The British Geriatrics Society Amulree Essay Prize 2015

Dietary restriction and its impact on the neuropathology of Alzheimer’s disease

Charlotte Boreham
Newcastle University Medical School

Word Count: 6,250 (excluding references)
ABSTRACT
Ageing is the main risk factor for developing Alzheimer's disease (AD). Mitochondrial dysfunction increases with age and produces damaging levels of reactive oxygen species (ROS), leading to cellular oxidative stress. Oxidative stress plays a fundamental role in brain ageing. Extensive scientific evidence indicates that dietary restriction (DR), the reduction of caloric intake without malnutrition, protects the brain against oxidative injury. DR increases the resistance of neurons to degeneration and death, induces neurogenesis, and attenuates behavioural and cognitive impairments in experimental animal models of AD. The mechanisms underlying the neuroprotective properties of DR are proposed to involve enhanced expression of neurotrophic factors and stress proteins which suppress ROS production, and the attenuation of protein deposits in neurons, characteristic of AD. Moreover, the deacetylase sirtuin proteins appear to mediate the protective effects of DR against neuronal loss in AD. Advances in non-human primate studies have revealed further insights into the neuroprotection provided by DR, complementing the evidence from numerous rodent studies. Human clinical trials are currently getting underway and may provide a greater understanding of the cellular and molecular pathways involved in the beneficial effects dietary modifications have on AD. This review assesses the existing literature relating to the impact of DR on the neuropathological features of AD and evaluates its potential clinical application as an effective therapeutic intervention for patients with AD.

Key words: ageing, Alzheimer’s disease, β-amyloid, dietary restriction, hippocampus, neurodegeneration, neuron, neurotrophic factor, oxidative stress, SIRT1, stress protein.
TABLE OF CONTENTS

TITLE PAGE................................................................................................................. i
ABSTRACT.................................................................................................................... ii
TABLE OF CONTENTS............................................................................................... iii
INTRODUCTION........................................................................................................... 1
THE OXIDATIVE STRESS HYPOTHESIS OF ALZHEIMER’S DISEASE.................. 2
DIETARY RESTRICTION PROMOTES NEUROGENESIS AND PROTECTS NEURONS................................................................................................................... 4
THE GLUCOCORTICOID DEBATE............................................................................... 8
DIETARY RESTRICTION ATTENUATES Aβ PEPTIDE DEPOSITION...................... 10
THE ROLE OF SIRTUINS IN ALZHEIMER’S DISEASE......................................... 13
DIETARY RESTRICTION AS A THERAPY FOR ALZHEIMER’S DISEASE.......... 17
CONCLUSIONS........................................................................................................... 18
BIBLIOGRAPHY......................................................................................................... 19
INTRODUCTION

Dietary restriction (DR) refers to a moderate reduction in caloric intake without malnutrition (Masoro, 1988; Weindruch & Walford, 1988). The relationship between DR and lifespan was first introduced by McCay et al. (1935). Since then, it has been repeatedly demonstrated that decreasing dietary intake can extend lifespan in many organisms, including yeast (Lin et al., 2000), Caenorhabditis elegans (Klass, 1977), Drosophila melanogaster (Chapman & Partridge, 1996), rodents (Weindruch et al., 1986; Weindruch & Walford, 1988; Yu et al., 1982) and primates (Colman et al., 2009; Lane et al., 2001). The mechanisms underlying DR-induced longevity are not well understood but it is widely believed that DR reduces cellular oxidative stress by decreasing the rate of free radical production by mitochondria (Sohal & Weindruch, 1996; Wachsman, 1996).

Extensive evidence indicates that DR, in addition to its impact on longevity, can decrease the incidence of several age-related diseases including diabetes (Kennedy & Lyons, 1997; Roth et al., 2001) and cancer (Ames, 1989; Hursting et al., 2003). DR is also implicated in retarding the onset of neurodegenerative disorders such as Alzheimer’s disease (AD) (Zhu et al., 1999) and Parkinson’s disease (PD) (Duan & Mattson, 1999). The brain is particularly vulnerable to ageing because neurons are post-mitotic, and so are unable to divide (Emerit et al., 2004). Moreover, the high oxygen consumption rate of the brain (Coyle & Puttfarcken, 1993) means that neuronal cells are continually subjected to enhanced levels of oxidative stress (Markesbery, 1997), accumulating protein damage (Orgel, 1963; Ryazanov & Nefsky, 2002; Sohal, 2002) and metabolic defects (Harman, 1956). Neuronal degeneration causes cognitive impairment and behavioural disturbances which are early clinical manifestations of AD (Martin et al., 2006; Mattson, 2004).

Experimental and epidemiological data consistently supports the neuroprotective effects of DR. Dietary modifications have received significant attention due to the successful attenuation of age-associated cognitive deficits and AD-type neuropathology in rodents (Patel et al., 2005; Wang et al., 2005) and non-human primates (Qin et al., 2006a) maintained on DR regimens. DR promotes neurogenesis and neuron survival (Lee et al., 2002a), as well as enhancing the expression of neurotrophic factors (Duan et al., 2001; Lee et al., 2000a), stress proteins (Yu & Mattson, 1999), and sirtuin enzymes (Kim et al., 2007; Qin et al., 2006b). Moreover, a study of New York City residents found that those with the lowest dietary intake had the smallest risk of developing AD (Luchsinger et al., 2002). Thus, there is widespread interest in elucidating the mechanisms underlying DR and its relationship with AD (Bruce-Keller et al., 1999; Duan & Mattson, 1999).
THE OXIDATIVE STRESS HYPOTHESIS OF ALZHEIMER’S DISEASE

Ageing is the greatest risk factor for developing AD (Alzheimer’s Association, 2012a). An estimated 35 million people suffer from AD worldwide (World Health Organisation & Alzheimer’s Disease International, 2012), with this figure projected to rise to over 115 million by 2050 (Alzheimer’s Disease International, 2011). Figure 1 provides a diagrammatic outline of the complex neurodegenerative process involved in AD. AD is linked to abnormalities in the mitochondrial electron transport chain (Blass, 2000; Castellani et al., 2002), with the endogenous production of reactive oxygen species (ROS) during respiration consistently associated with brain ageing (Butterfield et al., 2001; Markesbery & Lovell, 1998; Rosenberg, 2000; Sohal & Weindruch, 1996).

ROS are essential regulators of cellular function (Finkel, 1998). Normally, damage by these oxygen radicals is controlled by antioxidant systems (Smith et al., 2000), but under pathological conditions, increased mitochondrial metabolism leads to irreversible oxidative damage in cells (Gershman et al., 1954; Harman, 1972). Neurons in the hippocampus and neocortex constantly

Figure 1: A schematic overview of the proposed mechanisms involved in AD onset.
This model is consistent with the key role of Aβ and free radical oxidative stress in the pathogenesis of AD.
(Adapted from: Varadarajan et al., 2000, p.199)
endure ROS-induced oxidative injury. As they are major sites of learning and memory (Mattson, 2004), this leads to the progressive loss of higher brain functioning typical of AD (Burns et al., 2001; Nunomura et al., 2006). Oxidative protein damage (Harman, 1956; Irvine et al., 2008) and the lipid peroxidation product, 4-hydroxynonenal (Keller et al., 1997) promote protein aggregation in nerve cell bodies (Goedert et al., 1991) forming the classic neuropathological hallmarks of AD; senile plaques and neurofibrillary tangles (NFTs) (Cummings, 2004; Emerit et al., 2004).

Although AD is unique to humans, the underlying pathological mechanisms have been investigated using animal models (Keller et al., 2005; Markesbery, 1997; Oddo et al., 2003). AD has a strong genetic component. Identification of the AD-specific genes: amyloid precursor protein (APP) (Tanzi et al., 1987), presenilins PS-1 (Sherrington et al., 1995; Tanzi et al., 1996) and PS-2 (Levy-Lahad et al., 1995), and APOE-ε4 (Corder et al., 1993; Saunders et al., 1993), enabled the development of animal models which have provided an invaluable insight into the effects of DR on brain ageing (Guo et al., 1999a).

APP transgenic mice are often used in AD studies (e.g. Callahan et al., 2001; Reddy et al., 2004). APP is continually produced and metabolised in the brain (Bateman et al., 2006; Lee et al., 2008). By blocking import channels, APP impairs the activity of complex IV, the terminal enzyme of the mitochondrial electron transport chain (Devi et al., 2006). This promotes membrane depolarisation and renders neurons vulnerable to apoptotic cell death (Mark et al., 1995; Mattson et al., 1992). Sequential cleavage of APP by β- and γ-secretase enzymes generates the insoluble and highly aggregable amyloid-β (Aβ)-42 (Mattson, 2004; Yankner et al., 1990). Aβ42 is neurotoxic and promotes neuronal degeneration by inducing lipid peroxidation (Mark et al., 1997; Mattson et al., 1993; Reddy et al., 2004). Mutations in APP, PS-1 and PS-2 are associated with the abnormally early and abundant accumulation of Aβ42-containing plaques (Duff et al., 1996; Guo et al., 1999b; Scheuner et al., 1996).

Evidence suggests that Aβ42 aggregation precedes NFT formation in AD (Götz et al., 2001; Oddo et al., 2003). The amyloid cascade hypothesis proposes that neuronal dysfunction in AD is initiated by the production of amyloid-containing senile plaques (Hardy & Allsop, 1991), with the microtubule-associated protein tau acting as a downstream mediator of ROS-induced Aβ neurotoxicity (Behl, 2012). This leads to AD-related cytoskeletal pathology (Grundke-Iqbal et al., 1986) and NFT generation (Emerit et al., 2004). Aβ42 not only enhances oxidative stress levels (Martin et al., 2006; Prolla & Mattson, 2001), but also activates multiple caspases (Rissman et al., 2004; Rohn & Head, 2009) and kinases (Lee et al., 2000a). Caspases regulate apoptosis (Roth, 2001) while both caspases (Chung et al., 2001; Rohn et al., 2002) and a diverse number of kinases (Lee et al., 2000a; Tabira, 2004) are involved in tau phosphorylation. Abnormal hyperphosphorylation of tau
destabilises microtubules, leading to neuron disintegration and NFT production (Iqbal et al., 2005). However, substantial evidence indicates that DR protects neurons from degeneration (Bruce-Keller et al., 1999; Zhu et al., 1999). Given the inextricable link between brain function and AD, the proposed reduction of AD neuropathology using DR is of considerable interest to researchers in the field of ageing.

**DIETARY RESTRICTION PROMOTES NEUROGENESIS AND PROTECTS NEURONS**

Neural progenitor cells (NPCs) are highly abundant in the hippocampal region of the adult mammalian brain (Gage, 2000). These undifferentiated cells can divide and act as a reservoir to replace lost neurons (Liu et al., 1998). This is known as neurogenesis (Gage, 2000). It has long been known that neural cells in the rat hippocampus are capable of continual division and differentiation into functional neurons (Altman, 1966; Altman & Das, 1965), but only relatively recently was neurogenesis definitively shown to occur in the adult mammalian brain (Eriksson et al., 1998). The brain’s capacity for neurogenesis decreases during ageing (Kuhn et al., 1996), leading to a progressive loss of neurons and learning and memory impairment (Shors et al., 2001). Neuronal cell death plays a key role in the pathobiology of AD (Guo et al., 1999a; Mattson et al., 1993). Animal models of AD indicate that DR enhances the expression of chaperone proteins and neurotrophic factors, as well as increasing neurogenesis and neuron survival (Duan & Mattson, 1999; Yu & Mattson, 1999). This improves behavioural outcomes and ameliorates the decline in cognitive function that is widely associated with AD (Bruce-Keller et al., 1999; Duan et al., 2001; Ingram et al., 1987; Wang et al., 2005).

Mutant presenilin-1 (PS-1) increases the vulnerability of neurons to dysfunction and death (Guo et al., 1999a; Guo et al., 1999c) by altering the proteolytic processing of APP and producing neurotoxic Aβ42 (Duff et al., 1996). Zhu et al. (1999) discovered that a 3-month DR regimen protected the neurons of 6-week old mice expressing the AD-linked PS-1 M146V gene mutation against apoptotic cell death. Oxidative stress levels following kainate administration were lower in the hippocampus of DR mice compared to the *ad libitum*-fed controls. Kainate is an excitotoxin often used to induce oxidative stress in AD models (Zheng et al., 2011). It selectively damages hippocampal neurons by binding to and stimulating nerve cell receptors, causing cellular damage and apoptosis (Prolla & Mattson, 2001). The extent of kainate-induced neuronal damage increased by 50-60% in the PS-1 mutant mice compared to the wild-type mice, whilst kainate damage was significantly reduced in both the PS-1 mutant and wild-type mice maintained on DR (Zhu et al., 1999). As shown previously (Good et al., 1996; Smith et al., 1997), AD-associated neurodegeneration involves enhanced ROS production and lipid peroxidation. When Zhu et al.
(1999) immunostained the PS-1 mutant mice with an antibody recognising 4-hydroxynonenal-modified proteins, they found DR had attenuated the mutant PS-1-induced increase in lipid peroxidation in hippocampal neurons (Figure 2).

![Graph showing relative levels of 4-hydroxynonenal in the hippocampus of wild-type and PS-1 mutant knock-in mice.](image)

**Figure 2:** Graph showing relative levels of 4-hydroxynonenal in the hippocampus of wild-type and PS-1 mutant knock-in mice. There is a marked suppression of kainate-induced lipid peroxidation in mice maintained on DR compared to the *ad-libitum* (AL) fed control mice. (Zhu et al., 1999, p.227)

Similarly, Bruce-Keller et al. (1999) maintained rats on an alternate-day feeding regimen which resulted in a 30% overall decrease in normal dietary intake and, in agreement with Zhu et al. (1999), they discovered that the hippocampal neurons of DR rats exhibited an enhanced resistance to kainite-induced degeneration. Furthermore, these rodents demonstrated a remarkable preservation of learning and memory skills when tested in a water maze spatial learning task (Figure 3) (Bruce-Keller et al., 1999). The consistency of these results with previous research implies that short-term DR significantly decreases neuronal vulnerability to degeneration and attenuates memory deficits. Moreover, it indicates that the prevention of oxidative injury may be one way in which DR exerts its neuroprotective effects on the brain, and supports the proposal that neuronal dysfunction induced by AD-specific gene mutations could be counteracted by dietary regulation.

The ability of DR to increase neuronal resistance correlates with the upregulated expression of certain genes, such as those coding for chaperone proteins and neurotrophic factors (Prolla & Mattson, 2001, p.S28). DR induces a mild stress response in neurons due to decreased energy availability, resulting in the production of these proteins (Duan & Mattson, 1999). Numerous studies
show that enhanced levels of the chaperone proteins HSP70 (Lowenstein et al., 1991) and GRP78 (Yu et al., 1999) in the hippocampal and cortical brain regions of DR rats compared with ad libitum-fed rats promote neuronal resistance to oxidative injury. This suggests that the upregulated expression of these proteins may contribute to the neuroprotection provided by DR (Duan & Mattson, 1999; Yu & Mattson, 1999), preventing the progressive cognitive impairment associated with AD (Mattson, 2004). The view that a moderate stress response induced in neurons can account for the protective properties of DR is further supported by studies involving animal models of PD (Duan & Mattson, 1999) and stroke (Yu & Mattson, 1999).

As well as chaperone proteins, the protective effects of neurotrophic factors are also well documented in animal studies of AD (Lee et al., 2002b). Neurotrophins such as brain-derived neurotrophic factor (BDNF) and neurotrophin-3 (NT-3) are proteins which promote the survival of NPCs (Lowenstein & Arsenault, 1996) and newly generated neurons (Cheng & Mattson, 1994; Mattson et al., 1995). BDNF protects NPCs against oxidative injury (Mattson & Lindvall, 1997) by inducing the expression of genes encoding antiapoptotic enzymes which suppress oxidative stress and prevent apoptosis by stabilising calcium homeostasis (Mattson, 2000a). In addition, introduction of a BDNF-blocking antibody into rat brains strongly attenuated the beneficial effects induced by DR (Duan et al., 2001), supporting the role played by neurotrophic factors in neuroprotection.

![Figure 3](image.png)

**Figure 3:** Graph showing that DR increases the resistance of neurons to degeneration in rodents. Rats were maintained on DR or *ad libitum* (AL) feeding regimens for 3 months. Injection of kainate caused severe impairment in learning and memory in the AL group but not in the DR group.

(Bruce-Keller et al., 1999, p.12)
Lee et al. (2002b) sought to further establish how BDNF mediates the effects of DR on neurogenesis. They maintained heterozygous BDNF +/- mice and wild-type mice on a DR regimen or fed *ad libitum* for 3 months, then injected BrdU stain to label neurons and euthanised the mice. The number of labelled cells in the hippocampal region of the BDNF+/- *ad libitum*-fed mice was significantly lower than the number in the wild-type *ad libitum*-fed controls. In the DR group, the wild-type mice exhibited an increase in hippocampal neurons and, to a lesser extent, in the BDNF +/- mice. This replicated the results of a previous study by Lee et al. (2002a) which reported that DR increased neurogenesis and cell survival in the hippocampus of adult mice. They attributed this to the enhanced levels of neuronal BDNF and NT-3 which were present in the DR mice.

The evidence provided by Lee and his colleagues implies that neurotrophic factors play a major role in mediating neuron survival in the hippocampus as well as suggesting that it may be possible to enhance brain function and increase resistance to developing neurodegenerative diseases by controlling dietary intake in humans. However, distinct differences in brain ageing between mice and humans cause translatability issues (Loerch et al., 2008). For instance, mice do not live long enough to develop AD naturally so it has to be experimentally induced. Also, APP transgenic mice fail to fully recapitulate all aspects of AD, and do not form NFTs despite the presence of AD-type amyloid plaques (Ashe & Zahs, 2010). This raises concerns over whether the findings from transgenic mice studies can be safely and reliably extrapolated to humans (Colman & Anderson, 2011; Mattson et al., 2002). Furthermore, not all DR studies report the same outcomes.

In contrast to previous studies detailing increased neurogenesis in rodents on DR, research by Bondolfi et al. (2004) found that DR had no effect on neurogenesis or cell survival. One possible explanation for these conflicting results is the use of different methods to restrict dietary intake. Bondolfi et al. (2004) implemented a constant reduced intake whereas Lee and his colleagues employed an alternate day feeding method to achieve DR. Increased activity in the rats searching for food on fasting days may partly explain the discrepant results as evidence shows that increased physical activity enhances neurogenesis (van Praag et al., 1999). Furthermore, Bondolfi et al. (2004) used older mice that were maintained on a DR regimen for longer than the younger mice used by Lee and his colleagues maintained on DR for a maximum of 3 months. This brings into question whether the effects of long-term DR somehow counteract the initial benefits witnessed in the brain, as well as raising concerns over the feasibility of using DR in humans to prevent or treat AD, especially as AD typically onsets later in life (Alzheimer’s Association, 2012b). Glucocorticoids, which have a bimodal effect on neurons and cognitive function (Patel & Finch, 2002, p. 707; Reagan & McEwen, 1997), may provide a better explanation for these inconsistent results.
THE GLUCOCORTICOID DEBATE

Glucocorticoids - corticosterone (CORT) in rodents and cortisol in primates, including humans - are stress-induced hormones secreted by the adrenal cortex (Qiu et al., 2012; Reagan & McEwen, 1997). Chronic exposure to glucocorticoids is associated with hippocampal degeneration and cognitive impairment (Landfield et al., 1981a; Patel & Finch, 2002), with increased glucocorticoid efficacy reported in early-stage AD patients (Landfield et al., 2007). Numerous studies detail how reduced cortisol levels in humans decrease hippocampal atrophy and improve performance on memory tasks (Lupien et al., 1998; Seeman et al., 1997). Moreover, raising glucocorticoid levels enhances neuronal degeneration in both rodents (Landfield et al., 1978) and non-human primates (Sapolsky et al., 1990). However, an apparent paradox arises (Patel & Finch, 2002). Glucocorticoids enhance neurodegeneration, and both acute and chronic DR markedly increases plasma glucocorticoid levels (Sabatino et al., 1991; Wan et al., 2003), yet there is abundant evidence detailing the neuroprotective effects of DR in the brain.

The brain has a very high demand for glucose making it particularly vulnerable to alterations in energy supply (Patel & Finch, 2002, p.712). Glucocorticoids inhibit glucose uptake by hippocampal neurons (Horner et al., 1990; Virgin et al., 1991), rendering them susceptible to oxidative injury (Tombaugh et al., 1992). This leads to neuron loss (Kerr et al., 1991; Sapolsky et al., 1985) and brain atrophy (Watanabe et al., 1992; Woolley et al., 1990). Moreover, human clinical studies have revealed that prolonged exposure to glucocorticoids causes neuronal cell death in the CA3 region of the hippocampus (Sheline et al., 1996; Starkman et al., 1992). Conversely, decreasing CORT levels by adrenalectomy in DR rats attenuates learning and memory deficits (Figure 4) (Qiu et al., 2012) and decreases neuron dysfunction (Landfield et al., 1981a; Landfield et al., 1981b; Meaney et al., 1988). Thus, due to the chronic elevation of glucocorticoid levels it induces, DR is assumed to have a negative effect on the brain. However, this is distinctly contrasted by abundant evidence which supports the neuroprotective effects of DR. For instance, Bruce-Keller et al. (1999) found that transient increases in glucocorticoids can enhance cognitive performance and protect against neuronal degeneration. In addition, Ábrahám et al. (2000) discovered that moderately elevated levels of CORT in rat plasma are correlated with the attenuation of Aβ accumulation in the brain. Furthermore, one study found that an absence of CORT for 3 days in rat brains following adrenalectomy resulted in extensive hippocampal neuron death (Gould et al., 1990).
The seemingly paradoxical situation that DR provides significant neuroprotection whilst simultaneously inducing chronic elevations in glucocorticoid levels poses a problem for understanding the DR-mediated benefits on brain ageing and the pathology of AD, especially as the adverse effects of glucocorticoids on neurons and cognitive function are well established (Landfield et al., 1981a; Sapolsky et al., 1990). Collectively, research suggests that DR increases the resistance of neurons by promoting expression of stress proteins (Duan & Mattson, 1999; Yu & Mattson, 1999) and neurotrophic factors (Lee et al., 2000a). Patel and Finch (2002) proposed that the adverse effects of elevated CORT levels are well-compensated for by these neuroprotective mechanisms, which are also induced by DR and attenuate ROS-mediated damage and inhibit apoptosis (Mattson, 2000a; Qiu et al., 2012). Determining whether glucocorticoid alterations are implicated in AD pathogenesis is important as they may represent a potential pharmacological target in the prevention and treatment of AD (Landfield et al., 2007; Reagan & McEwen, 1997).
**DIETARY RESTRICTION ATTENUATES Aβ PEPTIDE DEPOSITION**

The progressive accumulation of Aβ42 in neurons plays a central role in the pathogenesis of AD (Selkoe, 2001; Walsh & Selkoe, 2004). Oxidative stress is a key mediator of Aβ neurotoxicity (Goodman et al., 1996; Goodman & Mattson, 1994) and abnormal amyloid deposition resulting from altered proteolytic cleavage of APP due to oxidative damage initiates an amyloid cascade which leads to AD (Cummings, 2004; Hardy & Selkoe, 2002; Selkoe, 1996). Exposure to neurotoxic Aβ42 increases the amount of oxidative injury to neurons (Mattson, 2000b). Therefore, preventing Aβ42 production or enhancing amyloid plaque clearance from neurons using dietary modifications is an attractive mechanism for delaying the onset of AD (Tabira, 2004).

Mutant forms of the AD-specific proteins APP, PS-1, and PS-2, increase the generation of neurotoxic Aβ42 (Duff et al., 1996; Guo et al., 1999b; Scheuner et al., 1996). PS-1 is a major component of γ-secretase (Yang et al., 2012) which, along with β-secretase, cleaves APP and produces Aβ42 (Mattson, 2004). PS-1 mutations enhance the vulnerability of hippocampal neurons to oxidative damage both in vivo (Grilli et al., 2000; Guo et al., 1999a; Guo et al., 1999b) and in vitro (Guo et al., 1999a). There is extensive scientific evidence substantiating the neuroprotective properties of DR in relatively young mice (under 7 months old) with transgenic APP or double transgenic APP/PS-1 mutations which induce the formation of amyloid brain plaques histologically identical to those found in AD patients (Borchelt et al., 1997; Callahan et al., 2001; Holcomb et al., 1998). Patel et al. (2005) reported that 40% DR for 6 weeks in APP mutant mice led to a 40% reduction in Aβ accumulation compared with the *ad libitum*-fed control group. Likewise, a study by Wang et al. (2005) involving longer-term DR observed an 85% reduction in neocortical Aβ deposits in 3-month old APP mutant mice maintained on 30% DR for 9 months compared to the *ad libitum*-fed controls. Their results, shown in Figure 5, detailed a similar reduction in amyloid plaque burden in the hippocampal region. More recent studies have assessed whether DR can attenuate AD-type neuropathology in older mice with significant neuronal Aβ deposits analogous to those observed in humans who develop AD later in life. Mouton et al. (2009) reported that 40% DR significantly attenuated Aβ deposition in 13-14 month-old double transgenic APP/PS-1 mutant mice, mirroring the results from an earlier study by Patel et al. (2005) in younger APP/PS-1 mice. This is a promising result which indicates that implementing a similar dietary regimen in middle-aged humans could delay the onset and progression of AD (Mouton et al., 2009, p.188).
Although rodent studies present DR as an attractive treatment opportunity for AD, the gap in genetic background and differences in brain ageing between rodents and humans remains a major hurdle (Qin et al., 2006a, p.418). Non-human primates, on the other hand, share many genetic similarities with humans (Walker & Cork, 1999) such as a predisposition to developing AD-type amyloid brain plaques (Kimura et al., 2003; Walker et al., 1990) and increasing memory deficits with age (Moore et al., 2006). Consequently, focus has shifted onto investigations into the effect of DR on longevity and age-related disease in non-human primates, namely squirrel monkeys (Qin et al., 2006a) and rhesus monkeys (Colman et al., 2009). In one such study, the preservation of motor function in elderly rhesus macaques maintained on a DR regimen matched the results previously observed in similar rodent studies (Kastman et al., 2012). Thus, non-human primates represent a fundamental link between laboratory studies of AD and real-life clinical applications (Colman & Anderson, 2011).

Qin et al. (2006a) conducted research into the effect of DR on AD-associated brain amyloidosis in squirrel monkeys. They maintained 3 male monkeys on a dietary regimen with a 30% lower intake than the control group, and supplemented with around 40% higher than recommended essential micronutrients to avoid deficiencies occurring, for their entire lives. Post-mortem brain tissue analysis revealed that the neocortical Aβ42 concentration was significantly lower in the brains of the DR monkeys relative to the control group (Qin et al., 2006a). This was the first study to demonstrate that DR attenuates AD-type brain amyloidosis in non-human primates.
Wang et al. (2005) proposed that the attenuation of Aβ plaque deposition was the result of DR-induced upregulation of the anti-amyloidogenic enzyme, α-secretase (Figure 6). This was subsequently confirmed by Qin et al. (2006a) in squirrel monkeys. Oxidative stress alters the proteolytic cleavage of APP, generating neurotoxic Aβ42 which is deposited in the brain (Fukui et al., 2005; Praticò, 2008). These senile plaques further enhance oxidative stress levels, thus ROS appears to activate a positive feedback mechanism which increases Aβ toxicity in the brain (Yang et al., 2012; Ye & Zhang, 2012, p.48). However, inhibition of either β- or γ-secretase prevents Aβ42 generation (Bonda et al., 2011). Conversely, non-amyloidogenic APP cleavage by α-secretase produces a soluble segment of APP which has neuroprotective properties (Kojro & Fahrenholz, 2005). When upregulated by DR, this alternative pathway competes with the amyloidogenic processing of APP (Postina et al., 2004) by intervening in the amyloid feedback system (Yang et al., 2012) and diminishing Aβ42 formation (Qin et al., 2006a).

Figure 6: DR promotes α-secretase enzyme activity in APP mutant mice. Fluorimetric assessment of α-secretase activity in the brains of APP transgenic mice fed *ad libitum* (AL) or maintained on a DR regimen for 9 months.

(Wang et al., 2005, p.661)

Although non-human primate studies have provided momentum for further exploration into DR as a therapy for AD, some issues remain to be resolved. For instance, in the study by Qin et al. (2006a), the monkeys maintained on DR died, on average, 5-7 years earlier than the control group. This is significant as it suggests that long-term DR may induce other health problems, making it unsuitable for clinical use. Furthermore, a study by Kerr et al. (2011) found equivalent levels of neurotoxic Aβ in both DR and *ad libitum*-fed flies, which conflicts with previous studies reporting that DR attenuates Aβ42 accumulation in neurons (Patel et al., 2005; Wang et al., 2005) and improves cognitive function (Wu et al., 2008). These discrepant results are most likely due to the use
of different experimental organisms, but it reinforces the issue of translatability (Piper & Partridge, 2007) and highlights the essentiality of conducting thorough human clinical trials before the implementation of novel therapies. Kerr et al. (2011) did, however, report that DR led to a complete reversal of the defects resulting from tau hyperphosphorylation in neurons, supporting the commonly held view that DR alters oxidative stress levels upstream of both Aβ and tau pathology in AD (Drake et al., 2003; Praticò et al., 2001).

Recent evidence indicates that the sirtuin, SIRT1, plays a crucial role in mediating the neuroprotective effects of DR. SIRT1 upregulation is implicated in Aβ peptide attenuation (Donmez et al., 2010; Julien et al., 2009; Qin et al., 2006b) and SIRT1 transgenic mice exhibit phenotypes resembling those observed in DR mice (Bordone et al., 2007). This suggests that SIRT1 is involved in regulating the neuropathology of AD.

THE ROLE OF SIRTUINS IN ALZHEIMER’S DISEASE

Sirtuins are NAD⁺-dependent deacetylase enzymes which are closely linked to ageing (Balazs et al., 2011) and are upregulated by DR (Bordone & Guarente, 2005; Lamming et al., 2004; Lombard et al., 2005). The relationship between sirtuins, DR, and ageing was first shown in yeast. DR extends the lifespan of yeast, but this increase in longevity was not observed in yeast lacking the sirtuin, Sir2 (Lin et al., 2000). SIRT1, the human ortholog of Sir2 (Michán et al., 2010), enhances the tolerance of cells to oxidative stress (Cohen et al., 2004; Chen et al., 2005; Tang, 2006), reduces amyloid deposition (Luo et al., 2001; Patel et al., 2005; Qin et al., 2006b), and blocks neuronal apoptosis (Kume et al., 2006). Moreover, inhibition of SIRT1 is detrimental to neurons (Chong et al., 2005).

Neuronal SIRT1 is proposed to mediate the protective properties of DR regimens in preventing AD-type amyloid neuropathology (Figure 7) (Cohen et al., 2009; Kim et al., 2007; Nisoli et al., 2005; Qin et al., 2006b). Activated sirtuins deacetylate and activate the peroxisome proliferator-activated receptor γ coactivator 1α (PGC1α), a transcriptional coactivator which is vital for preventing mitochondrial dysfunction and neurodegeneration (Cui et al., 2006; Lagouge et al., 2006; Onyango et al., 2010). Mounting evidence suggests that the promotion of sirtuin activity, either by DR itself or via DR mimetics (Karuppagounder et al., 2009; Marambaud et al., 2005; Vingtdeux et al., 2008), increases neuronal resistance to cellular stress and, as demonstrated in Figure 7, can regulate the onset of neurodegenerative diseases such as AD.
Figure 7: An overview of the SIRT1-mediated neuroprotective effects induced by DR on AD and other neurodegenerative disorders.

(Outeiro et al., 2008, p.365)

SIRT1 is a crucial regulator of α-secretase activity (Haigis & Guarente, 2006). In APP transgenic mice, deacetylation by SIRT1 suppresses Rho-associated protein kinase 1 (ROCK1) expression (Donmez et al., 2010), subsequently increasing the non-amyloidogenic processing of APP by upregulating α-secretase activity in vitro (Qin et al., 2006b). Recent in vivo data agrees with these findings. Double transgenic mice overexpressing APP and SIRT1 displayed a marked reduction in Aβ42 accumulation (Figure 8A) along with enhanced α-secretase activity (Figure 8B), whilst cognitive deficits were exacerbated in SIRT1 knockout mice (Donmez et al., 2010). Similarly, DR increased SIRT1 levels and significantly reduced amyloid plaque burden in the brains of squirrel monkeys (Qin et al., 2006a). The negative correlation between Aβ accumulation and SIRT1 levels implies that DR-induced SIRT1 expression is effective in regulating AD pathogenesis. Moreover, in vitro models of AD tauopathy report that SIRT1 activation promotes neuron survival in the hippocampus due to the increased deacetylation of PGC-1α by SIRT1 (Kim et al., 2007).

SIRT1-mediated deacetylation of PGC-1α also increases gene transcription (Anderson et al., 2008) and induces mitochondrial biogenesis (Gan & Mucke, 2008). Metabolic perturbations play a central role in AD and thus are an important target for SIRT1 activity (Barbato et al., 2012). DR enhances the phosphorylation of endothelial nitric oxide synthetase (eNOS) in the brain (Nisoli et al., 2005; Fusco et al., 2012). Subsequent activation of a downstream nitric oxide-sensitive pathway mediates mitochondrial biogenesis (Cerquiera et al., 2012; Nisoli et al., 2003). DR induces SIRT1 expression in wild-type mice but not in eNOS knockout mice (Nisoli et al., 2005), and the beneficial effects of DR on mitochondrial function were largely attenuated in the knockout mice. This suggests
that nitric oxide is involved in controlling SIRT1 expression and mediates the mitochondrial protection provided by DR, despite reports that nitric oxide can exert deleterious effects on the brain (Moro et al., 2004).

The attenuation of AD-associated mitochondrial dysfunction in the brain by DR can be reproduced using DR mimetics, such as the polyphenol resveratrol (Borra et al., 2005; Hu et al., 2011; Pallàs et al., 2009). With the ability to cross the blood-brain barrier, resveratrol replicates the physiological responses of DR (Ingram et al., 2006, p.99; Marambaud et al., 2013) without requiring a reduction in food intake (Ingram et al., 2004). Lagouge et al. (2006) reported that resveratrol improves mitochondrial function by inducing SIRT1 expression and PGC-1α deacetylation. Similarly, Porquet et al. (2013) showed that resveratrol not only increased SIRT1 expression, and thus decreased apoptotic neuronal death in the cortex and hippocampus, but also exhibited a neuroprotective role, decreasing both tau hyperphosphorylation and levels of Aβ plaques in the brains of mouse models genetically modified to over-produce APP. However, a similar study using the same mouse model concluded that resveratrol neither improved cognitive function nor increased the expression of SIRT1 (Chang et al. 2012). This may be accounted for by methodological differences between the two studies, including the age of the mice when the diet was started, duration or treatment, and the dose of resveratrol administered, although an earlier study by Karuppagounder et al. (2009) also found no evidence of SIRT1 activation in the brains of mice supplemented with dietary resveratrol. They did, however, note a significant reduction in Aβ plaque pathology following

Figure 8: SIRT1 attenuates Aβ42 accumulation and upregulates α-secretase activity in the brains of AD transgenic mice models. A) Assessment by Aβ ELISA assay of Aβ42 levels in the whole brains of transgenic mice models. B) α-secretase activity in the brains of transgenic mice models assessed using an activity assay.

(Adapted from: Donmez et al., 2010, p. 324)
resveratrol supplementation. Currently, the exact mechanisms underlying this decrease in amyloid plaque load induced by resveratrol are unknown, but it does not appear to be the result of altered APP processing (Karuppagounder et al., 2009). Marambaud et al. (2005) reported that resveratrol had no effect on the activity of the APP cleavage enzymes β- or γ-secretase and did not inhibit Aβ42 production. Instead they found that resveratrol reduced amyloid plaque formation via intracellular degradation by the proteasome, thus suggesting that additional SIRT1-independent processes are at work in DR (Dasgupta & Milbrandt, 2007).

Despite extensive evidence detailing the neuroprotective effects of SIRT1, some studies report contrasting results. For instance, Li et al. (2008) reported a reduction in oxidative stress levels and enhanced neuroprotection in the brains of SIRT1 knockout mice. This could be a consequence of the NAD⁺-dependence which inextricably links SIRT1 to the metabolic activity of cells and can render neurons vulnerable to oxidative damage (Onyango et al., 2010). In addition, DR-enhanced SIRT1 expression in the brain appears to be region specific (Figure 9) (Chen et al., 2008a; Chen et al., 2008b). This means that pharmacological interventions regulating sirtuin activity may only mimic DR in certain areas (Haigis & Guarente, 2006). Moreover, some studies found that although resveratrol treatment increased the survival of mice on a high-calorie diet, it had no effect on the survival of mice on a standard intake (Baur & Sinclair, 2006; Pearson et al., 2008) which further implies that resveratrol may only partially act as a DR mimetic (Barger et al., 2008). Further studies

![Bar graph showing regional differences in SIRT1 expression level in the brain.](image)

**Figure 9: Regional differences in SIRT1 expression level in the brain.** SIRT1 expression is downregulated in the cerebellum and midbrain, but enhanced in the hippocampus and cortex of DR mice compared with *ad libitum* (AL)-fed mice.

(Chen et al., 2008b, p.1093)
are necessary to resolve these experimental disparities and to fully elucidate the role of sirtuins in brain ageing. Nevertheless, SIRT1 represents a promising drug target in the treatment of diseases, such as AD, which are tied to the harmful effects of oxidative stress (Chong et al., 2012; Donmez, 2012).

**DIETARY RESTRICTION AS A THERAPY FOR ALZHEIMER’S DISEASE**

Dietary modifications continue to emerge as a highly attractive strategy for the successful management of the neuropathology of AD. However, the application of DR in a clinical setting is complicated because the development of novel therapies needs to be based on evidence from both experimental and epidemiological research, as well as from controlled human trials (Pasinetti et al., 2007). Although the lack of validated biomarkers of ageing has previously hindered the progress of research in humans (Warner, 2004), epidemiological studies provide substantial evidence of the neuroprotective properties of DR in human populations (Hendrie et al., 2001; Luchsinger et al., 2002; Mayeux et al., 1999). In addition, data from controlled human DR studies is beginning to emerge (Buchowski et al., 2012; Rickman et al., 2011; Rochon et al. 2010; Stewart et al., 2012).

A key issue when developing novel therapies is determining their safety (Donmez, 2012), hence the need for comprehensive clinical trials. Unfortunately, in practice, human studies assessing dietary intake are plagued by methodological difficulties. It is often impractical to conduct longitudinal dietary studies in human populations (Omodei & Fontana, 2011), especially due to a widespread inability to commit to a prolonged DR regimen (Levenson & Rich, 2007). Moreover, long-term DR is accompanied by a number of adverse side-effects which may prevent its application in a clinical setting. These include fatigue and distracting hunger (Johnstone, 2007), and excessive loss of body fat and muscle mass (Dirks & Leeuwenburgh, 2005). DR mimetics bypass some of these issues and have thus emerged as a potential AD therapy (Fontán-Lozano et al., 2008). Trials monitoring the effects of resveratrol in AD patients are ongoing (Clinicaltrials.gov, 2008a, 2008b, 2010), although creating a DR mimetic able to cross the blood–brain barrier has proven problematic (Donmez, 2012, p.500). In addition, the high degree of heterogeneity in AD, such as variable rates of disease progression (Komarova & Thalhauser, 2011) and differing patient responses to treatments (Wattmo et al., 2011), has further challenged the development of an effective, universal AD therapy.

Although current understanding has enabled researchers to widely demonstrate the clinical potential of DR in attenuating AD neuropathology, further research into the neuroprotective properties of DR is essential. For instance, the possible discovery of a common mechanism behind the beneficial effects of DR on both longevity and AD could represent a promising step towards the development a novel therapy for AD.
CONCLUSIONS

The beneficial effects of DR on longevity and brain ageing, and its proposed role in protecting neurons from mitochondrial ROS-induced oxidative damage, are receiving considerable attention in AD research. Modern technology has provided an exciting new insight into the molecular mechanisms underlying AD pathogenesis and has enabled researchers to conduct more comprehensive investigations into the development of a safe, effective treatment for AD. As with other age-related diseases, studies consistently indicate that a moderate reduction in dietary intake can attenuate AD neuropathology.

Investigations involving experimental animal models of AD provide conclusive evidence of the neuroprotective properties of DR. By upregulating the expression of neurotrophic factors, stress proteins, and SIRT1 in the hippocampal and cortical regions of the brain, DR promotes neuron survival and increase resistance to oxidative stress. This is achieved by a number of mechanisms including enhanced mitochondrial biogenesis and the reduction of neurotoxic Aβ42 deposition in neurons. Furthermore, promising data from non-human primate studies indicates that DR induces the same physiological response in primates as it does in rodent models of AD, attenuating the neuropathological features of AD. However, it remains to be established whether the results obtained from laboratory-based animal studies can be extrapolated to humans.

Existing research has not only provided greater insights into the mechanisms underlying DR, but it has also revealed that DR could play a crucial role in the clinical management of AD. Ultimately, it is likely that a range of therapeutic approaches will be used in combination to treat AD patients. Clarification of the ways in which DR exerts its neuroprotective effects on AD pathogenesis may aid the development of a novel therapeutic intervention for AD patients involving lifestyle modifications. Nevertheless, existing evidence consistently indicates that DR attenuates the neuropathological features of AD and thus it remains an area of significant interest within the field of ageing.
BIBLIOGRAPHY


Chen, D., Bruno, J., Easlon, E., Lin, S. J.,


Sohal, R. S. (2002). Role of oxidative stress and proteins oxidation in the aging process. *Free Radical Biology & Medicine, 33*(1): 37-44.


