

REVIEW

# Meal size and frequency affect neuronal plasticity and vulnerability to disease: cellular and molecular mechanisms

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## Abstract

Although all cells in the body require energy to survive and function properly, excessive calorie intake over long time periods can compromise cell function and promote disorders such as cardiovascular disease, type-2 diabetes and cancers. Accordingly, dietary restriction (DR; either caloric restriction or intermittent fasting, with maintained vitamin and mineral intake) can extend lifespan and can increase disease resistance. Recent studies have shown that DR can have profound effects on brain function and vulnerability to injury and disease. DR can protect neurons against degeneration in animal models of Alzheimer's, Parkinson's and Huntington's diseases and stroke. Moreover, DR can stimulate the production of new neurons from stem cells (neurogenesis) and can enhance synaptic plasticity, which may increase the ability of the brain to resist aging and restore function following injury. Interestingly, increasing the time interval between meals can have beneficial effects on the brain and overall health of mice that are independent of cumulative calorie intake. The beneficial effects of DR, particularly those of intermittent fasting, appear

to be the result of a cellular stress response that stimulates the production of proteins that enhance neuronal plasticity and resistance to oxidative and metabolic insults; they include neurotrophic factors such as brain-derived neurotrophic factor (BDNF), protein chaperones such as heat-shock proteins, and mitochondrial uncoupling proteins. Some beneficial effects of DR can be achieved by administering hormones that suppress appetite (leptin and ciliary neurotrophic factor) or by supplementing the diet with 2-deoxy-D-glucose, which may act as a calorie restriction mimetic. The profound influences of the quantity and timing of food intake on neuronal function and vulnerability to disease have revealed novel molecular and cellular mechanisms whereby diet affects the nervous system, and are leading to novel preventative and therapeutic approaches for neurodegenerative disorders.

**Keywords:** Alzheimer's disease, brain-derived neurotrophic factor (BDNF), caloric restriction, chaperone, neurogenesis, stem cells.

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## Diet and the nervous system

The influence that dietary factors have on the function of the nervous system, and on its susceptibility to disease, is an active and important area of biomedical research. Studies have identified several specific dietary components that are critical for proper development of the nervous system. For example, folate deficiency during pregnancy can result in neural tube defects in babies (Refsum 2001), choline deficiency during brain development may result in learning and memory problems (Zeisel 1997), and certain fatty acids are critical for optimum brain function in the adult (Chalon *et al.* 2001). Recent findings suggest that folate deficiency (and a consequent increase in the levels of homocysteine)

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**Abbreviations used:** AD, Alzheimer's disease; ALS, amyotrophic lateral sclerosis; APP, amyloid precursor protein; BDNF, brain-derived neurotrophic factor; BrdU, bromodeoxyuridine; CNTF, ciliary neurotrophic factor; 2-DG, 2-deoxy-D-glucose; DR, dietary restriction; HD, Huntington's disease; HSP, heat-shock protein; IRS-1, insulin receptor substrate-1; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; NT-3, neurotrophin-3; PD, Parkinson's disease; SOD, superoxide dismutase.

may increase the risk of Alzheimer's disease (AD), Parkinson's disease (PD), stroke and psychiatric disorders (Duan *et al.* 2002; Kruman *et al.* 2002; Seshadri *et al.* 2002). Folate plays a critical role in one-carbon metabolism by facilitating the remethylation of methionine from homocysteine (Fenech 2001). By increasing homocysteine levels and impairing DNA synthesis, methylation and repair, folate deficiency can damage cells including neurons (Kruman *et al.* 2000, 2002). Other examples of dietary supplements that may improve brain function and/or protect against age-related disease include antioxidants such as vitamin E (Halliwell 2001), Ginkgo biloba extract (Youdim and Joseph 2001) and creatine (Mattson 2000a).

While dietary vitamins, minerals and antioxidants may improve the healthspan of the brain somewhat, a more fundamental aspect of diet is emerging as a major factor in brain health. This factor, which is the focus of the present review article, is caloric intake. Both the number of calories consumed over time and the time interval between feedings affect the physiology of brain cells in quite profound ways. The dietary restriction (DR) regimens used in the animal studies described involve a reduction in overall calorie intake, and/or an increase in the intermeal interval, with maintenance of the composition of the diet in terms of vitamins, minerals, protein, etc.

### The impact of calorie intake on lifespan and susceptibility to disease

The mean and maximum lifespans of a range of organisms including yeast, roundworms, rodents and monkeys can be increased by up to 50% simply by reducing their calorie intake (Weindruch and Sohal 1997; Lin *et al.* 2000; Sze *et al.* 2000; NIA primate study, unpublished data). Caloric restriction reduces the incidence of age-related cancers, cardiovascular disease and deficits in immune function in rodents (Weindruch and Sohal 1997). Conversely, overeating is a risk factor for cardiovascular disease, many types of cancers, type-2 diabetes and stroke (Leibovitz 1999; Levi 1999; Brochu *et al.* 2000). Less appreciated is evidence suggesting that DR reduces disease risk and extends lifespan in individuals that are not 'overweight' (Roth *et al.* 2002; Walford *et al.* 2002). The latter studies provide strong associations between biomarkers of caloric restriction and lifespan extension in humans. The carbohydrates, fats and proteins in food are metabolized to glucose which is then utilized as the major source for ATP production in cells. Although such biochemical energy production is required to sustain cell viability and functions, excessive energy production may cause cells to become damaged and 'spoiled', and thereby susceptible to disease. Because organisms evolved in environments in which food supplies were present in varied locations and amounts, the organisms had to adapt to such changing food availability. One such adaptation is the

ability to store energy reserves in the forms of glycogen and lipids. When food supplies are scarce, the cells of organisms are faced with an energetic stress that may induce changes in gene expression that result in adaptive changes in cellular metabolism and the increased ability of the organism to resist stress. When food supplies are plentiful, as in most laboratory animal colonies and human populations in industrialized countries, individuals consume more calories than are necessary for the maintenance of their health.

Two different paradigms of DR have been widely employed because of their highly reproducible abilities to increase lifespan in rats and mice. In one paradigm the animals receive food daily, but are limited to a specified amount which is typically 30–50% less than the *ad libitum* consumption of the control group. The second paradigm involves periodic fasting in which the animals are deprived of food for a full day, every other day, and are fed *ad libitum* on the intervening days. Analyses of various physiological parameters in animals maintained on these two different DR regimens have revealed several similar changes including decreased body temperature, decreased heart rate and blood pressure, and decreased glucose and insulin levels (Table 1). These DR regimens have also been shown to have beneficial effects on the brain. For example, DR retards age-related increases in the levels of glial fibrillary acidic protein and oxidative damage to proteins and DNA (Dubey *et al.* 1996; Major *et al.* 1997). Analysis of the levels of mRNAs encoding thousands of proteins in the brains of young and old rats, which had been fed either *ad libitum* or DR diets, revealed numerous age-related changes in gene expression that were attenuated by DR (Lee *et al.* 2000c). The genes in which expression was affected by aging and counteracted by DR included those involved in oxidative stress responses, innate immunity and energy metabolism (Table 2). These kinds of studies are providing novel insight into how DR affects the function and plasticity of the nervous system.

**Table 1** Physiological responses to dietary restriction

Parameter	Daily CR	Periodic fasting
Body weight	decrease	decrease or no change
Body fat	decrease	decrease
Body temperature	decrease	decrease
Blood pressure	decrease	decrease
Heart rate	decrease	decrease
Blood glucose	decrease	decrease
Blood insulin	decrease	decrease
IGF-1 levels	decrease	increase
$\beta$ -hydroxybutyrate	no change	increase
HDL	increase	increase
Homocysteine	decrease	decrease

Taken from data cited in Weindruch and Sohal (1997) and Lane *et al.* (1999) and from the author's unpublished data.

**Table 2** Examples of the effects of dietary restriction on changes in gene expression in the brain during aging

Gene	Change during aging	
	Usual diet	Dietary restriction
<i>Energy-related</i>		
Cytochrome oxidase	decreased expression	little or no change in expression
Glucose-6-phosphatase	decreased expression	no change in expression
Fructose-1,6-bisphosphatase	increased expression	no change in expression
Creatine kinase	increased expression	increased expression
<i>Stress-related</i>		
HSP-70	no change or decrease	no change or increase
GRP-78	no change or decrease	no change or increase
Gadd153	increased expression	increased expression
Proteasome z subunit	decreased expression	decreased expression
<i>Inflammation-related</i>		
GFAP	increased expression	little or no change in expression
Complement C1q	increased expression	little or no change in expression
Complement C4	increased expression	small increase in expression
<i>Plasticity-related</i>		
NMDA receptor NR1	decreased expression	little or no change in expression
BDNF	decreased expression	little or no change in expression
TrkB	decreased expression	not determined
$\alpha$ -Synuclein	decreased expression	decreased expression

Taken from data in, or cited in, Lee *et al.* (2000c, 2002d) and Duan and Mattson (1999). In the study of Prolla and colleagues (Lee *et al.* 2002c) analyses were performed on brain tissue samples from 24-month-old mice that had been maintained throughout their adult life on a diet with a 30% reduction in calories. In the studies of Mattson and colleagues (Duan and Mattson 1999; Lee *et al.* 2002d) analyses were performed on brain tissue samples from young adult mice that had maintained on an every-other-day fasting regimen for 3 months.

### Effects of dietary restriction on synaptic plasticity and neurogenesis

Because deficits in learning and memory, motor control and other behaviors occur during aging, some of the earliest studies of the impact of DR on the nervous system involved testing the function of the nervous systems of old rodents that had been maintained on *ad libitum* or calorie-restricted diets during their adult lives (Table 3). Mice maintained on a diet with a 40% reduction in calories beginning at the time of weaning did not exhibit the deficits in motor coordination and spatial learning seen in control mice fed *ad libitum* (Ingram *et al.* 1987). DR beginning at 3 months of age prevented age-related deficits in a radial maze learning task in mice (Idrobo *et al.* 1987). Similarly, life-long caloric restriction prevented age-related deficits in the performance of rats in radial arm maze and Morris water maze learning and memory tasks (Stewart *et al.* 1989). DR retarded age-associated deficits in sensorimotor coordination and avoidance learning in mice (Dubey *et al.* 1996). Long-term potentiation of synaptic transmission is believed to be a cellular correlate of learning and memory. Aged rats exhibit a deficit in long-term potentiation in the hippocampus, and this deficit is largely abolished in age-matched rats that are fed a reduced calorie

diet during their adult life (Hori *et al.* 1992; Eckles-Smith *et al.* 2000). Beneficial effects of DR were evident in aged (22-month-old) mice in which caloric restriction was initiated in mid-life (14 months of age); strength and coordination were preserved and age-related changes in spontaneous alternation behavior and altered responses to enclosed alleys were preserved (Means *et al.* 1993).

A few studies have directly examined synapses from animals that had been maintained on DR feeding regimens. In one study, neocortical synaptosomes were isolated from rats that had been maintained for 3 months on a periodic fasting (alternate-day fasting) feeding regimen and from control rats fed *ad libitum*. The synaptosomes from the rats on the DR regimen exhibited improved glucose transport and mitochondrial function following exposure to oxidative and metabolic insults (Guo *et al.* 2000), demonstrating that DR has local beneficial effects on synapses. In another study, presumptive inhibitory and excitatory synapses in layer 2 of the somatosensory cortex of 29-month-old rats that had been maintained for 25 months on control and calorie-restricted diets were quantified by analysis of electron micrographs. Caloric restriction did not prevent the age-related decrease in the density of inhibitory synapses and, surprisingly, decreased the density of excitatory synapses (Shi *et al.*

**Table 3** Effects of dietary restriction on the nervous system

Effect	Reference
Mouse	
Enhanced learning in aged animals	Ingram <i>et al.</i> (1987)
Enhanced motor function in aged animals	Ingram <i>et al.</i> (1987)
Slows age-related loss of spiral ganglion neurons	Park <i>et al.</i> (1990)
Reduces oxidative stress in brain cells of aged animals	Dubey <i>et al.</i> (1996)
Protects against MPTP-induced damage to dopaminergic neurons and preserves motor function	Duan and Mattson (1999)
Counteracts adverse effects of an Alzheimer's mutation in presenilin-1	Zhu <i>et al.</i> (1999)
No benefit in Cu/Zn-SOD mutant ALS mice	Pedersen <i>et al.</i> (1999)
Induces BDNF production and enhances neurogenesis	Lee <i>et al.</i> (2002a,c)
Suppresses injury-induced microglial activation	Lee <i>et al.</i> (2002d)
Rat	
Enhanced spatial learning in aged animals	Stewart <i>et al.</i> (1989)
Attenuates age-related loss of cortical dendritic spines	Moroi-Fetters <i>et al.</i> (1989)
Enhances dopamine overflow in striatum	Diao <i>et al.</i> (1997)
Attenuates age-related increases in GFAP levels	Major <i>et al.</i> (1997)
Attenuates age-related decrease in cardiac synaptic terminal norepinephrine uptake	Snyder <i>et al.</i> (1998)
Protects against seizure-induced hippocampal damage and memory impairment	Bruce-Keller <i>et al.</i> (1999)
Protects striatal neurons against mitochondrial toxins	Bruce-Keller <i>et al.</i> (1999)
Protects against focal ischemic brain injury and improves functional outcome in a stroke model	Yu and Mattson (1999)
Protects synapses against oxidative and metabolic stress	Guo and Mattson (1999)
Protects thalamic neurons against thiamine deficiency	Calingasan and Gibson (2000)
Prevents age-related deficit in hippocampal LTP	Eckles-Smith <i>et al.</i> (2000)
Enhances hippocampal neurogenesis	Lee <i>et al.</i> (2000a, 2002b)

2002). The effects of DR on synaptic numbers, structure and function may therefore be complex and may differ in different regions of the nervous system. Effects of DR on neurotransmitters have also been documented. For example, DR prevented age-related alterations in the levels of serotonin and dopamine in the cerebral cortex of rats (Yeung and Friedman 1991), and enhanced evoked dopamine accumulation in the striatum of aged rats (Diao *et al.* 1997). Preservation of neurotransmitter signaling is likely to be critical for the ability of DR to maintain the function of the nervous system during aging.

The adult brain contains populations of cells that are capable of dividing and then differentiating into neurons (neurogenesis) or glial cells (gliogenesis) (Gage 2000). In mammals, including humans, neural stem cells are most abundant in the subventricular zone and the dentate gyrus of the hippocampus. Stem cells in the adult brain may provide a cellular reserve to replace neurons and glia that die as the result of various injuries and diseases; evidence suggesting that neurogenesis can be stimulated by ischemic and excitotoxic brain injuries is consistent with the cellular reserve hypothesis (Parent *et al.* 1997; Liu *et al.* 1998). Interestingly, more subtle physiological signals can regulate neurogenesis, suggesting that neural stem cells may be continually responding to functional demands placed upon neuronal circuits. For example, raising rats or mice in an 'enriched' environment or increasing their level of physical

exercise can enhance neurogenesis (Kemperman *et al.* 1997; Nilsson *et al.* 1999; van Praag *et al.* 1999). In addition, neurogenesis and synaptic connections are affected by changes in the levels of the sex steroids testosterone and estrogen (Alvarez-Buylla and Kim 1997; McEwen 2001).

We recently reported that DR (periodic fasting) can increase neurogenesis in the brains of adult rats and mice (Lee *et al.* 2000a, 2002b,d). Animals that had been maintained on a periodic fasting regimen and control animals fed *ad libitum* for 3 months were given five daily injections of the DNA precursor bromodeoxyuridine (BrdU), and were killed either 1 day or 3–4 weeks after the last BrdU injection. Numbers of BrdU-positive (newly generated) cells in the hippocampal dentate gyrus were quantified by unbiased stereological methods. There was no difference in BrdU-labeled cells between DR and control animals at the 1-day time point indicating that DR does not affect the proliferation rate of the neural stem cells. However, there was a significant increase in the number of BrdU-positive cells remaining at the 3- and 4-week time points in the animals on DR, suggesting that DR promotes the survival of newly generated neural cells (Lee *et al.* 2000a, 2002d). Many of the newly generated cells became neurons as indicated by their expression of neuron-specific antigens and by their localization within the granule cell layer of the dentate gyrus.

Does increased neurogenesis contribute to the improved learning and memory ability in rodents maintained on DR

regimens? Shors *et al.* (2001) administered a drug that inhibits cell proliferation to adult rats, and compared their performance in a hippocampal-dependent trace conditioning task to that of control rats (Shors *et al.* 2001). The drug-treated rats exhibited decreased hippocampal neurogenesis and were impaired in the associative learning task, suggesting a requirement for neurogenesis in this form of learning and memory. Another study involved exposure of rats to a low dose of gamma irradiation that inhibits neurogenesis. Three weeks later, electrophysiological analyses of synaptic plasticity in the dentate gyrus revealed that long-term potentiation of synaptic transmission was blocked in rats that had been irradiated (Snyder *et al.* 2001). However, these studies did not rule out the possibility that the adverse effects of the anti-mitotic drug and the radiation on learning and memory were a result of direct effects on neurons that are unrelated to neurogenesis. In another study it was shown that the selective deletion of the presenilin-1 gene in the mouse forebrain results in a deficiency in enrichment-induced neurogenesis in the dentate gyrus, but does not cause any detectable learning deficits (Feng *et al.* 2001). Interestingly, additional analyses in the latter study suggested that neurogenesis is required for the elimination of 'outdated' memory traces.

### Neuroprotective effects of dietary restriction

The number of people with age-related neurodegenerative conditions such as AD, PD, stroke, and hearing and vision loss is increasing rapidly as life expectancy continues to increase. The severe impact of age-related neurodegenerative 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. Different, but overlapping, populations of neurons degenerate in AD, PD and stroke. Neurons in brain regions involved in learning and memory processes, such as the hippocampus and cerebral cortex, are affected in AD (Ray *et al.* 1998). In PD, dopaminergic neurons in the substantia nigra degenerate resulting in motor dysfunction (Jenner and Olanow 1998). A stroke occurs when a cerebral blood vessel becomes occluded or ruptured resulting in the degeneration of neurons in the brain tissue supplied by that vessel (Schulz and Dichgans 1999). Overeating is a well-established risk factor for stroke, and recent epidemiological data suggest that individuals with high calorie intakes may also be at increased risk of AD and PD (Bronner *et al.* 1995; Logroscino *et al.* 1996; Mayeux *et al.* 1999).

Striking neuroprotective effects of DR in animal models of neurodegenerative disorders suggest that it may be possible to reduce their incidence and severity by changes in diet. In one of the first studies to document a neuroprotective effect of DR, it was shown that DR attenuates the age-related loss of spiral ganglion neurons in C57BL/6 mice (Park *et al.* 1990). A series of recent studies have employed animal

models of neurodegenerative disorders to directly determine the effects of DR on neuronal vulnerability and functional outcome; the models are based upon genetic and environmental factors that may initiate or promote the neurodegenerative process in the corresponding human disorder. AD models include transgenic mice expressing mutant forms of human amyloid precursor protein and/or presenilin-1 that cause early onset inherited AD (Games *et al.* 1995; Duff *et al.* 1996; Hsiao *et al.* 1996; Guo *et al.* 1999) and infusion of amyloid  $\beta$ -peptide and excitotoxins into the brains of rats and mice (Geula *et al.* 1998; Bruce-Keller *et al.* 1999). PD models include administration of the toxins 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), 6-hydroxy-dopamine or rotenone to rodents or monkeys resulting in the selective degeneration of substantia nigra dopaminergic neurons and associated motor dysfunction (Duan *et al.* 1999), and transgenic mice expressing mutant human  $\alpha$ -synuclein, which exhibit degeneration of dopaminergic neurons and a behavioral phenotype that mimicks several features of PD (Masliah *et al.* 2000). A stroke can be induced in rodents by transient or permanent occlusion of the middle cerebral artery (Dirnagl *et al.* 1999; Yu and Mattson 1999). Many of the neuronal deaths that occur in these animal models are believed to involve a form of programmed cell death called apoptosis (Mattson 2000b).

Rats maintained on DR for 2–4 months exhibit increased resistance of hippocampal neurons to excitotoxic degeneration in a model relevant to the pathogenesis of epilepsy and AD; this neuroprotection resulted in a preservation of learning and memory ability that is normally compromised in this model (Bruce-Keller *et al.* 1999). In addition to its neuroprotective actions, DR may be beneficial for epilepsy patients by reducing seizure incidence and severity. Thus, fasting reduced the incidence and severity of audiogenic seizures in magnesium-deficient rats (Mahoney *et al.* 1983) and caloric restriction reduces the incidence of seizures in a mouse model of idiopathic epilepsy (Greene *et al.* 2001). Fasting and ketogenic diets (which are also low in calories) have been shown to reduce seizure incidence in epilepsy patients (Yudkoff *et al.* 2001).

In other studies, presenilin-1 mutant knockin mice and amyloid precursor protein (APP) mutant transgenic mice were maintained on DR or *ad libitum* control diets. Studies had 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 (Guo *et al.* 1999). The vulnerability of hippocampal CA1 and CA3 neurons to excitotoxic injury was decreased in presenilin-1 mutant mice that had been maintained on a DR regimen compared with mice fed *ad libitum* (Zhu *et al.* 1999). The latter study further showed that levels of oxidative stress in the hippocampus of presenilin-1 mutant mice were decreased in mice that had been maintained on DR, suggesting that

suppression of oxidative stress is one mechanism whereby DR protects neurons (Zhu *et al.* 1999). Another study was aimed at determining whether DR can reduce amyloid deposition in the brains of APP mutant mice. Surprisingly, when APP mutant mice (Hsiao *et al.* 1996) were placed on a periodic fasting (alternate day feeding) regimen they died within 2–3 weeks (Pedersen *et al.* 1999). The APP mutant mice became severely hypoglycemic during the days they were without food, and likely succumbed to the hypoglycemia. The APP mutant mice exhibited abnormalities in their neuroendocrine responses to stress including altered glucocorticoid and blood glucose regulation in response to restraint stress. However, it is not clear that the increased stress sensitivity was a direct result of the APP mutation, as similar alterations were not observed in a different APP mutant transgenic line of mice (Borchelt *et al.* 1996; W. A. Pedersen and M. P. Mattson, unpublished data).

The vulnerability of nigro-striatal dopaminergic neurons to MPTP toxicity was decreased in mice maintained on DR; more dopaminergic neurons survived exposure to MPTP, and deficits in motor function were markedly decreased (Duan and Mattson 1999). The striatal pathology in Huntington's disease (HD) patients can be partially reproduced in rats by administration of the succinate dehydrogenase inhibitor (mitochondrial toxin) 3-nitropropionic acid. When rats were maintained on a periodic fasting DR regimen for several months prior to administration of the toxin, more striatal neurons survived exposure to the toxin and their motor function was improved dramatically (Bruce-Keller *et al.* 1999).

The kinds of studies described above suggest that DR can protect neurons against an array of insults relevant to the pathogenesis of several prominent neurodegenerative disorders. However, there are exceptions. One example comes from studies of a mouse model of amyotrophic lateral sclerosis (ALS), a disorder involving degeneration of spinal cord motor neurons resulting in progressive paralysis and death. Although most cases of ALS are sporadic, some cases result from mutations in the antioxidant enzyme Cu/Zn-superoxide dismutase (SOD). Overexpression of mutant human Cu/Zn-SOD in transgenic mice results in a phenotype similar to ALS with progressive motor neuron degeneration, paralysis and death. We determined the age of onset of paralysis and the time until death of mice expressing the G93A familial ALS mutation that were maintained on either a periodic fasting DR regimen or an *ad libitum* control diet. Disease onset was unaffected by DR, and once the mice became symptomatic the progression of the disease was accelerated (Pedersen and Mattson 1999). Although it is not known why DR did not overcome the pathogenic action of the Cu/Zn-SOD mutation, it is possible that the neurodegenerative cascade in this mouse model is fundamentally different than that in the AD, PD, HD and stroke models, or that not all populations of neurons benefit equally from

DR. Another possibility is that the mutant Cu/Zn-SOD exerts an action that blocks the neuroprotective mechanism of action of DR. One specific mechanism is suggested by our finding that DR increases the expression of heat-shock protein-70 (HSP-70) in neurons (Duan and Mattson 1999; Yu and Mattson 1999) and the recent report that mutant Cu/Zn-SOD sequesters HSP-70 (Okado-Matsumoto and Fridovich 2002). The mutant enzyme may therefore eliminate a major neuroprotective mechanism of DR.

The ability of DR to improve outcome after a stroke was demonstrated in a rat 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 (Yu and Mattson 1999). When rats were maintained on a periodic fasting regimen for several months they exhibited reduced brain damage and improved behavioral outcome following transient focal ischemia. The latter findings suggest that, in addition to reducing the risk of suffering a stroke, DR may improve outcome after a stroke by increasing the survival of neurons in the ischemic penumbra.

Interestingly, DR can also counteract adverse effects of deficiencies of certain nutrients. Thiamine deficiency impairs oxidative metabolism and can cause degeneration of neurons in certain susceptible brain regions such as the thalamus. Rats maintained on a DR regimen are able to tolerate thiamine deficiency such that damage to thalamic neurons was greatly decreased (Calingasan and Gibson 2000). It will be of considerable interest to determine whether DR can also protect neurons against the adverse effects of folic acid deficiency, which has recently been linked to the pathogenesis of AD, PD and stroke (Duan *et al.* 2002; Kruman *et al.* 2002; Seshadri *et al.* 2002).

The possibility that DR can reduce the risk of neurodegenerative disorders is supported by epidemiological studies of human populations. When the average daily food intake of different populations throughout the world are compared with the incidence of AD in those populations, there is a correlation such that low food intake is associated with decreased disease incidence (Grant 1997). Thus, people in China and Japan have relatively low calorie intakes (1600–2000 calories/day) as compared with people in the United States and Western Europe (2500–3000 calories/day), and the incidence of AD in China and Japan is approximately half that seen in the United States and Western Europe. Although there are potential confounders in such analyses (e.g. per capita food consumption is a very poor measure of energy intake, and disease diagnosis may differ among the countries), they are consistent with a protective effect of low calorie diets against age-related neurodegenerative disorders. Data from a prospective study of a well-characterized cohort in New York City in urban settings, suggest that individuals with a low calorie intake may have reduced risk for AD and PD (Logroscino *et al.* 1996; Mayeux *et al.* 1999). More

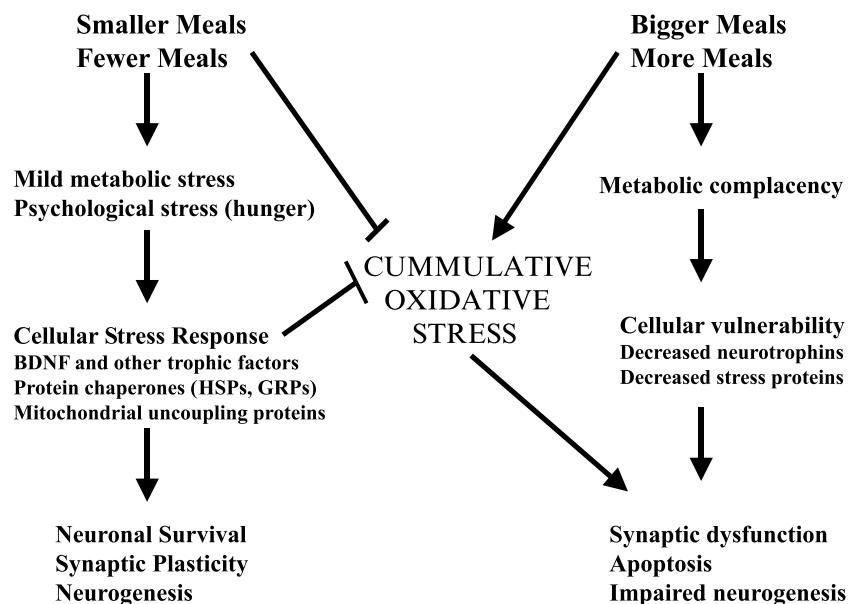
recently, Hendrie *et al.* (2001) reported the intriguing finding that a cohort of people who originally lived in a community in Africa exhibited an increased incidence of AD after moving to the United States. Although the reason that their risk of AD increased remains to be determined, one major change is that their food intake increased considerably compared with the low calorie intake of their relatives that remained in Africa. Although the extent to which caloric intake affects the risk of AD and PD remains to be determined, the data suggesting that overeating is a major risk factor for stroke is compelling (Bronner *et al.* 1995). Thus, it seems a safe bet that if people would incorporate a Spartan approach to food intake into their lifestyle, this would greatly reduce the incidence of AD, PD and stroke, three devastating disorders that currently plague our society.

### Cellular and molecular mechanisms by which dietary restriction affects the nervous system

The ability of DR to increase lifespan and protect against age-related disease suggests that DR counteracts the fundamental aging processes at the molecular and cellular levels. Widespread consequences of aging include increased oxidative damage to proteins, lipids and nucleic acids, and dysregulation of various homeostatic and functional biochemical pathways. Studies of neurodegenerative disorders have identified disease-specific genetic and environmental factors that can initiate the neurodegenerative process, but have also revealed a shared cascade of biochemical alterations that ultimately kills the neurons in each disorder. Major alterations in the shared cascade include increased oxidative stress, perturbed cellular calcium homeostasis and impaired energy metabolism (Mattson 1997; Mattson 2000a;

Calabrese *et al.* 2001). The later alterations render neurons vulnerable to apoptosis, a form of cell death that involves a regulated series of molecular interactions mediated by proteins such as Par-4, members of the Bcl-2 family and caspases (Mattson 2000b). The neurodegenerative cascade in AD may be initiated by the aging process in combination with a specific genetic predisposition (e.g. apolipoprotein E4 allele) and/or certain environmental factors (e.g. head trauma or a high calorie diet). As a consequence there is increased production and extracellular deposition of amyloid  $\beta$ -peptide, a neurotoxic proteolytic peptide product of APP. Amyloid  $\beta$ -peptide promotes neuronal apoptosis and excitotoxicity by a mechanism involving membrane lipid peroxidation and impairment of ion-motive ATPases and glucose and glutamate transporters (Mattson 1997). In PD, dopamine metabolites and iron may induce oxidative stress in dopaminergic neurons, rendering them dysfunctional (Jenner and Olanow 1998); environmental toxins as well as genetic factors may trigger the neurodegenerative cascade. The degeneration of neurons in HD and stroke also involves oxidative stress and perturbed cellular calcium regulation (Dirnagl *et al.* 1999; Grunewald and Beal 1999).

The ability of DR to increase the resistance of neurons to dysfunction and death in models of neurodegenerative disorders suggests that DR modifies a step or steps in the degenerative process that is common to each disorder. Experimental findings support the latter hypothesis. For example, neurons in the brains of rodents maintained on DR exhibit improved mitochondrial function and reduced levels of oxidative stress (Guo *et al.* 2000). We have obtained considerable evidence that DR exerts its neuroprotective effects by inducing the expression of proteins that promote cell survival (Fig. 1). Two major classes of such survival

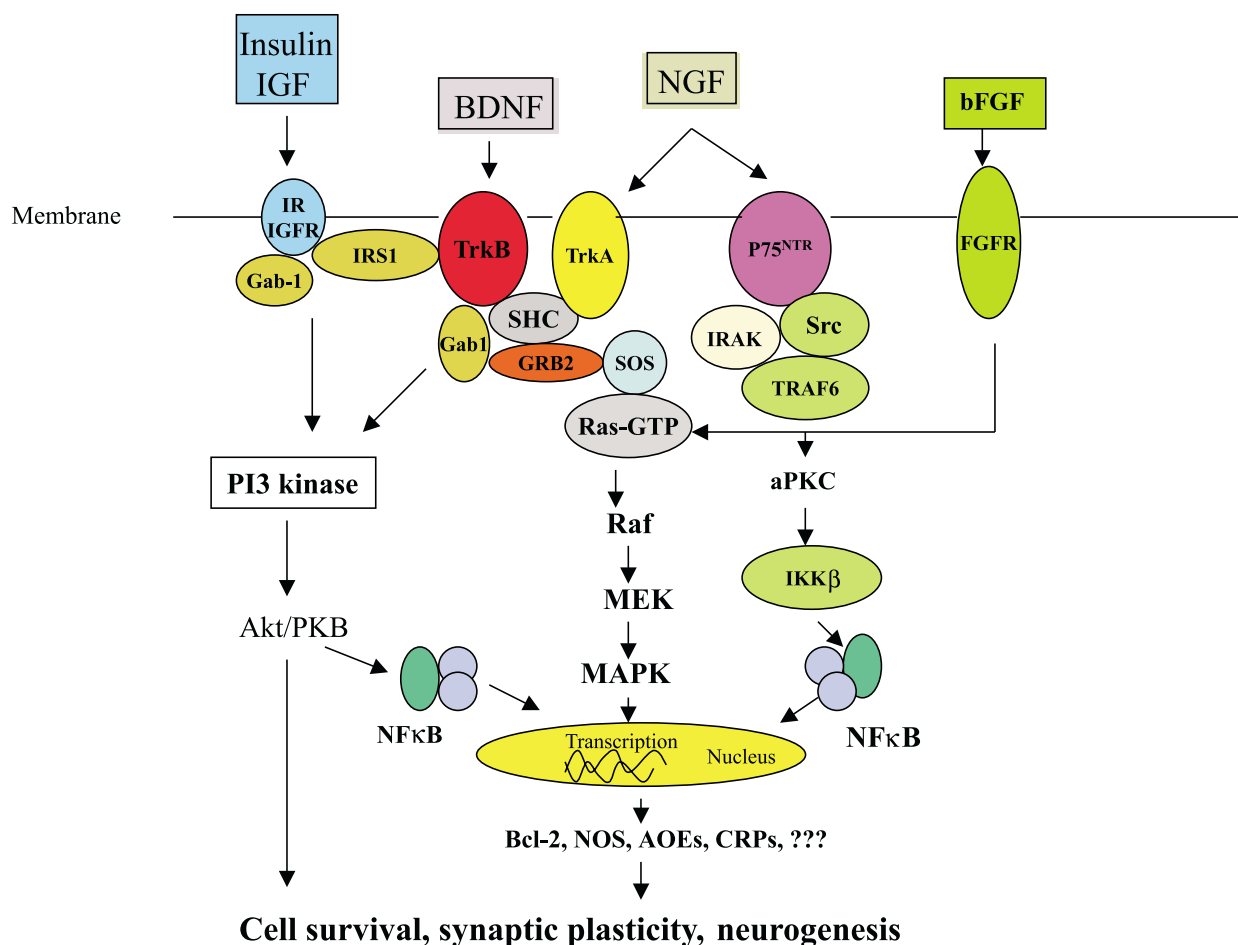


**Fig. 1** Model of the mechanisms whereby meal size and frequency affect the plasticity of the nervous system and its vulnerability to neurodegenerative disorders.

proteins are protein chaperones and neurotrophic factors. Analyses of brain tissue from rats maintained on a periodic fasting DR regimen revealed that levels of HSP-70 and glucose-regulated protein-78 (GRP-78) were increased in cortical, striatal and hippocampal neurons, whereas levels of HSP-60 were unchanged. HSP-70 and GRP-78 are so-called protein chaperones that play important roles in regulating protein folding and degradation, and that can also stabilize cellular calcium homeostasis. Studies have shown that HSP-70 (Lowenstein *et al.* 1991) and GRP-78 (Yu *et al.* 1999) can protect neurons against excitotoxic and oxidative insults, suggesting that their increased levels contribute to the neuroprotective effects of DR.

Studies performed in many different laboratories, including our own, have documented neuroprotective activities of several different neurotrophic factors including neurotrophins such as nerve growth factor, brain-derived neuro-

trophic factor (BDNF), fibroblast growth factors and insulin-like growth factors (for a review see Mattson and Lindvall 1997). 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) (Figs 2 and 3). We recently discovered that levels of BDNF are increased in the neurons of rats and mice maintained on a DR regimen (Lee *et al.* 2000a; Duan *et al.* 2001a). BDNF appears to play a particularly important role in the excitoprotective effect of DR because infusion of a BDNF blocking antibody into the lateral ventricles of rats and mice significantly attenuated the neuroprotective effect of DR in the kainate model of seizure-induced hippocampal damage (Duan *et al.* 2001a,b). In a recent study of mice with reduced BDNF levels (BDNF heterozygous knockout mice) it was shown that neurogenesis

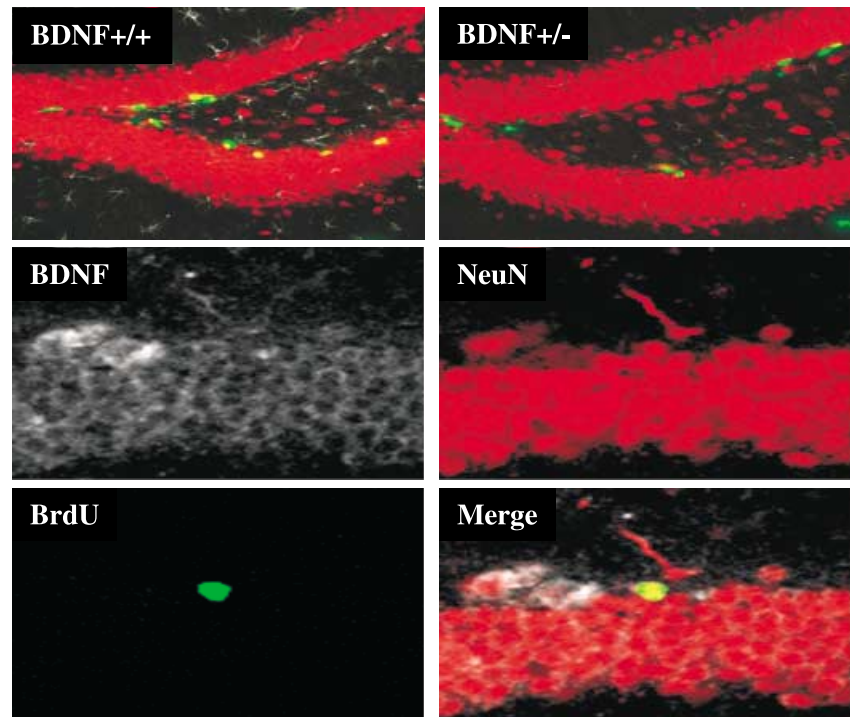


**Fig. 2** Signal transduction pathways relevant to the actions of dietary restriction on the nervous system. The PI3 kinase–Akt pathway, the MAP kinase pathway, and the transcription factor NF- $\kappa$ B appear to be important mediators of the beneficial effects of dietary restriction on the nervous system. Definitions: antioxidant enzymes (AOEs); atypical protein kinase-C (aPKC); basic fibroblast growth factor (bFGF); brain-

derived neurotrophic factor (BDNF); calcium-regulating proteins (CRPs); insulin-like growth factor (IGF); insulin receptor substrate-1 (IRS-1); mitogen-activated protein kinase (MAPK); nerve growth factor (NGF); nitric oxide synthase (NOS); low affinity neurotrophin receptor (p75<sup>NTR</sup>); tumor necrosis factor associated factor-6 (TRAF6).



**Fig. 3** Hippocampal neurogenesis is decreased in mice with reduced BDNF levels. BDNF<sup>+/+</sup> and BDNF<sup>+/-</sup> mice were maintained for 3 months on *ad libitum* or DR (periodic fasting) feeding regimens. The mice were then given five daily injections of BrdU and were killed either 1 day or 4 weeks later. The upper two micrographs show bromodeoxyuridine (BrdU; green)–NeuN (red) double-label confocal images of the dentate gyrus of *ad libitum* fed BDNF<sup>+/+</sup> and BDNF<sup>+/-</sup> mice; note reduced number of BrdU-labeled cells in the BDNF<sup>+/-</sup> mouse. The bottom four micrographs show triple labeling for the indicated antigens in the dentate gyrus of a BDNF<sup>+/+</sup> mouse killed 4 weeks after BrdU injection; in this section a single BrdU-labeled cell was NeuN-positive. DR enhances neurogenesis by a mechanism involving BDNF-mediated survival of newly produced neurons. Modified from Lee *et al.* (2002b).



is impaired, consistent with an important role for BDNF in mediating the stimulation of neurogenesis by DR (Lee *et al.* 2002b) (Fig. 3). DR may also increase the expression of other neurotrophic factors including nerve growth factor (Duan *et al.* 2001a) and glial cell line-derived neurotrophic factor (W. Duan and M. P. Mattson, unpublished data).

Based upon the kinds of findings described above, it appears that a major contribution of the beneficial effects of DR on neurons comes from a cellular stress response in which levels of protein chaperones and neurotrophic factors are increased. The cellular stress response may be induced by a mild metabolic stress associated with DR and/or by 'psychological' stress resulting from hunger (Fig. 1). However, although glucocorticoid levels are increased in rodents maintained on DR, the stress associated with DR appears to be fundamentally different than that induced by other stressors such as psychosocial stress, restraint stress, etc. As evidence, the changes in expression of corticosteroid receptors in neurons in the brains of rats maintained on DR are different than the changes that occur in rats subjected to uncontrollable stressors. DR results in a decrease in the levels of glucocorticoid receptors (GR), whereas levels of mineralocorticoid receptors are maintained (Lee *et al.* 2000b). In contrast, stressors that have been reported to have deleterious effects on neurons cause a decrease in the levels of mineralocorticoid receptor in neurons (Vazques *et al.* 1996). Moreover, uncontrollable physiological and psychosocial stressors have been reported to decrease levels of BDNF in the brain (Smith *et al.* 1995), a change opposite to the increase in BDNF levels in the brains of animals maintained on DR (Lee *et al.* 2000a; Duan *et al.* 2001a).

The adverse effects of overeating on the nervous system are likely to result, in part, from increased levels of cumulative oxidative stress as the result of increased glucose metabolism and consequent superoxide production (Fig. 1). In addition, hypercaloric diets may place neural cells in a state of 'metabolic complacency' wherein their defenses against stress are reduced and their vulnerability to dysfunction and degeneration is increased.

The death of neurons in AD, PD, HD and stroke are likely to begin with alterations in synaptic terminals that result in synaptic dysfunction and activation of apoptotic and excitotoxic cascades (Mattson *et al.* 1998). Studies of cortical synaptosomes prepared from rats maintained on calorie-restricted or control diets have shown that caloric restriction can increase the resistance of synapses to oxidative and metabolic insults, as indicated by the relative preservation of glucose and glutamate transport and mitochondrial function (Guo *et al.* 2000). 2-Deoxy-D-glucose (2-DG) administration exerted similar beneficial effects on synapses (Guo and Mattson 2000). The levels of HSP-70 and GRP-78 were increased in the synaptosomes taken from calorie-restricted rats and rats given 2-DG, demonstrating that energy restriction bolsters the ability of synapses to cope with the oxidative and metabolic stress associated with aging. BDNF and other neurotrophic factors have also been shown to modulate synaptic plasticity in ways that facilitate learning and memory (Guo and Mattson 1999; Jankowsky and Patterson 1999).

Finally, our hormesis hypothesis for the beneficial effects of DR in the brain also provides a satisfactory explanation for

the increased neurogenesis observed in mice and rats maintained on DR. *In situ* hybridization analysis showed that the expression of BDNF and neurotrophin-3 (NT-3) are increased in subpopulations of neurons in the hippocampus of mice maintained on DR; BDNF levels increase in CA3 and CA1 pyramidal neurons and NT-3 levels increase in dentate granule neurons (Lee *et al.* 2002d). Previous findings suggest that the increased levels of BDNF and NT-3 could account for the increased neurogenesis; BDNF promotes the survival and differentiation of hippocampal neural progenitor cells (Lowenstein and Arsenault 1996; Shetty and Turner 1998) and their newly generated neuronal progeny in culture (Cheng and Mattson 1994; Mattson *et al.* 1995; Cheng *et al.* 1997). In addition, stimuli such as seizure activity and enriched environments that increase neurogenesis in the dentate gyrus also increase BDNF expression (Parent *et al.* 1997; Lowenstein and Arsenault 1996; Lee *et al.* 1997; Cameron *et al.* 1998; Young *et al.* 1999). NT-3 and BDNF each promote neuronal differentiation of embryonic (Sah *et al.* 1997) and adult (Takahashi *et al.* 1999) hippocampal neural progenitor cells. Mice lacking NT-3 exhibit decreased survival of certain populations of neural progenitor cells and their progeny (El Shamy *et al.* 1998; Kahn *et al.* 1999). It is therefore likely that the increased expression of BDNF and NT-3 induced by DR is critical for the enhanced neurogenesis.

The parallels between the cellular signal transduction pathways affected by DR in peripheral organs, such as muscle and liver cells, and the pathways activated in brain cells are intriguing. A prominent effect of DR in muscle and liver cells is to enhance their insulin sensitivity. Insulin receptors are coupled to a protein called insulin receptor substrate-1 (IRS-1), which is essential for activation of the PI3 kinase–Akt pathway (Table 3). The high-affinity BDNF receptor *trkB* is also coupled to the IRS-1, PI3 kinase–Akt pathway, as are insulin-like growth factor (IGF) receptors that are expressed by neurons. In addition, neurotrophins and basic fibroblast growth factor (bFGF) activate the MAP (mitogen-activated protein) kinase pathway, as well as the transcription factor NF- $\kappa$ B. By increasing insulin-like and neurotrophin signaling pathways, DR induces the expression of genes that encode proteins which promote cell survival and adaptive plasticity. A consideration of the evolution of these signaling pathways in the context of regulation of food acquisition and energy metabolism was recently published (Mattson 2002).

#### Meal size versus meal frequency: beyond calorie intake

It has been assumed that all of the benefits of DR feeding regimens are the result of a reduction in cumulative calorie intake (Weindruch and Sohal 1997). However, we have recently documented a clear dissociation between caloric

**Table 4** Evidence that decreased meal frequency, without caloric restriction, can exert anti-aging and neuroprotective effects in C57BL/6 mice

Parameter	Change compared with mice fed <i>ad libitum</i>	
	Intermittent fasting	Caloric restriction
Food intake	little or no change	decreased by > 30%
Body weight	little or no change	decreased by > 30%
Blood glucose	30% decrease	30% decrease
Blood insulin	80% decrease	70% decrease
Blood $\beta$ -hydroxybutyrate	100% increase	50% decrease
Neuronal vulnerability*	large decrease	modest decrease

\*Degeneration of hippocampal CA3 and CA1 neurons in the kainic acid seizure model. Data taken from Anson *et al.* (submitted).

intake and beneficial effects of DR in a study that compared the effects of periodic fasting (alternate day feeding) and limited daily feeding on various physiological parameters and neuronal vulnerability to excitotoxicity in C57BL/6 mice (Anson *et al.*, submitted). We had noted that, in contrast to Sprague–Dawley rats which lose weight when maintained on a periodic fasting regimen, C57BL/6 mice did not lose weight. Measurement of food intake revealed that on the days they had access to food the C57BL/6 mice on the periodic fasting regimen consumed twice as much food as mice fed *ad libitum* (Table 4). Remarkably, however, the mice on periodic fasting exhibited ‘anti-aging’ physiological changes equal to or greater than those maintained on the reduced calorie diet, including decreased plasma insulin and glucose levels, and reduced body temperature. Moreover, levels of the ketone body  $\beta$ -hydroxybutyrate were increased in the mice on the periodic fasting regimen, but not in the mice on the limited daily feeding regimen, suggesting a change in cellular energy metabolism pathways (Anson *et al.*, submitted). Periodic fasting was more effective than limited daily feeding in protecting hippocampal neurons against excitotoxic injury. These findings suggest that increasing the time interval between meals is beneficial, even when the size of the meals are increased to a level that results in no overall decrease in caloric intake.

The findings just described, while surprising, provide strong support for the hypothesis that many of the beneficial effects of DR are the result of a mild cellular stress response. Indeed, we have found that periodic fasting is much more effective than limited daily feeding in increasing the expression of HSP-70 and neurotrophic factors in the brain (W. Duan, Z. Guo and M. P. Mattson, unpublished data).

#### Dietary restriction mimetics

Food addiction is now the major cause of disease and death in the United States, as well as in several other industrialized countries. In theory, this problem could be solved by simply

communicating the consequences of overeating to physicians and the public. In practice, however, it has proven very difficult to successfully implement prolonged DR regimens. In light of the inability of many people to reduce their food intake, research efforts are in progress to identify ways to either reduce food intake or mimic the beneficial effects of DR using drugs, dietary supplements and even gene therapy approaches. Food intake is regulated by complex neuroendocrine systems. The hypothalamus plays an important role in regulating feeding by sensing glucose levels and levels of hormones such as leptin (Elmqvist 2001). However, more complex cognitive and emotional factors also influence ingestive behaviors. Appetite can be altered by drugs acting on various neurotransmitter systems with serotonin and dopamine being particularly important (Halford 2001). The activation of hypothalamic receptors for leptin and ciliary neurotrophic factor (CNTF) appears to be a particularly promising approach for reducing food intake and body weight (Halford 2001; Lambert *et al.* 2001; Mattson 2001). Although initial studies involved peripheral administration of leptin or CNTF, more recent efforts have employed gene therapy strategies in which hypothalamic cells are infected with viral vectors containing leptin or CNTF. For example, it was reported that such leptin gene therapy can reduce body weight and fat levels in normal rats and can block high fat diet-induced hyperlipidemia, hyperinsulinemia and weight gain (Dube *et al.* 2002). It will be of considerable interest to determine whether this gene therapy approach also results in the kinds of neuroprotective effects conferred by DR.

Because many of the beneficial effects of DR may result from a preconditioning effect and/or a decreased production of reactive oxygen species, we designed experiments aimed at determining whether agents that impair glucose metabolism can induce a beneficial cellular hormesis response in animals fed *ad libitum*. We first screened various agents that affect energy metabolism to identify those that induced a cellular stress response and that were also neuroprotective in primary neuronal cultures; our first positive results were obtained with 2-DG, a non-metabolizable analog of glucose. When rats or mice were administered 2-DG for two weeks they exhibited increased resistance of neurons in their brains to dysfunction and death in experimental models relevant to the pathogenesis of AD, PD and stroke (Duan and Mattson 1999; Lee *et al.* 1999; Yu and Mattson 1999). For example, hippocampal neurons in rats given 2-DG were more resistant to damage induced by the amnesic excitotoxin kainic acid, and this increased resistance was reflected in a preserved learning and memory ability (Lee *et al.* 1999). In a mouse model of PD, damage to dopaminergic neurons caused by the toxins MPTP and rotenone was significantly decreased in mice given 2-DG, and this neuroprotection was correlated with an amelioration of motor dysfunction (Duan and Mattson 1999). In a rat stroke model in which the middle cerebral artery is transiently blocked, the amount of damage

to cortical and striatal neurons was significantly decreased in rats that had been given 2-DG, and their behavioral outcome was significantly improved (Yu and Mattson 1999). 2-DG can be used as a neuroprotective dietary supplement. When incorporated into the food of rats and mice (0.2–0.4% w/w) 2-DG decreases the resistance of neurons to excitotoxic injury and improves behavioral outcome (Z. Guo and M. P. Mattson, unpublished data). Long-term dietary supplementation with these quantities of 2-DG results in several physiological changes similar to DR including decreased body temperature and decreased insulin levels (Lane *et al.* 1998).

The mechanism whereby 2-DG supplementation protects neurons may be similar to that of DR because levels of HSP-70 and GRP-78 are increased in neurons of rats and mice given 2-DG (Duan and Mattson 1999; Lee *et al.* 1999; Yu and Mattson 1999). As was the case with DR (Guo *et al.* 2000), when synaptosomes were isolated from the cerebral cortex of rats given 2-DG, they exhibited increased resistance to dysfunction caused by oxidative stress and amyloid  $\beta$ -peptide (Guo and Mattson 2000). The later study further showed that levels of HSP-70 and GRP-78 were increased in synaptosomes from rats given 2-DG, suggesting that 2-DG protects the synaptic terminals by increasing the levels of protein chaperones. These findings suggest that it may be possible to derive benefits from DR without reducing food intake, although it remains to be determined whether long-term dietary supplementation with 2-DG has adverse side-effects.

Two additional agents that alter cellular energy metabolism were also found to exert neuroprotective effects. Iodoacetate, an inhibitor of glyceraldehyde-3-phosphate dehydrogenase, protected hippocampal neurons against death induced by glutamate, iron and trophic factor withdrawal (Guo *et al.* 2001). Levels of the stress proteins HSP-70 and HSP-90, and of the anti-apoptotic protein Bcl-2, were increased in neurons treated with iodoacetate, consistent with a metabolic stress-based preconditioning effect. Phenformin and metformin are drugs that have been used to treat patients with type-2 diabetes because of their ability to affect glucose metabolism and promote weight loss (Bailey 1992). Neurons treated with phenformin exhibit increased resistance to excitotoxicity; the mechanism involves a down-regulation of *N*-methyl-D-aspartate receptors and reduced calcium influx (Lee *et al.* 2002c). It remains to be determined whether dietary supplementation with such energy modulating agents will be effective and safe and, indeed, side-effects of phenformin and metformin are common and sometimes severe and life-threatening.

Finally, it may be useful to consider the contribution of caloric restriction to popular diets that have been touted for their purported ability to reduce body weight and improve health. One such diet is the Atkins diet which has a very low level of carbohydrate, with the majority of calories being obtained from fats. The Atkins diet is ketogenic resulting in reduced appetite and therefore a reduced calorie intake;

individuals who can comply with the diet may therefore exhibit some physiological changes observed in rodents and monkeys subjected to caloric restriction including reduced body weight, and decreased insulin and glucose levels. However, very few individuals are able to comply with the Atkins diet (Landers *et al.* 2002) and such ketogenic diets are high in fat and cholesterol and may therefore promote atherosclerotic vascular disease (Anderson *et al.* 2000).

### Future directions

There is much to learn about the effects of food intake (how much and how often) on the cellular and molecular biology of the nervous system, and its functional capabilities. Progress in this important area of investigation would be bolstered by the use of invertebrates such as *Caenorhabditis elegans* and *Drosophila* in which genes that mediate effects of food intake on the nervous system might be rapidly identified (Wolkow 2002). Gene expression analyses of neural tissues from normal rodents maintained on various DR and overeating regimens, and of rodent models of obesity, may reveal novel genes upon which to focus future research efforts. Studies of the effects of food intake on the cellular and molecular pathogenesis of neuronal degeneration in models of neurodegenerative disorders should continue, as well as parallel epidemiological and clinical investigations in humans. Being able to control food intake using pharmacological and gene therapy approaches is a current focus of translational research in the obesity field, but should also be pursued from the standpoint of neurodegenerative disorders. Finally, an all-out campaign to educate the public about the devastating consequences of overeating should be deployed. The available data suggest that the nervous system is highly vulnerable to excessive calorie intake, just as is the case with the cardiovascular systems and most other organ systems. When extrapolated to humans, the data obtained from the kinds of animal studies described above suggest that a daily calorie intake in the range of 1800–2200 calories for moderately active adults may dramatically reduce the risk of age-related disorders of the nervous system including AD, PD and stroke. Foregoing one or two meals a day might be an alternative to reducing meal size.

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