Age and Energy Intake Interact to Modify Cell Stress Pathways and Stroke Outcome

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Objective: Age and excessive energy intake/obesity are risk factors for cerebrovascular disease, but it is not known if and how these factors affect the extent of brain damage and outcome in ischemic stroke. We therefore determined the interactions of age and energy intake on the outcome of ischemic brain injury, and elucidated the underlying mechanisms.

Methods: We utilized a novel microchip-based immunoaffinity capillary electrophoresis technology to measure a panel of neurotrophic factors, cytokines, and cellular stress resistance proteins in brain tissue samples from young, middle-aged, and old mice that had been maintained on control or energy-restricted diets prior to middle cerebral artery occlusion and reperfusion.

Results: Mortality from focal ischemic stroke was increased with advancing age and reduced by an intermittent fasting (IF) diet. Brain damage and functional impairment were reduced by IF in young and middle-aged mice, but not in old mice. The basal and poststroke levels of neurotrophic factors (brain-derived neurotrophic factor and basic fibroblast growth factor), protein chaperones (heat shock protein 70 and glucose regulated protein 78), and the antioxidant enzyme heme oxygenase-1 were decreased, whereas levels of inflammatory cytokines were increased in the cerebral cortex and striatum of old mice compared with younger mice. IF coordinately increased levels of protective proteins and decreased inflammatory cytokines in young, but not in old mice.

Interpretation: Reduction in dietary energy intake differentially modulates neurotrophic and inflammatory pathways to protect neurons against ischemic injury, and these beneficial effects of IF are compromised during aging, resulting in increased brain damage and poorer functional outcome.

ANN NEUROL 2010;67:41–52

Stroke, a major cause of disability and mortality in the elderly, occurs when a cerebral blood vessel is occluded and/or ruptured, resulting in ischemic damage and death of neurons.1 The neurodegenerative mechanism involves metabolic and oxidative stress, excitotoxicity, apoptosis, and inflammatory processes, including activation of glial cells and infiltrating leukocytes.2–4 Studies using cell culture and animal models have identified several different proteins and signaling pathways that can protect neurons against ischemic injury, including: neurotrophic factors, such as brain-derived neurotrophic factor (BDNF) and basic fibroblast growth factor (bFGF); protein chaperones, including heat shock protein 70 (HSP70) and glucose regulated protein 78 (GRP78); and antioxidant enzymes, such as superoxide dismutases and heme oxygenase-1 (HO-1).5–9 When young rodents are sub-

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Published online in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/ana.21798

Received Apr 22, 2009, and in revised form Jun 16, 2009. Accepted for publication Jun 26, 2009.

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Additional Supporting Information may be found in the online version of this article.
jected to brief mild cerebral ischemia prior to a stroke, the extent of brain damage is reduced, and functional outcome is improved; this “preconditioning” effect of mild ischemia involves increased expression of neurotrophic factors and protein chaperones. The existence of the ischemic preconditioning mechanism suggests that it may be possible to activate a similar adaptive neuroprotective response with a noninvasive mild energetic stress.

Dietary energy restriction, by daily calorie reduction (CR) or intermittent fasting (IF), extends lifespan and decreases the development of age-related diseases, including diabetes, cardiovascular disease, and cancers. In humans, CR and IF can reduce circulating markers of oxidative stress and inflammation, and can improve cardiovascular disease risk and symptoms in asthma patients. Dietary energy restriction may also benefit neurons, as suggested by data showing that CR and IF protect neurons against dysfunction and degeneration in animal models of epileptic seizures, and Alzheimer, Parkinson, and Huntington diseases. Recent findings suggest the possibility that IF may promote neuronal survival and plasticity, in part by inducing the expression of BDNF and HSP70. However, energy restriction did not increase BDNF levels in the brains of old rats. Because aging is a major risk factor for stroke, and stroke outcome is poorer in the elderly, we tested the hypothesis that aging impairs the ability of brain cells to respond adaptively to IF and so to survive a stroke. A novel microchip-based immunoaffinity capillary electrophoresis-based analytical technology was used to quantify levels of a battery of neurotrophic factors, protein chaperones, and cytokines in several brain regions after experimental stroke. We show that multiple neuroprotective pathways are activated and inflammatory pathways are suppressed by IF in young animals, and that aging impairs the ability of IF to modulate these pathways adaptively.

Materials and Methods

Animals and Blood Collection and Measurements of Glucose and Insulin Concentrations

Male C57BL6 mice of 3 initial ages (3, 9, and 16 months) were obtained from the Aging Colony at the National Institute on Aging. On arrival, all mice were maintained with a standard NIH-07 diet (Harlan-Teklad, Indianapolis, IN) and free access to water, and a 12-hour light/12-hour dark cycle (lights on at 0600). Two weeks after arrival, mice were randomly assigned to either ad libitum (AL) or intermittent dietary energy restriction (IF; alternate day fasting) diets. Body weights were recorded weekly; for mice in the IF group, the body weight was recorded on 2 consecutive days (a feeding day and a fasting day). Mice were maintained on the diets for at least 3 months prior to the experimental manipulations. Blood samples were taken after an overnight fast (for both diet groups). Glucose concentrations (mg/dl) were determined from whole blood using a glucose meter (FreeStyle, TheraSense, Alameda, CA). All mice were euthanized at 72 hours after cerebral ischemia or sham operation, at which time plasma and brain tissue samples were collected and stored at −80°C. The levels of plasma insulin was measured using an ultrasensitive insulin enzyme-linked immunoassay kit (ALPCO, Salem, NH). All animal procedures were approved by the National Institute on Aging Animal Care and Use Committee.

Focal Ischemic Stroke Model and Evaluations of Functional Outcome and Brain Damage

Mice were subjected to transient middle cerebral artery occlusion/reperfusion, as reported previously and described in the Supplementary Methods. The functional consequences of focal cerebral ischemia/reperfusion (I/R) injury were evaluated using a 5-point neurological deficit score (0, no deficit; 1, failure to extend right right paw; 2, circling to the right; 3, falling to the right; and 4, unable to walk spontaneously) and were assessed in a blinded fashion. Two-millimeter-thick coronal brain sections taken after 72 hours of reperfusion were stained with 2% 2,3,5-triphenyltetrazolium chloride and evaluated for infarct size using standard methodology.

Cerebral Blood Flow Measurement

Each animal’s head was placed in a fixed frame after it had been anesthetized and prepared for middle cerebral artery occlusion. Craniotomy performed to access the left middle cerebral artery was extended to allow positioning of a 0.5mm Doppler probe (PeriFlux System 5000, Perimed, Jarfalla, Sweden) over the underlying parietal cortex approximately 1mm posterior to the bregma and 1mm lateral to the midline. Laser-Doppler recordings are expressed as percentages of the preischemic baseline and averaged over 30-minute periods during 1 hour of ischemia, and 30, 90, and 180 minutes of posts ischemic reperfusion.

Measurements of Neurotrophic Factor, Stress Protein, and Cytokine Concentrations

On sacrifice, cerebral cortical and striatal tissue samples were rapidly removed from both the ipsilateral and contralateral hemispheres and were flash frozen and stored at −80°C. Levels of BDNF, bFGF, HSP70, GRP78, HO-1, tumor necrosis factor (TNF)-α, interleukin (IL)-1β, IL-6, IL-10, and IL-17 were quantified by immunoaffinity capillary electrophoresis using methods described previously and detailed in the Supplementary Methods. Briefly, the frozen samples were hand homogenized in 100mM phosphate buffer and clarified by centrifugation at 10,000 g before being injected into the immunoaffinity port of a glass microchip (Mircalyze, Edmonton, Alberta), which contained immobilized antibodies (R & D Systems, Minneapolis, MN; Bachem Biochemicals, King of Prussia, PA), thus capturing the analytes of interest, and removing them from the rest of the tissue extract. The bound analytes were labeled in situ, eluted in acid buffer (pH 1.0), separated by
electrophoresis, and measured by on-line laser-induced fluorescence detection.

**Statistical Analysis**

Several general linear model procedures, including multivariate repeated measures and 1-way or 2-way analysis of variance (ANOVA), were applied to the data analyses in which the appropriate models were applicable. The neurological data were analyzed with the generalized estimating equations approach, and fit a marginal model that accounts for the repeated measures in the data set. The model analyzed ordinal multinomial data using a cumulative logit. Details of the analyses performed are available in the Supplementary Methods.

**Results**

**Dietary Energy Restriction Protects Against Cerebral Ischemia-Induced Mortality, Brain Cell Death, and Functional Deficits in a Mouse Stroke Model**

Groups of C57BL/6 mice at 3 ages (3, 9, and 16 months) were maintained on AL or IF diets for 4–5 months prior to the experimental stroke. As expected from previous studies, young and middle-aged mice on the IF diet maintained significantly lower body weight (on both feeding and fasting days) compared with mice on the AL diet (Supplementary Fig 1). The body weights of old AL-fed mice were not different from those of old IF mice on feeding days, but were significantly greater than the body weights of IF mice on fasting days. Blood glucose concentrations were significantly lower in mice on IF (75–80mg/dl) compared with mice on the AL diet (90–100mg/dl) regardless of age (Supplementary Fig 2). Plasma insulin concentrations were significantly lower in young mice on the IF diet compared with those on the AL diet, and there was a trend toward lower plasma insulin concentrations in middle-aged and old mice on the IF diet (Supplementary Fig 2). The effects of IF on glucose and insulin concentrations are consistent with improved insulin sensitivity, with a stronger effect of the diet in young compared with older mice.

To assess the effect of IF in experimental stroke, we used a mouse model that mimics the most common type of human stroke, transient I/R. The experimental group consisted of 3 age groups of C57BL/6 mice maintained on either an AL or an IF diet for 4–5 months prior to experimental stroke (n = 30–40 mice in each age group). After 1 hour of ischemia, we evaluated the mortality rate during a 72-hour reperfusion period. Mortality was af-

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The neurological data were analyzed with generalized estimating equations fitting a marginal model that account for the repeated measures. The significances of the main effects are as follows. Age: df = 2; chi-square = 20.43; p < 0.0001. Time: df = 2; chi-square = 6.7; p < 0.05. Diet: df = 1; chi-square = 30.46; p < 0.0001.

AL = ad libitum; IF = intermittent fasting.
fected by both age and diet. Mortality was greater in older mice (35%), compared with young (13%) and middle-aged (23%) mice, and in mice on the AL diet compared with mice on the IF diet for all 3 age groups (Supplementary Table 1). The extent of anatomical and functional brain damage was also greatly reduced by IF in young and middle-aged animals. Neurological impairment during a 3-day poststroke period was significantly less in young and middle-aged IF mice compared with AL-fed mice of the same ages (Table 1). However, in old mice IF had no significant effect on neurological deficit. Measurements of brain infarct volume showed a highly significant decrease in infarct volume in IF young and middle-aged mice compared with mice on the AL diet (Fig 1). In contrast, IF had no significant effect on infarct volume in old mice (Fig 1). Stroke-induced mortality, neurological deficits, and brain infarct volume were increased with advancing age in both the AL and IF diet groups (Supplementary Table 1; Table 1; Fig 1). Laser-Doppler measurements of cerebral blood flow prior to, during, and for 180 minutes after I/R showed that middle cerebral artery occlusion was effective in reducing cerebral blood flow in mice of all ages and diet groups, and that there were no significant effects of age or diet on postocclusion cerebral blood flow (Supplementary Fig 2).

**Levels of Cortical and Striatal Neurotrophic Factors Are Reduced During Aging, and Increased by IF and Ischemic Stroke in an Age-Related Manner**

Previous findings have demonstrated that BDNF and bFGF can reduce brain damage and improve functional outcome in stroke models. Using a novel microchip-based immunoaffinity capillary electrophoresis technology, we quantified levels of BDNF and bFGF in tissue samples from both the ipsilateral (stroke side) and contralateral striatum and cortex of mice in all age and diet groups (Fig 2; Supplementary Table 3). Concentrations of BDNF and bFGF were significantly lower in both the cortex and striatum of old mice compared with young and middle-aged mice; BDNF levels declined progressively, whereas bFGF levels dropped from young to middle-age with no further decline in old mice (Fig 2; Supplementary Table 3). IF resulted in increased levels of cortical and striatal BDNF in mice of all 3 ages, although the absolute levels of BDNF were 4- and 2-fold greater in young and middle-age mice, respectively, compared with old mice (Supplementary Table 3). Mice of all 3 ages exhibited increased levels of bFGF in the cortex and striatum when maintained on IF compared with AL diets, although this effect of IF was greater in young compared with older mice. In AL-fed mice, ischemia did not significantly affect BDNF levels in the cortex and striatum of young or old mice, but did cause a significant although small increase in BDNF levels in both the ipsilateral and contralateral cortex and striatum of middle-aged mice (Supplementary Table 3). In IF mice, BDNF levels were significantly increased by ischemia in both the ipsilateral and contralateral cortex and striatum of middle-aged mice, but not in young or old mice. In young mice, but not in middle-aged or old mice, ischemia induced an increase in bFGF levels in both the cortex and striatum. (The major statistical results using multivariate ANOVA with repeated measures for BDNF and bFGF, as well as stress response proteins and cytokines in the following sections, are summarized in Supplementary Table 7.)

**Levels of Cortical and Striatal Stress Response Proteins Are Reduced During Aging, and Increased by IF and Ischemic Stroke in an Age-Dependent Manner**

Previous studies have shown that the expression of the protein chaperones HSP70 and GRP78, and the antioxidant enzyme HO-1, are induced by cerebral ischemia and can increase the resistance of neurons to ischemia. We therefore measured levels of HSP70, GRP78, and HO-1 in samples of cerebral cortex and striatum in mice from all age
and diet groups (Fig 3; Supplementary Table 4). There were large reductions in levels of all 3 stress proteins in both the cortex and striatum with advancing age; in the cases of HSP70 and GRP78, the reductions were progressive, whereas HO-1 levels decreased from young to middle-aged with no further reduction in old mice. Levels of HSP70 and GRP78 were increased by IF in both brain regions and in all age groups. HO-1 levels were increased in response to IF in young and middle-aged mice, but not in old mice. After a stroke in AL-fed mice, levels of all 3 stress proteins were lowest in the cortex and striatum of old mice, compared with young and middle-aged mice (Fig 3b, d, and f; Supplementary Table 4). In IF mice, the poststroke levels of all 3 stress proteins were significantly greater in the cortex and striatum of young and middle-aged mice compared with the poststroke levels in AL-fed mice. The ability of brain cells to respond to IF by increasing levels of HSP70 and HO-1 was significantly attenuated in old mice compared with young and middle-aged mice. However, old mice subjected to a stroke did exhibit a significant increase in GRP78 levels compared with AL-fed mice subjected to a stroke.

FIGURE 2: Cortical and striatal levels of brain-derived neurotrophic factor (BDNF) and basic fibroblast growth factor (bFGF) decrease during aging and are increased by intermittent fasting (IF). (a) BDNF levels were significantly decreased in middle-aged and old sham animals compared with young sham animals (n = 10 in each group). ***p < 0.0001. IF sham animals had significantly increased BDNF compared with ad libitum (AL)-fed sham controls in all age groups. ***p < 0.0001. (b) IF animals had significantly increased brain BDNF levels compared with AL-fed controls in all age groups following cerebral ischemia/reperfusion (I/R). ***p < 0.0001. (c) bFGF levels were significantly decreased in middle-aged and old sham animals compared with young sham animals (n = 10 in each group). p < 0.05 ***p < 0.0001. IF sham animals had significantly increased bFGF compared with AL-fed sham controls in all age groups. ***p < 0.0001. (d) Following cerebral I/R, IF animals had significantly increased bFGF compared with AL-fed I/R controls in all young and middle age groups. ***p < 0.0001. Values are the mean and standard error of the mean.
Age- and Stroke-Related Alterations in Pro- and Anti-Inflammatory Cytokines Are Modulated by Dietary Energy Restriction

Considerable evidence suggests that inflammation contributes to neuronal dysfunction and degeneration both chronically during aging and acutely after a stroke.32 Three proinflammatory cytokines (PICs) implicated in ischemic brain injury are TNF-α,33,34 IL-1β,35 and IL-6.36 To elucidate possible roles for changes in proinflammatory cytokines in the ameliorative effects of IF on stroke outcome in the context of advancing age, we therefore measured levels of TNF-α, IL-1β, and IL-6 in the cortical and striatal tissue samples. In AL-fed sham control mice, levels of TNF-α and IL-6 in the cortex and striatum increased from young to middle-aged and remained elevated in old mice (Fig 4a; Supplementary Table 5). Levels of TNF-α and IL-6 were significantly lower in the cortex and striatum of mice on the IF diet compared with the AL diet. In response to ischemic stroke, the levels of both TNF-α and IL-6 increased significantly in both the ipsilateral and contralateral cortex and striatum, with the magnitude of the elevations being greatest in young mice and least in old mice (Fig 4a–d; Supplementary Table 5). IF suppressed the ischemia-induced increases of TNF-α and IL-6 levels, with this dietary effect being greatest in young mice and least in old mice.

Surprisingly, changes in levels of the PIC IL-1β in response to age, diet, and stroke were strikingly different compared with TNF-α and IL-6. In both the cortex and striatum of AL-fed control mice, the levels of IL-1β were 30- to 40-fold greater in young mice compared with middle-aged and old mice (Fig 4e; Supplementary Table 5). IF resulted in a significant reduction in cortical and striatal IL-1β levels in young mice, whereas IF increased IL-1β in middle-aged and old mice. In AL-fed mice, ischemic stroke caused an increase of IL-1β levels in the cortex and striatum (both ipsilateral and contralateral) of young mice, while having little or no effect on IL-1β levels in middle-aged and old mice (Fig. 4f; Supplementary Table 5). IF suppressed the stroke-induced IL-1β increase.
in young mice, while increasing levels of IL-1β in middle-aged and old mice.

IL-10 most often serves an anti-inflammatory function, whereas IL-17A produced by lymphocytes is generally proinflammatory. Levels of IL-17A decreased significantly with advancing age and were increased by IF in both the cortex and striatum (Fig 4g, h; Supplementary Table 6). Ischemia caused an approximately 2-fold increase in IL-17A levels in both the ipsilateral and contralateral cortex and striatum in mice of all 3 ages. The greatest levels of IL-17A were found in the ipsilateral ischemic cortex of mice on the IF diet (Supplementary Table 6). There was a striking progressive increase in IL-10 levels with advancing age in both the cortex and striatum of young mice, but did not affect IL-10 levels in middle-aged or old mice. In AL-fed mice, ischemia induced a significant increase of IL-10 levels in both the ipsilateral and contralateral cortex and striatum of young and middle-aged mice, but not old mice (Supplementary Table 6). Young and old, but not middle-aged mice on the IF diet exhibited elevated postischemia IL-10 levels in the cortex and striatum compared with the postischemia IL-10 levels in mice on the AL diet. Notably, the effects of age and diet on IL-10 levels and IL-1β levels were inversely related (Supplementary Tables 5 and 6).

**Neuroprotective and Inflammatory Proteins Are Differentially Regulated by Dietary Energy Restriction**

We next sought to establish relationships among and between neuroprotective factors (neurotrophic factors, protein chaperones, and HO-1) and proinflammatory cyto-

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**FIGURE 4:** Dietary energy restriction decreases proinflammatory cytokines tumor necrosis factor (TNF-α), interleukin (IL)-6, and IL-1β and increases anti-inflammatory cytokines IL-17A and IL-10 in the brain. Proinflammatory cytokines TNF-α (a) and IL-6 (c) levels were significantly increased in middle-aged and old sham animals compared with young sham animals. Intermittent fasting (IF) significantly reduced both TNF-α and IL-6 (c) levels compared with ad libitum (AL)-fed animals in all 3 age groups. ***p < 0.0001. TNF-α (b) and IL-6 (d) levels were significantly decreased in middle-aged and old ischemia/reperfusion (I/R) animals compared with young I/R animals. **p < 0.0001. IF animals also had decreased levels of TNF-α (b) and IL-6 (d) compared with AL-fed animals in all 3 age groups. **p < 0.0001. (e, f) IL-1β levels were significantly decreased in both middle-aged and old sham animals compared with young sham animals. ***p < 0.0001. (e, f) IF young animals had significantly reduced IL-1β levels compared with AL-fed young animals in the sham and I/R groups. **p < 0.0001. (g, h) Anti-inflammatory cytokine IL-17A levels were decreased with aging. Middle-aged and old animals had significantly reduced IL-17A levels compared with young animals. ***p < 0.0001. IF animals had significantly increased levels of IL-17A compared with AL-fed animals in all age groups in sham animals and following cerebral I/R. ***p < 0.0001. (i, j) IL-10 levels were significantly increased with aging in middle-aged and old animals compared with young animals. ***p < 0.0001. Young IF animals had a significant increase in IL-10 in the sham group (*p < 0.01), and young as well as old IF animals had a significant increase in IL-10 following cerebral I/R compared with AL-fed I/R animals. ***p < 0.0001.
Noninflammatory cytokines (TNF-α, IL-6, and IL-1β) in brain tissue samples from mice in all age and diet groups, under control and stroke conditions. To achieve this goal, we employed nonlinear mixed-effects regressions to model the data and to assess the product of the slopes for the concentrations of protein pairs in tissue samples from the ipsilateral cerebral cortex (Table 2). A significant negative relation (the product of the slopes was < 0) indicated that an age-, diet-, or ischemia-dependent elevation in 1 particular factor was associated with a reduction in another factor. In contrast, a significant positive relation indicated that both factors were altered in the same direction.

To accomplish this goal, the linear mixed-effects model was fit using a nonlinear mixed-effects regression model that allowed for the assessments on the product of the slopes. This revealed that many of the changes in neurotrophic factors, protective protein chaperones, and inflammatory products under the influences of dietary energy restriction, aging, and ischemic lesion were highly significant positive associations between different neuroprotective factors, and negative associations between neuroprotective factors and proinflammatory cytokines, in mice on the IF diet. This was the case in both nonischemic and ischemic brain tissue samples. In control and ischemic tissue samples from mice maintained on IF, there were significant positive associations between: BDNF and bFGF; BDNF and HSP70; BDNF and GRP78; BDNF and HO-1; bFGF and HSP70; bFGF and GRP78; and bFGF and HO-1 (Table 2). In mice fed
AL, there were also significant positive associations for most pairs of neuroprotective factors in control and ischemic cortical tissue samples for BDNF and HSP70, BDNF and GRP78, and BDNF and HO-1. However, in the case of bFGF, there were either weak or no significant associations with protein chaperones and HO-1 in mice fed AL.

In control and ischemic cortical tissue samples from mice maintained on IF, there were highly significant negative associations between neurotrophic factors and proinflammatory cytokines in all cases (BDNF and TNF-α; BDNF and IL-6; BDNF and IL-1β; bFGF and TNF-α; bFGF and IL-6; bFGF and IL-1β). In contrast, associations between neurotrophic factors and proinflammatory cytokines were, in many cases, not significant in control and ischemic cortical tissue samples from mice fed AL (Table 2). Interestingly, and in contrast to the negative association between BDNF and proinflammatory cytokine levels in cortical tissue from mice on IF, BDNF and proinflammatory cytokines were positively correlated in ischemic cortical tissue from AL-fed mice. Collectively, these findings suggest that IF enhances the ability of brain cells to protect neurons against ischemic injury by a mechanism involving the coordinate upregulation of multiple neuroprotective proteins (neurotrophic factors, protein chaperones, and antioxidant enzymes) and downregulation of proinflammatory cytokines.

Discussion

Our findings suggest that aging compromises the ability of energy restriction to protect the brain against ischemic injury and improve functional outcome in a mouse model of stroke. The neuroprotective effect of IF was robust in young mice (infarct volume and neurological deficits were decreased by >50%), was diminished in middle-aged mice, and was lacking in old mice. Aged mice that had been fed AL did not show increased infarct volume compared with young and middle-aged AL-fed animals. We believe the main reason for the latter result is that we occluded the middle cerebral artery for a relatively long time period (1 hour), which causes maximal or near-maximal damage to the striatum and cortex in young animals. The rationale for using this amount of ischemia was so that we would be more likely to detect robust protective effects of the intermittent fasting diet compared with the ad libitum diet in the young animals, and indeed this was the case. However, our study shows that aged mice are more vulnerable to ischemic stroke-induced brain injury, as their mortality was significantly greater than that of the young mice, and the functional outcome of those that survived was worse than that of young or middle-aged mice. However, we did not evaluate functional outcome beyond 72 hours of reperfusion, as mortality was high in the aged mice on the ad libitum diet (~35% mortality). It will be of interest to evaluate long-term outcome, as well as the efficacy of poststroke dietary energy restriction on recovery from stroke in future study.

Our analysis of neurotrophic factors, stress resistance proteins, and cytokines suggests mechanisms by which aging impairs the ability of IF to protect brain cells against a stroke. Levels of BDNF and bFGF were diminished in the cortex and striatum of old mice compared with young mice. The amounts of BDNF and bFGF were increased by IF to much higher levels in young compared with middle-aged and old mice. Both BDNF and bFGF have previously been shown to protect neurons against ischemic injury. We found that at 72 hours after the stroke, cortical and striatal bFGF levels were increased 10-fold in young IF mice, with little or no change in bFGF levels in AL-fed young mice or IF middle-aged and old mice. Therefore, elevated bFGF levels were strongly associated with improved stroke outcome, suggesting an important role for bFGF in the protective effects of youth and IF against ischemic brain injury.

Levels of all 3 cellular stress proteins examined (HSP70, GRP78, and HO-1) were elevated in cortex and striatum in response to IF and a stroke in young mice, but with greatly diminished responses in middle-aged and old mice. Particularly striking was the robust poststroke upregulation of HSP70 and GRP78 in mice on the IF diet compared with mice fed AL. These findings suggest that aging impairs the ability of brain cells to engage adaptive stress responses to both a beneficial environmental factor (IF) and an acute severe insult (stroke). HSP70 and GRP78 function as major protein chaperones in the cytosol and endoplasmic reticulum, respectively, whereas HO-1 is an antioxidant enzyme. All 3 of these stress proteins have previously been shown to protect neurons against stroke in vivo and can directly protect neurons against other insults relevant to stroke, including excitotoxicity and oxidative stress.

Changes in cortical and striatal levels of TNF-α and IL-6 in response to aging, IF, and stroke were tightly cor-
related. TNF-α and IL-6 levels increased during aging, and decreased in response to IF, particularly in young and middle-aged mice, among sham control mice. After a stroke the TNF-α and IL-6 levels increased by 2- to 3-fold in AL-fed mice, and these cytokine responses to stroke were attenuated by IF, particularly in young mice. Both TNF-α and IL-6 are proinflammatory cytokines that are implicated in neuronal degenerative processes, including ischemic brain injury.32–36 Our findings therefore suggest that suppression of TNF-α and IL-6 production may contribute to the reduction in brain damage and improved functional outcome in mice on IF compared with AL-fed control mice. Interestingly, IF increased IL-17A levels in the cortex and striatum of sham mice, but suppressed stroke-induced IL-17A production. The IL-17 antibody used in our study was directed against a peptide sequence common to all IL-17 subtypes (IL-17A–IL-17F). Our findings therefore implicate 1 or more IL-17 family members in brain cell responses to IF and stroke.

There was a striking inverse relationship between levels of IL-10 and IL-1β across diet and age groups. IL-10 levels increased with advancing age, whereas IL-1β levels decreased. IF increased IL-10 levels and decreased IL-1β levels in sham and stroke mice, but only in young animals. IL-10 is an anti-inflammatory molecule that can suppress microglial IL-1β production,47 and our data are therefore consistent with a role for IL-10 in protecting neurons against the damaging effects of IL-1β in ischemic stroke. Indeed, Vila and colleagues50 reported lower levels of IL-10 in stroke patients with clinical deterioration, relative to those who remained stable or improved during the first 2 days after the stroke.

Previous studies have shown that a stroke induces changes in cellular energy metabolism and the expression of stress-responsive genes that are not limited to the region of tissue damage, but instead also occur at distant sites, including the contralateral cerebral hemisphere.51–53 We found that the effects of age, IF, and stroke on levels of all neurotrophic factors, stress proteins, and cytokines were nearly identical in both hemispheres. Although there is no reason to believe that age or IF would differentially affect the expression of these proteins in right and left hemispheres, it is intriguing that stroke induced similar changes in their expression in both the damaged hemisphere and the undamaged contralateral hemisphere. Several mechanisms may underlie the upregulation of expression of neurotrophic factors, stress proteins, and cytokines in the contralateral hemisphere. One possibility is that stroke induces oxidative and metabolic stress in cells in the contralateral hemisphere, and indeed there is evidence to support this possibility.54,55 Another possibility is that proinflammatory cytokines in the ischemic tissue are transferred to the contralateral hemisphere via the vasculature and cerebrospinal fluid as the result of blood-brain barrier disruption, a possibility suggested by previous findings.56 Yet another mechanism involves increased transhemispheric excitability,57 which is known to increase the production of neurotrophic factors, stress proteins, and cytokines.58,59

IF increased levels of growth factors that promote neuronal survival and plasticity, as well as protein chaperones and an antioxidant enzyme, in the cortex and striatum, revealing a mechanism by which IF protects the brain against injury. The ability of IF to increase levels of neurotrophic factors and stress proteins was diminished with advancing age and, accordingly, IF was less effective in reducing ischemic brain injury in old compared with young animals. Aging also increased stroke mortality and compromised IF-mediated suppression of proinflammatory cytokines and induction of the anti-inflammatory cytokine IL-10. Our findings suggest that individuals with relatively low energy intake are more likely to survive and recover function after a focal ischemic stroke compared with those with a higher energy intake. This possibility is supported by a recent study showing that caloric restriction improves recovery from transient global cerebral ischemia in rats.60 Although stroke is relatively rare in children and young adults, traumatic injury to the central nervous system is common, and it was recently reported that IF initiated after spinal cord injury can improve recovery of function in rats.61 The present findings suggest that IF protects neurons against acute tissue injury and enhances brain function by simultaneously stimulating neurotrophic and neuroprotective pathways and suppressing inflammation.

This research was supported by the National Institute on Aging Intramural Research Program and the National Institute of Biomedical Imaging and Bioengineering Intramural Research Program of the National Institutes of Health.

References


