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Anti-Inflammatory Mechanisms of Dietary Restriction in Slowing Aging Processes

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Abstract

Dietary restriction (DR) remains the most powerful and general environmental manipulation of aging processes in laboratory animals with strong beneficial effects on most age-related degenerative changes throughout the body. Underlying the beneficial effects of DR is the attenuation of system-wide inflammatory processes including those occurring within the central nervous system. During normal aging a progressive neuroinflammatory state builds in the brain involving astrocytes and microglia, the primary cellular components of neuroinflammation. DR attenuates the age-related activation of astrocytes and microglia with concomitant beneficial effects on neurodegeneration and cognition. Increasing evidence suggests that common pathways are emerging that link many normal aging inflammatory processes with age-related diseases such as Alzheimer, cancer, diabetes and cardiovascular disease.

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Dietary restriction (DR) remains the most powerful and general manipulation of aging processes in laboratory animals. Evidence is now overwhelming that DR increases life span by slowing the Gompertz mortality rate acceleration. The Gompertz analysis of DR was first made by Berg [1] in 1976. This fundamental effect of DR has been amply verified [2, p. 508; 3].

Corresponding to slowed mortality rates, most spontaneous degenerative changes in aging are attenuated. In rodents, the age-related increases in tumor and organ-specific pathology are delayed by DR, according to the genotype [2, 4–7]. In the widely used F344 rats, for example, chronic renal disease, which may be the major cause of morbidity, is strikingly reduced [8]. Cardiomyopathy of F344 rats is also strongly associated with the severity of kidney degeneration, but the mechanisms may be different [9]. Nonetheless, we must confront the

puzzle in F344 rats that about 25% of old DR rats have no gross organ pathology at death [8]. We suggest the possibility of metabolic instability during DR below in lesion-free aging rodents.

Dietary Restriction Attenuates Neuroinflammatory Aspects of Aging

The first indication that DR is neuroprotective for aging came from a 1985 report [10]. In some rodent colonies, hind limb paralysis becomes increasingly common during aging in association with degeneration of spinal motor neurons (radiculoneuropathy) [10, 11]. The degeneration of myelin sheaths in spinal roots arises after sporadic axonal atrophy and is associated with segmental demyelination and local ballooning [12–14]. Hind limb paralysis was markedly attenuated by DR [10, 11].

Hind limb paralysis varies widely between colonies and is unfamiliar to current researchers of aging. The greatest incidence reported, 100%, was observed in colonies before the era of modern husbandry (specific-pathogen free). In the NIA contract colony at Charles River Laboratory, in 1978–1983, rats (9 genotypes, both sexes) had a 25% incidence, with a mean age at lesion of 31 months; the incidence in mice (12 genotypes) was <0.1% [15]. These major differences are puzzling and not easily attributed to improved husbandry and health. Early rodent colonies before 1970 often carried a much higher load of infections than the present specific-pathogen-free colonies.

While most attention has been given to the biochemical, metabolic and genomic effects of DR, evidence is growing for the importance of system-wide anti-inflammatory effects of DR in attenuating aging [16–18]. Our laboratory is focused on neuroinflammatory changes of aging in rodents, primates and humans. These generalized aging changes arise in the absence of specific neurodegeneration [16, 17]. In aging rodents, the main brain aging changes are glial activation (microglia and astrocytes)¹ [17] and synaptic atrophy [19–24]. These changes are progressive during middle age into old age and arise in the absence of disease. The type and extent of change are selective and differ extensively between even closely connected brain systems. The opposing glial and synaptic changes span a range of about 50% but are much larger than changes in cell number. In fact, several exacting studies have looked for but did not detect age changes in the total numbers of neurons [25] or glia [26]. Thus, in

¹Microglia are bone-marrow-derived monocytes which are constantly repopulated in adult brains. Astrocytes are of neural crest origin and share the same stem cell precursors as neurons.

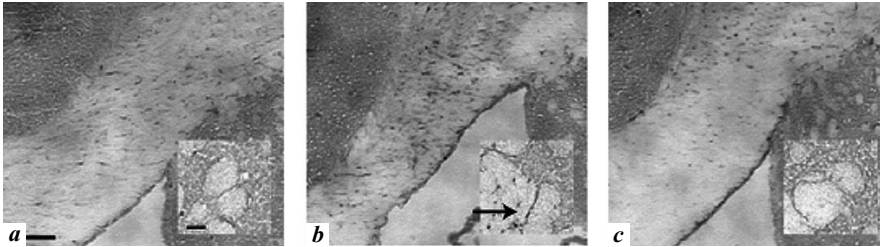


Fig. 1. The age-related increase in macrosialin (CD68) expression is attenuated by DR. Macrosialin immunoreactivity in the corpus callosum and corticostriatal bundles (insets) of 4-month ad libitum (**a**), 24-month ad libitum (**b**) and 24-month calorie-restricted (**c**) C57BL/6NNia mice. The arrow identifies macrosialin immunostaining at the periphery of a corticostriatal bundle in 24-month ad libitum mice. Bars = 100 μm for micrographs, 30 μm for insets. Reprinted from Wong et al. [35], with permission from Elsevier.

aging rodents and perhaps in humans, the main brain aging changes represent a type of plasticity that remodels cell cytoarchitectonic relationships without cell death. DR has a remarkable ability to attenuate these changes. White matter myelinated tracts are a robust example of the plasticity of neuroinflammatory aging.

Age-Related Microglial Activation: White Matter Degeneration

Macroscopically, magnetic resonance imaging studies on aging humans and monkeys show subtle structural changes in the corpus callosum, striatum and other white-matter-rich tracts [27–29]. These changes may be caused by the focal degeneration of myelin sheaths and differ by brain region. The later-myelinated regions are more susceptible to demyelination during normal aging and Alzheimer disease (AD) [30].

White matter aging is accompanied by increased microglial activation [31, 32], but cause and effect are unclear. Aging rodent models show robust increases in markers of microglial activation, e.g. CR3 (complement receptor) and MHC class II antigens (antigen presentation by macrophages) [33, 34]. These changes are attenuated by DR [33]. Most recently, we found that the scavenger receptor macrosialin (CD68), a member of the lysosomal/endosomal-associated membrane glycoprotein family, shows the greatest age-related increase in the corpus callosum of C57BL/6NNia mice; again, this is attenuated by DR (fig. 1) [35]. Because macrosialin is increased in peripheral macrophages by oxidized lipids (low-density lipoproteins) [36] and because oxidized lipids generally promote inflammation [37, 38], we hypothesize that the oxidation of white matter lipids is a factor in microglial activation. In fact, we showed that oxidized low-density

lipoproteins induced CD68 in BV-2 microglial cells [35]. Moreover, CD68 is induced by inflammatory stimuli (lipopolysaccharide plus γ -interferon) in BV-2 cells [35]. Therefore, CD68 serves as an inflammatory marker as well as an indicator of oxidative damage during normal brain aging.

Because DR clearly attenuates age-related increases in inflammatory genes such as CD68, CR3 and MHC class II antigens, we hypothesize that DR will protect against age-related demyelinating events. An ongoing study of DR in rhesus monkeys has not given definitive information for technical reasons. After 11–13 years of DR, middle-aged (<24 years old) and old monkeys (>24 years old) had smaller putamen volumes than ad libitum fed animals [39]. However, there were no initial magnetic resonance imaging data to establish the baseline (before or at the beginning of DR). Thus, it is unresolved if the smaller putamen volumes in DR animals resulted from DR, or if the volume differences were present at the beginning of the study.

Age-Related Astrocytic Activation: Glial Fibrillary Acidic Protein

Astrocytes are an important source of neurotrophic factors, axonal guidance molecules and extracellular matrix molecules crucial for neuron survival and sprouting. In response to injury or disease, astrocytes take on an activated phenotype that is characterized by cell hypertrophy and upregulation of the intermediate filament proteins, glial fibrillary acidic protein (GFAP) and vimentin, as well as inflammatory mediators and extracellular matrix molecules [40, 41]. However, during normal aging astrocytes become activated with concomitant increases in GFAP and vimentin in the absence of overt pathology [42–44]. This age-related astrocytic activation [45, 46] contributes to age-related increased inflammatory and oxidative damage [44, 47], decreased neurogenesis [48] and synaptic atrophy [19].

We are investigating the hypothesis that the increase in GFAP expression is a primary cause in synaptic atrophy and impaired synaptogenesis during normal aging [46]. We have developed a heterochronic cell culture model to test this hypothesis. In brief, test neurons (E18 cortex) are seeded on monolayers of primary cultures of astrocytes from young adult or aging rat cerebral cortex. The old-rat-derived astrocytes retain the high GFAP per cell [49] as observed in vivo [33]. Moreover, the E18 neurites outgrow poorly on old-rat-derived astrocytes. These age impairments in neurotrophic support are rapidly reversed by downregulating GFAP by small interfering RNA [46]. The mechanism involves an inverse relationship between GFAP expression and secretion of laminin, a critical component of the extracellular matrix that guides neurite outgrowth. Additional support for the critical role of GFAP comes from studies on mice lacking both GFAP and vimentin which have improved synaptic regeneration and increased neurogenesis [50, 51].

Just as age-related microglial activation is reduced by DR (discussed above), DR is also effective at attenuating many of the genotypic and phenotypic changes that astrocytes undergo during aging. The age-related increase in GFAP is attenuated by DR [43, 44, 52] and this occurs at the transcriptional level [33, 53]. Microarray profiling confirmed the effects of DR on GFAP [17, 44]. Although neuropathologists have long used GFAP as a marker of neurodegeneration, our work clearly shows that the age increase in GFAP arises in the absence of neuron cell death and may be an upstream factor in synaptic atrophy during aging. Because of the concurrent activation of microglial inflammatory markers, we provisionally consider that GFAP is embedded in a neuroinflammatory network. The beneficial effect of DR on glial activation may underlie DR's ability to attenuate age-related declines in synaptic plasticity and neurogenesis [54–58]. Ongoing studies are evaluating if DR improves the neurotrophic support of aging glia.

Age-Related Neurodegenerative Disease: Experimental Rodent Models

DR also protects against neurodegenerative processes in experimental rodent models. For example, DR protects neurons from many toxins, including methylphenyltetrahydropyridine [59], kainic acid [60], 3-nitropropionic acid and malonate [60, 61]. AD-like changes do not arise in aging rodents, possibly because the rodent β -peptide has several amino acid substitutions that decrease its aggregation into fibrillar amyloids that are characteristic of AD [62]. However, mice carrying human transgenes for early-onset familial AD develop fibrillar amyloids and various other specific AD-like neuropathological changes during aging. We have recently shown that DR attenuated brain deposits of brain amyloid by 50% within the short time of several months [63] (fig. 2a). These changes were accompanied by a reduction in GFAP in astrocytes surrounding the plaque (fig. 2b). We demonstrated these beneficial effects of DR in two transgenic mouse models of AD, APP^{swe/ind} and APP+PS1 [63]. These effects of DR also extend to a third genotype, Tg2576 [64].

Low-energy diets in humans are being considered as an approach to lowering AD risk, because in retrospective studies, AD victims tended to have higher calorie intake [65, 66]. Of course, it is much harder to establish causality of diet in humans, because individuals who adopt special diets also often pursue other health-promoting activities, such as exercise which may protect against cognitive declines in normal aging [67, 68].

Another example of DR providing age-related neuroprotective activities is in the experimental model of retinal ischemia/reperfusion [69]. As observed in cortical and hippocampal regions (see above), microglia and astrocytes become progressively activated in the aged retina [69]. Further glial activation occurs when the aged retina is subjected to ischemia/reperfusion with concomitant

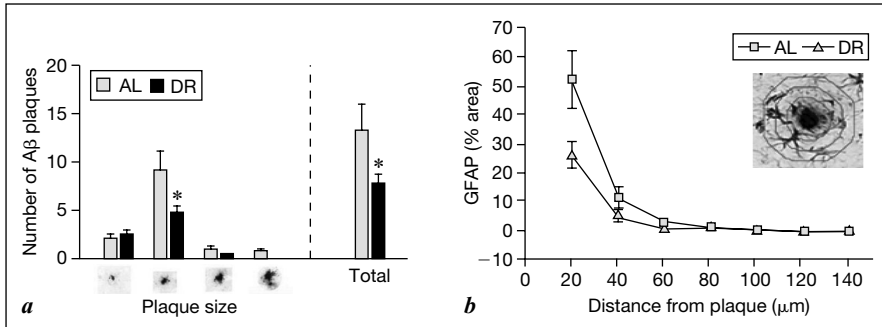


Fig. 2. DR reduces β -amyloid (A β) number and β -amyloid-associated astrocyte activation relative to ad libitum (AL) feeding. **a** Plaque size and total β -amyloid plaques were reduced by DR in APPsw/ind (* $p < 0.05$, $n = 7-8$). **b** Sholl analysis of concentric rings around β -amyloid plaques (inset) showed reduced GFAP immunoreactivity nearest to plaques in DR versus ad libitum feeding ($p < 0.05$). Reprinted from Patel et al. [63], with permission from Elsevier.

neuronal damage. In this model of ischemia/reperfusion with individual eyes, DR attenuated retinal glial activation and neuronal damage [69]. In fact, these authors suggest that the beneficial effects of DR are directly related to its effect on glial activation supporting the hypothesis that the anti-inflammatory actions of DR on glia may mediate neuroprotection.

Dietary Restriction Attenuates Inflammatory Processes

Microarray Profiling Highlights Anti-Inflammatory Effects of Dietary Restriction

The broad scope of inflammatory gene expression during brain aging has become clear through the numerous publications utilizing microarray gene expression profiling [44, 70, 71]. These studies showed that inflammation-related genes increased during aging. Importantly, DR attenuated the age-related increase in inflammatory genes [44]. In fact, DR prevented the age-related increased expression of 65% of those genes involved in the inflammatory response in the neocortex [44] suggesting that this is a primary mechanism underlying the beneficial effect DR has on brain aging processes.

Suppression of Inflammation in Acute Dietary Restriction

Inflammatory responses are attenuated by DR throughout the body [17, 72]. We begin with examples from the skin. In the classic pharmacological

model of footpad edema induced by subcutaneous injection, DR shortened the inflammatory responses in young mice on DR for 8 weeks [73]. In clinical studies, dermatitis was also improved by 8 weeks on a low-energy diet with micronutrient supplements. All patients responded to some degree, with the reductions of edema, oozing and skin sloughing (excoriation) being correlated with weight loss [74]. Inhibition of keratinocyte proliferation, an observed effect of DR in young mice [75], may contribute to the reduced excoriation.

In humans, serum C-reactive protein (CRP) was 80% lower in a self-selected group that had maintained DR for 8 years [76]. CRP is an acute-phase protein secreted by the liver in humans [77], which is an important host defense molecule by binding to Gram-negative bacteria and enhancing their clearance by phagocytosing macrophages. However, CRP also has major importance in vascular disease as a risk indicator and for its potential direct role in lipid accumulations by macrophages (foam cells) in atheromas. Serum CRP is elevated during obesity and, not surprisingly, short-term weight reduction decreased serum CRP by 30% [78, 79]. Here we confront the complexities of weight reduction. DR could enhance the host defense by lowering blood glucose [80–82], yet DR diminishes CRP and possibly other defenses.

Changes in gene expression in the liver during short-term DR (3–30 weeks) have been profiled by microarrays in several studies [83–87]. Agreement is emerging, despite differences in the choice of rodent genotypes, duration of DR and microarray technologies. Short-term DR induces and represses many mRNAs in the liver that mediate increased gluconeogenesis, increased protein and fatty acid catabolism, and decreased synthesis of cholesterol, fatty acids and triglycerides [84, 87]. The Krebs cycle (tricarboxylic acid cycle) drives these changes, with increased shunting of pyruvate to oxaloacetate in the liver by increased activity of pyruvate carboxylase [88]. The increased oxaloacetate feeds into gluconeogenesis after conversion by malate dehydrogenase, which is also increased by DR. Besides transcriptional changes in these genes, levels of activity in some enzymes are allosterically regulated, e.g. pyruvate carboxylase is activated by acetyl-CoA, which is increased by the β -oxidation of fatty acids liberated during lipolysis. Acute-phase response mRNAs are also decreased, including serum amyloid A4 and several complement system factors (mannose-binding lectin, C4-binding protein, C9) [84]. DNA repair is upregulated (*Rad51*), as are CYP450 family genes that mediate detoxification and decrease DNA damage. Again, there is impressive overlap of genes associated with lipid metabolism and vascular disease.

Overall, these 50–100 mRNA changes are a small subset (<1%) of all the genes active in the liver. The race is on to find transcription factors that are shared key regulators of these gene subsets. The effects of DR on many diseases of aging with inflammatory components give a basis to look for transcription

factors that could modulate inflammatory gene subsets implicated in AD, cancer, diabetes and vascular disease [17, 72]. Corton et al. [84] have shown in the liver that the transcription factors peroxisome proliferator-activated receptor (PPAR), liver X receptor and retinoid X receptor, which regulate many genes during DR, also have major roles in inflammation. Additional experimental models include PPAR knockout mice and drug antagonists, which induce mRNA changes that overlap with DR to some extent (see below).

Mechanisms Underlying Anti-Inflammatory Actions of Dietary Restriction

Glucocorticoids

DR increases blood glucocorticoids by 20% or more [18, 73, 89]. The increased glucocorticoids are a homeostatic response to increase the catabolism of fatty acids for energy (gluconeogenesis), while decreasing the synthesis of fatty acids and cholesterol. If the energy deficit is prolonged, protein catabolism is also increased. DR also decreases the tissue content of oxidatively damaged proteins and lipids, which are always present with enough food intake and which accumulate during aging. Importantly, glucocorticoids have broad anti-inflammatory effects mediated by the direct interaction between the glucocorticoid receptor and the transactivation domain of NF- κ B which serves as a key transcription factor in the regulation of inflammation [90, 91]. Because chronically elevated glucocorticoids are also broadly associated with neuronal damage and neuronal death, it is paradoxical that DR is neuroprotective [18].

Glucose and Advanced Glycation End Products

DR lowers blood glucose by about 10–15%. Blood glucose levels directly influence the formation of oxidation products, as was outlined two decades ago in Cerami's hypothesis of glucose as a mediator of aging [92]. Glucose and other reducing sugars react spontaneously (nonenzymatically) with free amino groups of proteins (e.g. $-\text{NH}_2$ of lysine) to form an initial 'glycation' product by the Amadori reaction, which is assayed as furosine. Then, Amadori glycation products become oxidized to 'glycoxidation products', assayed as pentosidine, which are also referred to as advanced glycation end products [93]. DR inhibits glycoxidation during aging in rodent skin, whereas diabetes and end-stage renal disease accelerate glycoxidation [93–95].

Advanced glycation end product adducts are recognized by a macrophage scavenger receptor, the RAGE (receptor for advanced glycation end products) of monocytes (macrophages, microglia) and other cells. RAGEs are also

activated by the β -amyloid peptide and other stress-associated proteins (S100/calgranulins). A working hypothesis is that advanced glycation end products and RAGEs mediate feed-forward loops of oxidative stress and inflammation that increase bystander molecular damage in atherosclerosis, AD and other chronic inflammatory diseases [96, 97]. In turn, RAGE activation enhances proinflammatory pathways that release cytokines (e.g. interleukin 6) and leukocyte adhesion factors (e.g. monocyte chemoattractant protein 1 and vascular cell adhesion molecule 1), and that induce the enzymatic synthesis of reactive oxygen species through NAD(P)H oxidases (e.g. gp91phox) and mitochondrial electron transport. Lastly, RAGE activation may stimulate feed-forward vicious cycles by autoinduction in the same cell [98–100]. RAGE downstream signaling pathways include phosphatidylinositol triphosphate kinase, NF- κ B and JAK/stat. Feedback loops include the induction of RAGE by tumor necrosis factor α through production of reactive oxygen species, mediated by NF- κ B [101]. RAGE-dependent processes are also implicated in AD.

The lower glucose may also be a risk factor in sudden death. Recall the puzzle that some DR rats died without evidence of gross pathology. We suggest the precedent of the sudden ‘dead-in-bed syndrome’ of humans. Transient hypoglycemia is implicated in sudden death from cardiac arrest in type 1 diabetics (insulin-deficient), who have 3-fold more unexpected death than healthy young individuals [102].

Peroxisome Proliferator-Activated Receptors

As discussed earlier, the nuclear hormone superfamily of PPARs may play a critical role in mediating many of the transcriptional effects of DR in peripheral systems. Indeed, in the rat kidney PPAR mRNA, protein and DNA binding activities are decreased with age and these changes are attenuated with DR [103]. While the PPARs show wide distribution among glia and neurons in the brain [104], the effects of age or DR have not been documented. Although PPARs are best known for their precise transcriptional control of metabolic events, certain subtypes (in particular, PPAR- γ) mediate inflammatory processes [105–107]. Regarding the brain, PPAR stimulation reduces neuroinflammation, both in vivo [108, 109] and in vitro [105, 110, 111]. Thus, PPAR mediation of the anti-inflammatory effects of DR in the brain seems likely.

Conclusion

DR attenuates many age-related inflammatory events in the CNS and periphery of experimental animal models in concert with increasing life span. In the aging brain, DR suppresses the activation of microglia and astrocytes which are

associated with demyelination, synaptic atrophy and neurodegeneration. These events are believed to be the underlying causes of age-related cognitive decline. Rodent models suggest that DR may also protect against age-related neurodegenerative diseases involving inflammation such as AD and ischemia/reperfusion.

Even short-term DR can attenuate inflammation and affect metabolic and DNA repair pathways. Mechanisms by which DR suppresses peripheral inflammation include the elevation of glucocorticoids, lowering of glucose and activation of PPARs. Although the effects of DR are less understood in the brain, common pathways are emerging that link many normal aging inflammatory processes with age-related diseases such as AD, cancer, diabetes and cardiovascular disease.

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