Neuroprotective signaling and the aging brain: take away my food and let me run¹

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Abstract

It is remarkable that neurons are able to survive and function for a century or more in many persons that age successfully. A better understanding of the molecular signaling mechanisms that permit such cell survival and synaptic plasticity may therefore lead to the development of new preventative and therapeutic strategies for age-related neurodegenerative disorders. We all know that overeating and lack of exercise are risk factors for many different age-related diseases including cardiovascular disease, diabetes and cancers. Our recent studies have shown that dietary restriction (reduced calorie intake) can increase the resistance of neurons in the brain to dysfunction and death in experimental models of Alzheimer’s disease, Parkinson’s disease, Huntington’s disease and stroke. The mechanism underlying the beneficial effects of dietary restriction involves stimulation of the expression of ‘stress proteins’ and neurotrophic factors. The neurotrophic factors induced by dietary restriction may protect neurons by inducing the production of proteins that suppress oxyradical production, stabilize cellular calcium homeostasis and inhibit apoptotic biochemical cascades. Interestingly, dietary restriction also increases numbers of newly-generated neural cells in the adult brain suggesting that this dietary manipulation can increase the brain’s capacity for plasticity and self-repair. Work in other laboratories suggests that physical and intellectual activity can similarly increase neurotrophic factor production and neurogenesis. Collectively, the available data suggest that dietary restriction, and physical and mental activity, may reduce both the incidence and severity of neurodegenerative disorders in humans. A better understanding of the cellular and molecular mechanisms underlying these effects of diet and behavior on the brain is also leading to novel therapeutic agents that mimic the beneficial effects of dietary restriction and exercise.

Theme: Development and regeneration

Topic: Genesis of neurons and glia

Keywords: Alzheimer’s disease; Calories; Heat shock protein; Oxidative stress; Mitochondria; Neurotrophic factor; Parkinson’s disease; Stroke

1. Low calorie intake and exercise: what’s good for the body is good for the brain

Clinical and epidemiological data unequivocally show that overeating and lack of exercise increase the risk for the most prevalent of age-related diseases including cardiovascular disease, diabetes and cancers [1,23,26]. Conversely, a decrease in calorie intake and an increase in exercise can reduce risk for the same diseases. Emerging data from our studies of animal models and from epidemiological data suggest that a similar scenario may apply to neurodegenerative disorders including Alzheimer’s disease (AD), Parkinson’s disease (PD) and stroke. Before describing the findings that support the latter statement, it is important to briefly review the extensive data that document the beneficial effects of caloric restriction in the retardation of aging and disease.

The lifespans of laboratory mice and rats can be increased by up to 50% simply by reducing their calorie intake with maintenance of micronutrient intake [42]. Such dietary restriction (DR) also reduces the development of age-related cancers and deficits in immune function. The cellular and molecular underpinnings of the beneficial effects of DR are not yet fully understood, but may involve reduced mitochondrial oxyradical production and/or increased expression of cytoprotective stress proteins. Might
DR also benefit the brain? Although benefits of DR on the cardiovascular, immune and endocrine systems have been demonstrated, its effects on the nervous system are only now being studied. Studies of rats and mice maintained on a DR feeding regimen suggest that DR may slow age-related molecular changes in the brain including increases in levels of glial fibrillar acidic protein and oxidative damage to proteins and DNA [8,11]. Moreover, DR attenuates age-related deficits in learning and memory ability and motor function in rodents [19,44]. Interestingly, physical activity (which can reduce body weight) may also counteract the adverse effects of aging and disease on the brain. Thus, mice allowed access to a running wheel exhibit increased neurogenesis and improved learning and memory compared to ‘couch potato’ mice [45]. In addition, when rodents are raised in an enriched environment in which they have many objects to play with and so on, neurogenesis is enhanced and learning and memory ability improved [22,37,47].

2. Experimental and epidemiological support for neuroprotective effects of dietary restriction

Because life expectancy is increasing, more and more persons will suffer from age-related neurodegenerative conditions with AD, PD and stroke being the most prevalent. AD results from degeneration and death of neurons in brain regions involved in learning and memory processes, such as the hippocampus and cerebral cortex [29,30,40]. Degeneration of dopaminergic neurons in the substantia nigra, with consequent motor dysfunction is the defining feature of PD [21,41]. Stroke results from occlusion or rupture of a cerebral blood vessel which damages the brain cells supplied by that vessel [4,10]. The impact of these disorders on our society is emphasized by the fact that more dollars are required to care for patients with AD, PD and stroke than are spent on care for patients with cardiovascular disease and cancer.

The identification of disorder-specific genetic and environmental factors that may initiate the neurodegenerative process in AD, PD and stroke has led to the development of useful animal models of these disorders. In the case of AD the models include transgenic mice overexpressing mutant forms of amyloid precursor protein which are known to cause early-onset inherited AD [12,18], transgenic and knockin mice expressing mutant forms of presenilin-1 linked to inherited forms of AD [9,15], and administration of amyloid β-peptide and/or excitotoxins into the brains of rats and mice [13,43]. Models of PD include administration of the toxin MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) to adult monkeys and mice which results in selective loss of substantia nigra dopaminergic neurons and associated motor dysfunction [6], and α-synuclein mutant mice which exhibit degeneration of dopaminergic neurons and a behavioral phenotype that mimicks several features of PD [28]. Stroke models include transient or permanent occlusion of the middle cerebral artery in rats and mice [4]. We have investigated the impact of DR on the neurodegenerative process in several of these animal models.

In many of our studies we employed an alternate day feeding DR regimen; this DR protocol results in an approximately 30% reduction in calorie intake over time and extends the lifespans of rats and mice by 30–40%. Maintenance of rats on the alternate day DR feeding regimen for 2–4 months results in resistance of hippocampal neurons to kainate-induced degeneration [3]. The reduced damage to hippocampal neurons is correlated with a striking preservation of learning and memory in a water maze spatial learning task. In order to determine whether DR might counteract the pathogenic actions of mutations in presenilin-1 and the amyloid precursor protein (APP), we maintained presenilin-1 mutant knockin mice and APP mutant transgenic mice on the alternate day feeding regimen. We had previously shown that presenilin-1 mutations increase the vulnerability of hippocampal and cortical neurons to excitotoxicity and apoptosis by a mechanism involving enhanced calcium release from the endoplasmic reticulum [15,34]. Presenilin-1 mutant knockin mice that had been maintained on DR for 3 months exhibited increased resistance of hippocampal CA1 and CA3 neurons to excitotoxic injury compared to mice fed ad libitum [51]. Levels of oxidative stress in the hippocampus following kainate administration were lower the DR mice compared to mice fed ad libitum, indicating that suppression of oxidative stress may be one mechanism underlying the neuroprotective effect of DR. Thus, the neurodegeneration-promoting effect of a mutation that causes AD can be counteracted by a reduced calorie diet.

APP mutant mice exhibit progressive age-dependent deposition of Aβ in their brains which is most prominent in the cerebral cortex and hippocampus [12,18]. In an initial test of the hypothesis that DR would suppress Aβ deposition in APP mutant mice, we obtained a quite surprising result. When APP mutant mice were placed on an alternate day feeding schedule, they died within 2–3 weeks [39]. The APP mutant mice became severely hypoglycemic during the days they were without food, and apparently succumbed to the hypoglycemia. Further analyses revealed marked abnormalities in regulation of the stress-responsive hypothalamic–pituitary–adrenal axis in the APP mutant mice characterized by abnormal glucocorticoid and blood glucose regulation in response to restraint stress, for example [39]. More recent findings suggest that there are abnormalities in corticotropin releasing hormone production in association with Aβ accumulation in the cerebral cortex, hippocampus and hypothalamus of the APP mutant mice that may contribute to their inability to ‘cope’ with stress. Interestingly, when the alternate day feeding regimen is begun in APP mutant mice that are less than 3 months of age they survive. We are now determin-
lying if, under the latter conditions, as well as in APP mutant mice maintained on a pair-feeding DR regimen, deposition of Aβ in the brain is decreased.

DR also has a beneficial effect in experimental models of the movement disorders. We have shown that the vulnerability of midbrain dopaminergic neurons to MPTP toxicity is decreased in mice maintained on DR [5]. More dopaminergic neurons survive exposure to MPTP, and deficits in motor function are markedly decreased in DR rats. Selective degeneration of neurons in the striatum of patients with Huntington’s disease (HD) is responsible for their inability to control body movements properly. An animal model of Huntington’s disease involves administration of the succinate dehydrogenase inhibitor (mitochondrial toxin) 3-nitropropionic acid (3NP) to rats and mice. Maintenance of rats on a DR regimen for several months prior to administration of 3NP results in increased resistance of striatal neurons to 3NP and improved motor function [3].

Amyotrophic lateral sclerosis (ALS) is a fatal disease characterized by progressive degeneration of spinal cord motor neurons resulting in progressive paralysis. A small percentage of cases of ALS result from mutations in the gene encoding the antioxidant enzyme Cu/Zn-superoxide dismutase. Transgenic mice expressing mutant Cu/Zn-SOD exhibit progressive motor neuron degeneration and a clinical phenotype remarkably similar to ALS patients [38]. We maintained Cu/Zn-SOD mutant mice on a DR regimen to determine the impact of a reduced calorie diet on age of disease onset and disease progression. In contrast to the beneficial effects of DR in the PD and HD models, Cu/Zn-SOD mutant mice do not benefit from DR [38]. DR did not delay disease onset and, once mice became symptomatic, disease progression was actually accelerated. These findings are important because they show that DR cannot overcome the pathogenic action of the Cu/Zn-SOD mutation suggesting that the neurodegenerative cascade in this mouse model is fundamentally different than that in the AD, PD and HD models. However, an alternative interpretation is that not all populations of neurons benefit equally from DR. From the clinical perspective these findings are also of interest because it is well known that increased energy intake has a beneficial effect on disease progression in ALS patients.

We have also determined whether DR can modify outcome in a rat stroke model in which the middle cerebral artery is transiently occluded resulting in damage to the cerebral cortex and striatum supplied by that artery, and associated motor dysfunction. Rats that had been maintained on DR for several months exhibited reduced brain damage and improved behavioral outcome following transient occlusion of the middle cerebral artery [49]. DR is also effective in reducing focal ischemic brain damage in mice (unpublished data). The experimental findings just described directly demonstrate profound neuroprotective effects of DR in animal models relevant to the pathogenesis of AD, PD, HD and stroke. The striking beneficial effects of DR in these experimental models suggest that DR may prove beneficial in reducing the incidence and/or severity of many different human neurodegenerative disorders. The following epidemiological data support the latter possibility.

Recent epidemiological data suggest that individuals with a low calorie intake may have reduced risk for AD and PD. Analyses of different populations throughout the world reveals a strong relation between per capita food consumption and risk for AD [14]. For example, persons in China and Japan have relatively low calorie intakes (1600–2000 calories/day) as compared to persons in the United States and Western Europe (2500–3000 calories/day). The incidence of AD in China and Japan is approximately half that in the United States and Western Europe, although it should be recognized that per capita food consumption is a very poor measure of energy intake. It may also be the case that AD is underdiagnosed in countries such as China, and therefore the kinds of relationships established in such cross-cultural comparisons are not conclusive. More compelling evidence that caloric restriction can protect against neurodegenerative disorders comes from prospective studies of a large cohort of people living in New York City. It was found that those with the lowest daily calorie intakes had the lowest risk for AD [35] and PD [27]. In both of these studies nutrient intake was assessed using a semi-quantitative food-frequency questionnaire which included 61 foods, use of vitamin and mineral supplements, types of breakfast cereals consumed, type of fats used for frying and baking, use of sugar and salt, and alcohol. Each food had a fixed portion size, and nutrient content of each portion was estimated from USDA food composition data. Subjects were asked how often the consumed each food, and were asked to report their usual dietary pattern over the last year. To control for possible disease-related changes in diet, the subjects were asked to report changes in their dietary habits in the last 10 years. Finally, the epidemiological data suggesting that overeating is a major risk factor for stroke is quite compelling [2]. Our experimental data described above further suggest that a high calorie intake can worsen outcome following stroke [49]. It remains to be determined whether reduced calorie intake will result in improved outcome following stroke in humans.

3. Training neurons to increase their endurance and power: the cellular and molecular mechanisms underlying the beneficial effects of dietary restriction and exercise

How does DR increase resistance of neurons to neurodegenerative disorders? In order to answer this question it is necessary to understand the biochemical cascades that occur in neurons that result in their dysfunction and death.
in aging and neurodegenerative disorders. Although the genetic and environmental factors that initiate the neurodegenerative process may differ among diseases, considerable evidence suggests that a common set of alterations ensues that ultimately kills the neuron. Prominent among the alterations are: increased oxidative stress involving oxyradical-mediated damage to proteins, lipids and nucleic acids; impaired ability of the neuron to regulate ion homeostasis resulting in aberrant increases in intracellular calcium levels; impaired energy metabolism that may result from and contribute to mitochondrial dysfunction; and activation of a cascade of molecular interactions called apoptosis that involves proteins such as Par-4, Bcl-2 family members and caspases [33]. In AD the neurodegenerative cascade can be initiated by the aging process, or by specific genetic mutations in APP, presenilin-1 or presenilin-2. In each case there is increased production and extracellular deposition of a neurotoxic proteolytic peptide product of APP called amyloid β-peptide (Aβ). Aβ promotes neuronal apoptosis and excitotoxicity by a mechanism involving membrane lipid peroxidation and impairment of ion-motive ATPases and glucose and glutamate transporters [30]. PD may be caused primarily by environmental factors, although a very small percentage of inherited cases have been linked to mutations in a synaptic protein called α-synuclein [41]. The neurodegenerative process may be triggered in dopaminergic neurons by factors that induce oxidative stress such as iron and dopamine metabolites. Oxidative stress and perturbed calcium regulation are also intimately involved in the neurodegenerative cascades that occur in HD and stroke. Because DR increases neuronal resistance to dysfunction and death in each of the just-mentioned disorders, it seems likely that the mechanism underlying this neuroprotective effect of DR somehow impinges upon the shared features of the neurodegenerative cascades.

Data from the animal studies described above clearly show that neurons in the brains of rats and mice maintained on a DR regimen exhibit increased resistance to oxidative, metabolic and excitotoxic insults. What accounts for resistance to this broad array of insults? We have begun to address this important question by measuring levels of expression of various proteins known to confer resistance of neurons to many different insults. Our first positive results came when levels of several different ‘stress proteins’ were measured in brain tissues from rats maintained for 3 months on either ad-libitum or DR diets. We found that levels of heat-shock protein-70 (HSP-70) and glucose-regulated protein-78 (GRP-78) were increased in cortical, striatal and hippocampal neurons of DR rats compared to rats fed ad libitum [5,24,49]. Levels of heat-shock protein-60 were unchanged. Previous studies in this and other laboratories had provided evidence that HSP-70 and GRP-78 can protect neurons against excitotoxic and oxidative injury [46,48], suggesting that they contribute to the neuroprotective effect of DR. These findings further suggested that DR may induce a mild stress response in neurons, presumably because of a reduced energy (glucose) availability to the neurons. Such a ‘preconditioning’ mechanism of action of DR is supported by our studies showing that metabolic stress induced by administration of 2-deoxyglucose (2-DG, a non-metabolizable analog of glucose) to animals fed ad libitum also increases resistance of neurons to injury. For example, rats receiving 2-DG exhibit reduced damage to hippocampal neurons and improved learning and memory ability following kainate administration [24], and reduced damage to cortical and striatal neurons and improved behavioral outcome following transient occlusion of the middle cerebral artery [49]. In addition, mice pretreated with 2-DG, exhibit decreased damage to dopaminergic neurons in the substantia nigra and marked attenuation of motor deficits [5]. 2-DG treatment resulted in increased expression of HSP-70 and GRP-78. In cultured neurons 2-DG pretreatment suppresses oxidative stress, preserves mitochondrial function, stabilizes calcium homeostasis and attenuates neuronal death following exposure to excitotoxic, metabolic and oxidative insults [24].

Considerable evidence suggests that the neurodegenerative process results from alterations in synaptic terminals that result in synaptic dysfunction and activation of apoptotic and excitotoxic cascades [33]. We have found that cortical synaptosomes prepared from rats maintained on DR exhibit increased resistance to oxidative and metabolic insults, as indicated by relative preservation of glucose and glutamate transport and mitochondrial function [16]. Synaptosomes from rats given 2-DG are also more resistant to various insults [17]. The content of HSP-70 and GRP-78 in the synaptosomes is increased suggesting that levels of neuroprotective proteins increase locally in synaptic compartments in response to DR [16,17]. Thus, DR bolsters the ability of synapses to cope with oxidative and metabolic insults.

Perhaps the most intriguing aspect of the effects of DR on the brain was revealed in our very recent studies showing that levels of several neurotrophic factors, the most notable of which is brain-derived neurotrophic factor (BDNF), are increased in brain cells of rats and mice maintained on a DR feeding regimen [7,25]. Studies performed in my laboratories during the past 12 years has documented the neuroprotective activities of several different neurotrophic factors in experimental models of neurodegenerative disorders [31,32]. In general, the neurotrophic factors protect neurons by inducing the expression of genes that encode proteins that suppress oxidative stress (antioxidant enzymes and Bcl-2) and stabilize cellular calcium homeostasis (calcium-binding proteins and glutamate receptor subunits). BDNF and other neurotrophic factors have also been shown to exert beneficial effects on synaptic plasticity and may thereby facilitate learning and memory [20]. BDNF may play a particularly prominent role in the neuroprotective effect of DR because infusion...
of a BDNF blocking antibody into the lateral ventricles of rats maintained on DR significantly attenuates the protective effect of DR [7].

Work performed during the past decade has established that the adult brain contains populations of cells that are capable of dividing and then differentiating into neurons or glial cells, a process called neurogenesis. In rodents and primates such neural stem cells are most abundant in the subventricular zone and the dentate gyrus of the hippocampus [36]. The presence of stem cells in the adult brain suggests that they may provide a reserve of neural cells that can be used to replace cells that die as the result of various injuries and diseases. Indeed, the proliferation of neural stem cells can be stimulated by traumatic, ischemic and excitotoxic brain injuries. Interestingly, an ‘enriched’ environment and physical exercise can also enhance neurogenesis [22,47] suggesting that the stem cells can respond not only to injury, but also to increased functional demands upon neural circuits. We have found that DR can modulate numbers of newly generated neural cells in the brains of rats [25]. Rats that had been maintained on ad libitum and DR (alternate day feeding) regimens for 3 months were given five daily injections of the DNA precursor bromodeoxyuridine (BrdU). Rats were killed either 1 day or 3 weeks after the last BrdU injection and numbers of newly generated cells in the dentate gyrus were quantified by unbiased stereological methods. There was no difference in numbers of newly generated cells at the 1 day time point. However, significantly more BrdU-positive cells remained at the 3 week time point in the DR rats compared to the ad libitum-fed rats suggesting that DR promotes survival of newly generated neural cells (Figs. 1 and 2). Although not yet established, it is conceivable that BDNF plays a role in the enhanced survival of newly-generated neural stem cells in the dentate gyrus of rats maintained on DR because BDNF is known to have a similar effect on other populations of neural stem cells [50].

4. Conclusions

In many different experimental animal models DR increases resistance of neurons to the kinds of adverse conditions believed to promote the neurodegenerative process. Findings from animal studies are supported by epidemiological data, and together strongly suggest that reduced calorie intake increases resistance of the nervous system to disease. DR may exert its beneficial effects by inducing a mild ‘stress response’ which results in the

![Diagram](image_url)

Fig. 1. Possible mechanism whereby dietary restriction increases resistance of neurons to aging, disease and injury. 2DG, 2-deoxy-D-glucose; ER, endoplasmic reticulum; ETC, electron transport chain; GRP78, glucose-regulated protein-78; HSP70, heat-shock protein-70; NTF, neurotrophic factor; ROS, reactive oxygen species.
expression of genes that encode proteins such as neurotrophic factors and heat-shock proteins that serve to suppress oxi-radical production and stabilize cellular calcium homeostasis. When extrapolated to humans, the data obtained from animal studies suggest that moderate levels of DR in adult life (in the range of 1800–2200 calories/day) may dramatically reduce the incidence and severity of AD, PD and stroke.

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