with a body mass index in the healthy range may benefit from CR. On the other hand, IF without CR had major health benefits in mice [6], suggesting the possibility that similar decreases in meal frequency might improve the health of humans regardless of their body weight. Clearly, well-controlled human studies are required to establish the health benefits that can be achieved by CR and IF in humans across a range of body mass indexes.

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