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Evaluation of dietary and lifestyle changes as modifiers of S100β levels in Alzheimer’s disease

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Abstract

There is a significant body of research undertaken in order to elucidate the mechanisms underlying the pathology of Alzheimer’s disease (AD), as well as to discover early detection biomarkers and potential therapeutic strategies. One such proposed biomarker is the calcium binding protein S100β, which, depending on its local concentration, is known to exhibit both neurotrophic and neuroinflammatory properties in the central nervous system. At present, relatively little is known regarding the effect of chronic S100β disruption in AD. Dietary intake has been identified as a modifiable risk factor for AD. Preliminary in vitro and animal studies have demonstrated an association between S100β expression and dietary intake which links to AD pathophysiology. This review describes the association of S100β to fatty acids, ketone bodies, insulin, and botanicals as well as the potential impact of physical activity as a lifestyle factor. We also discuss the prospective implications of these findings, including support of the use of a Mediterranean dietary pattern and/or the ketogenic diet as an approach to modify AD risk.

Keywords: S100B; Alzheimer’s disease; insulin; ketogenic diet; botanicals; nutrition; ageing; apolipoprotein E; APOE4

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Disclosure statement: All authors declare no conflict of interest

Acknowledgments:
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Introduction

Alzheimer's disease (AD) is an irreversible, multifactorial neurodegenerative disease that is characterised by progressive episodic memory loss and cognitive impairment (1-3). Pathologically, AD is indicated by severe neuronal apoptosis, excessive aggregation of extracellular amyloid-β (Aβ) in between neurons, abnormal hyperphosphorylated tau protein forming intraneuronal neurofibrillary tangles and a reduction in cerebral glucose metabolism (2, 4-7). At present, an estimated 1 in 10 adults over the age of 65 suffers from AD (8) with the number of individuals living with AD expected to rise to 106 million by the year 2050 (9, 10). Globally, between 2010 and 2015 the cost of dementia was estimated to have increased by 35% to total $818 billion (USD) (11). This increase has been mainly attributed to an overall increase in diagnosed cases and a growing per person treatment cost. The individual, societal and economic toll of AD is immense, and research to identify possible preventative and therapeutic strategies are vital in an ageing population.

While current pharmaceutical intervention has the potential to reduce symptoms, early diagnosis could potentially allow the slowing of AD disease progression (12), improve the quality of life (13) and lower the societal financial burden. The aetiology of AD remains unclear, with several mechanisms believed to play a role in the development of AD (Figure 1). Almost all cases of AD are considered sporadic, and without a single causal factor, the identification of several lifestyle-based risk factors have led to increased interest in lifestyle modification as a potential pathway to prevent or delay the disease. The common targets for intervention include dietary factors, physical activity, cognitive training, sleep quality and increased awareness of genetic vulnerabilities (3, 14, 15).

Dietary modifications have been identified as of interest for prevention of chronic diseases such as AD, although the optimal dietary patterns for individuals with AD have not been clearly defined (16). Specific dietary patterns, particularly the Mediterranean diet (MD), which is characterised by high consumption of omega-3 fatty acids and polyphenol-rich foods, such as olive oil, nuts, vegetables and fruits, has been associated with reduced risk of cognitive decline (17-20). In addition, the MIND diet (MD combined with the Dietary Approaches to Stop Hypertension (DASH) intervention for Neurodegenerative delay) has been associated with a delay in cognitive decline (21). The mechanisms through which specific dietary patterns impart their beneficial effects remain unclear as the majority of available evidence is qualitative and observational in nature, and nutritional research is impaired by a difficulty in isolating specific nutrients and their therapeutic benefits (22).
The purported advantages of a MD may in part be due to factors which differentiate it from the typical Western dietary pattern, characterised by high energy intake comprised heavily of refined carbohydrates and saturated and trans-unsaturated fatty acids (23, 24). In addition, those who traditionally subscribe to a Mediterranean dietary pattern are likely to derive benefits from the greater emphasis on social aspects of everyday meals (25). The prevalence of AD in Japan has increased concomitantly simultaneously with an influx of Western dietary influence and shift away from the traditional diet of vegetables, fish, and unprocessed foods (26). To test the impact of modifiable risk factors on AD, two small pilot studies have reported outcomes from individualised lifestyle interventions consisting of a nutrient-dense low carbohydrate diet, tailored supplement protocols and exercise advice (15, 27). The results revealed a reduction in AD symptoms based on neuropsychological tests and neuroimaging. These studies support mounting epidemiological evidence linking dietary patterns to reduced AD risk, contributing to a focus on the relationship between dietary components and biomarkers of brain health (28).

Table 1. Selection of theories of AD and modifiable risk factors.

<table>
<thead>
<tr>
<th>Theories of AD</th>
<th>Modifiable risk factors</th>
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<tbody>
<tr>
<td>β-Amyloid Cascade (6)</td>
<td>Western dietary patterns (23, 29)</td>
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<tr>
<td>Neurofibrillary Tangles (4)</td>
<td>Physical inactivity (30, 31)</td>
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<td>Mitochondrial dysfunction (32)</td>
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<td>Infectious agents (34)</td>
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<td>Brain insulin resistance (35)</td>
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<td>Immune system-mediated actions (36)</td>
<td>Hypertension (31)</td>
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<td>Blood-brain barrier dysfunction (37)</td>
<td>Social engagement (38)</td>
</tr>
<tr>
<td>Non-modifiable risk factors (age, gender, ethnicity &amp; genetics) (5)</td>
<td>Circadian rhythm disruption (39)</td>
</tr>
</tbody>
</table>

Figure 1. Selection of theories of AD and modifiable risk factors.

The prodromal phase of AD exists for some years and can be relatively long, approximately 12 years prior to the appearance of noticeable cognitive and functional declines required for a clinical diagnosis. Therefore there is a clear need for sensitive biomarkers to identify patients at risk of AD in
its earliest stages (40). The calcium-dependent regulatory protein S100β (S100β) has gained attention as a potential biomarker for a range of conditions, in particular, traumatic brain injury (TBI), and may function as an early detection biomarker and therapeutic target for AD (41). S100β is predominantly a glial-specific protein whereby excessive activity is considered a marker of Blood-Brain Barrier (BBB) dysfunction and neuronal damage (42), both of which are observed in AD. The association between S100β levels and AD has been preliminarily explored, yet the interaction between S100β and dietary factors remains unknown. The main aim of this review is to examine the evidence of potential moderating or mediating effects of dietary factors on S100β levels in the context of AD.

**S100β, origin, distribution, and pathogenesis**

The S100β protein was first discovered by Moore in 1965 as part of the S100 calgranulin protein family and has since attracted interest as a potential biomarker for a variety of cerebrovascular and neurodegenerative diseases (41). Approximately 80-90% of S100β is located in the brain, and its distribution is mostly related to white matter structures (43). Cellular S100β is predominantly expressed in the cytoplasm and/or nucleus of glial cells including astrocytes, oligodendrocytes, and Schwann cells (41), however, S100β is also found extra-cranially including in adipocytes (44) and skeletal myoblast cells (45). Therefore, quantitative measurements of S100β levels can be detected in biological fluids such as serum and plasma, urine, cerebrospinal fluids (CSF), saliva, amniotic fluid, and breast milk (41).

The S100β has dualistic effects, largely dependent upon its local concentration and the stage of human development. Elevated serum S100β in the first three months of life (mean=0.97±0.36µg/L) supports the development of the CNS (46), stimulating neuronal growth (47), glial cell proliferation (48), and increases neuronal survival and maturation (46). In healthy individuals, S100β levels stabilise between ages 2-16y (0.20µg/L) (46). However, elevated levels at other points in life correlate with several neurodegenerative and neuropsychological conditions, with the diagnostic potential of S100β mostly explored in relation to TBI. Increased S100β levels correlate with mild and severe TBI as well as predicted injury outcome (49, 50). In addition, S100β is also an initial marker of injury severity after major trauma independent of head injury and may help predict survival outcome (51). A systematic review by Mercier et al., (52) found a range of 1.38-10.50µg/L to be associated with mortality following moderate or severe traumatic brain injury (TBI). Additionally, testing S100β levels has recently been proposed as a cost reduction strategy in the determination of mild TBI (53). Elevated S100β levels have also been observed in individuals affected by ischemic stroke (54), subarachnoid haemorrhage (50), cardiac arrest (55), post-operative cognitive dysfunction (56), Down’s syndrome (57), Parkinson’s disease (PD) (58), multiple sclerosis (MS) (59), melanoma (60), sleep disruption (61), Tourette’s syndrome (62), fatigue (63) and sarcopenia (64).
The intracellular S100β functions as a regulator of cell proliferation, cell differentiation and calcium homoeostasis through attachment to intracellular membranes and cytoskeleton proteins (41, 65). Excessive secretion of S100β into the extracellular space, particularly by astrocytes and microglia, can result in reduced glucose metabolism (66). A comprehensive understanding of the mechanisms by which cells release S100β is not available; however, it was suggested that this occurs due to inflammation, cell stress and interactions with insulin signalling (44, 66). Extracellular levels of S100β exerts its biological effects through the receptor for advanced glycation end products (RAGE) which is a multi-ligand receptor known to induce inflammatory cell responses (67). RAGE is also upregulated during times of pathological stress and is itself a current therapeutic target in AD (68). RAGE is the primary S100β cell surface receptor, and their interaction triggers numerous signalling cascades including nuclear factor kappa-light-chain-enhancer of activated β cells (NF-κβ) resulting in the upregulation of inflammatory mediators such as inducible nitric oxide synthase and pro-inflammatory cytokines including, IL-1β, IL-6 and TNF-α (69, 70). These cytokines can pose neurotoxic effects including neuronal apoptosis, known to contribute to the progression of neurodegenerative and neuro-inflammatory conditions (41, 71). In part, due to these effects, measurement of S100β is considered a marker of neuroinflammation and BBB dysfunction.

**Alzheimer’s disease and S100β**

**Studies in humans**

The association between S100β levels and AD in humans is currently limited to a relatively small number of studies (Table 1) (72-76). Post-mortem examination of brain tissue in clinically diagnosed AD sufferers revealed over-expression of S100β compared with healthy individuals (77). This is likely due to the accumulation of S100β in the extracellular space following its release from activated astrocytes or death of the astrocyte (78). Higher CSF S100β levels correlate with a reduction in normalised brain volume in AD sufferers (72, 73). This is consistent with neuroimaging where it has been shown that there is accelerated brain atrophy during the early stages of AD (79). The S100β levels in the CSF have been found to be higher in mild and moderate AD patients as rated by the Clinical Dementia Rating Scale (CDR) when compared with severe cases and healthy controls (74). The mild to moderate AD group received mean Mini-Mental State Examination (MMSE) scores of 17±1 whereas the advanced group (CDR>3) had a mean MMSE score of 4±1. Higher CSF S100β was observed in patients with frontotemporal dementia (FTLD) and AD compared with both healthy controls and subjects with inflammatory diseases including MS (73). This study found a non-significant increase in CSF S100β levels with age and no link between severity of disease or disease onset. Overall, these results indicate a possible association between elevated CSF S100β, the onset of neuropsychological symptoms associated with AD and the presence of neuropathophysiological changes in the early stages of AD.
In contrast to the relatively consistent findings from studies of S100β levels in CSF, studies measuring serum S100β levels in individuals with AD have reported conflicting results. A study in 2010 by Chaves et al. (75) identified lower serum S100β levels in individuals with AD compared with healthy controls. On a three point CDR scale, serum S100β was lowest in mild AD and increased respectively in moderate and severe cases, findings that directly oppose those by Peskind et al. (74) in the CSF in 2001. In addition, a recent study by Bolayirli et al., (76) reported increased serum S100β in individuals with AD compared with controls. This increase was not observed in individuals with AD taking cholinesterase inhibitors (prevents acetylcholine breakdown in the brain), nor was it increased in individuals with diabetes alone. However, serum S100β was elevated in individuals with both AD and type 2 diabetes mellitus (T2DM) taking oral anti-diabetic agents (sulphonylurea). The difference in findings in the CSF and serum have been postulated to be related to the kinetics of the BBB in regulating the difference between intracranial and peripheral S100β (41). It has also been proposed that S100β is produced at low levels to protect against damage by Aβ but increases and contributes to neuronal damage as AD advances (80). In healthy older adults, serum S100β is related to cognitive performance supporting a beneficial role for S100β at normal physiological concentrations (0.24µg/L) (81). These results potentially suggest a link between elevated S100β in the CSF and the severity of AD; however, more investigation is required to determine the significance of serum S100β levels as they apply to AD.
<table>
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<th>Reference</th>
<th>Fluid</th>
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<th>S100β Finding</th>
<th>Significance</th>
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<tbody>
<tr>
<td>Petzold et al., 2002 (72)</td>
<td>CSF</td>
<td>Evaluate the clinical and/or pathological relationship between CSF S100β and brain atrophy.</td>
<td>CSF retrieved from individuals with AD (n=31, gender=15F&amp;16M, age=57±15.2), FTLD (n=36, gender=11F&amp;25M, age=60.1±7.1) and controls (n=49, gender=34F&amp;15M, age=57±8.9). MMSE and MRI was performed on all participants.</td>
<td>CSF S100β was higher in both individuals with AD (0.4ug/L, p&lt;0.001) and FTLD (0.42ug/L, p&lt;0.001) compared with healthy controls (0.25ug/L). In individuals with AD only, S100β correlated negatively with normalised brain volume (R²=-0.53, p&lt;0.001).</td>
<td>CSF S100β is increased in AD and FTLD, and increased S100β correlates with a decrease in brain volume in individuals with AD.</td>
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<tr>
<td>Green et al., 1997 (73)</td>
<td>CSF</td>
<td>Investigate whether elevated CSF S100β is found in AD and FTLD.</td>
<td>CSF collected from individuals with clinically-confirmed AD (n=16, gender=7F&amp;9M, age=60±20y) were retrospectively selected and compared with the CSF of healthy (n=19, age=26-78y) and inflammatory controls (n=29). MMSE was administered at the time of sampling and AD diagnosis.</td>
<td>CSF S100β was higher in individuals with AD (0.33ug/L, p&lt;0.05) and FTLD (0.49ug/L, p&lt;0.001) compared with control (0.24ug/L) and inflammatory controls (0.19ug/L). There was no correlation between CSF S100β and age at disease onset (R²=0.006) or MMSE scores (R²=0.12).</td>
<td>CSF S100β is increased in AD and FTLD and may reflect the degree of astrocytosis in some patients.</td>
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<tr>
<td>Peskind et al., 2001 (74)</td>
<td>CSF</td>
<td>Evaluate expression of CSF S100β in AD.</td>
<td>CSF collected from individuals with AD (n=68, gender=18F&amp;50M, age=69±3y) and healthy elderly (n=25, gender=11F&amp;14M, age=66±3y) and young subjects (n=63, gender=63M, age=26±3y). All subjects also undertook the MMSE and CDR.</td>
<td>CSF S100β was not significantly higher in AD (0.98ug/L) compared with healthy elderly (0.81ug/L, p=0.3). CSF S100β was significantly elevated in individuals with mild/moderate AD (1.17ug/L, p&lt;0.05) compared with healthy elderly and individuals with advanced AD (0.6ug/L). There was a significant positive association between CSF S100β and MMSE scores (r=0.322, p=0.05). CSF S100β was not significantly higher in the healthy elderly compared with healthy young subjects (0.61ug/L, p&gt;0.05).</td>
<td>CSF S100β is elevated in the mild/moderate stages of AD as measured by the CDR suggesting a link to increased S100β during the early onset of AD symptoms.</td>
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<tr>
<td>Chaves et al., 2010 (75)</td>
<td>Serum</td>
<td>Evaluate serum S100β and NSE levels in AD.</td>
<td>Serum from individuals with AD (n=54, gender=36F&amp;18M, age=77.1±5.7y) &amp; community-dwelling elderly (n=66, gender=46F&amp;20M, age=76.5±5.4y) was obtained and severity of AD assessed by CDR, MMSE and MRI.</td>
<td>Serum S100β was lower in AD group (0.08ug/L) compared with control group (0.21ug/L, p=0.008). In AD group, S100β positively correlated with CDR scores (rho=0.269, p=0.049) and negatively correlated with MMSE scores (rho=-0.33, p=0.048).</td>
<td>Levels of serum S100β may be lower in individuals with AD compared with controls, especially in mild cases of AD.</td>
</tr>
<tr>
<td>Bolayrli et al., 2016 (76)</td>
<td>Serum</td>
<td>Compare markers of oxidative stress and neurological markers in the relationship between AD and DM.</td>
<td>Participants (n=225) were assigned to one of 7 groups: control (n=25, gender=12F&amp;13M, age=73.1±10.6y), AD (n=30, gender=15F&amp;15M, age=73.2±10.2y), AD-CEI (n=55, gender=32F&amp;23M, age=72.4±9.6y), DM (n=25, gender=13F&amp;12M, age=70.5±15.5y), DM-OAD (n=30, gender 16F&amp;14M, age=72.5±14.2y), AD-CEI+DM (n=25, 13F&amp;12M, age=70.9±10.3y) and DM-OAD+AD (n=35, gender=18F&amp;17M, age=75.3±10.1y).</td>
<td>Serum S100β was higher in AD group compared with control (p&lt;0.05) and AD-CEI+DM, DM, AD+CEI groups (All p&lt;0.001). Serum S100β also negatively correlated with MMSE scores (p&lt;0.05).</td>
<td>Increased serum S100β may be present in individuals with AD. CEI medication may normalise serum S100β levels, but this is not observed in individuals with AD and DM using OAD medication.</td>
</tr>
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</table>

Key: CSF, cerebrospinal fluid; AD, Alzheimer’s disease; FTLD, frontotemporal lobe dementia; MMSE, Mini-mental state examination; CDR, clinical dementia rating; NSE, neuron-specific enolase; MRI, magnetic resonance imaging; DM, diabetes mellitus; CEI, cholinesterase inhibitor; OAD, oral anti-diabetic (sulfonyl urea).
**In vitro and Animal Models**

*In vitro*, overexpression of S100β by activated astrocytes resulted in an increase in Aβ Precursor Protein (β-APP) and formation of dystrophic neurites in the plaque of AD diagnosed human temporal lobe brain tissue (82). S100β was highly expressed by reactive astrocytes near deposits of Aβ which was shown to reduce neuronal survival (83, 84). The addition of S100β (10 and 100ng/ml) was shown to directly increase levels of β-APP and β-APP mRNA expression in a dose-dependent manner that can potentially result in increased Aβ production and deposition (85). Interleukin-1β (IL-1β), like S100β, was also present in and near plaque and may have contributed to increased levels of APP mRNA (86). In retinal ganglion cells, injection of IL-1β and Aβ1-42 resulted in reduced APP immunoreactivity, whereas S100β appeared to increase APP levels (86).

Mice engineered to either overexpress or knockout S100β have provided behavioural and pathological insights into the relationship between S100β and AD (Table 2) (83, 87-93). When compared with non-transgenic controls, transgenic mice with multiple copies of the S100β gene exhibit impairments in memory and learning in behavioural tests that assess cognitive function in animal models (87). Transgenic S100β mice also performed worse in a water maze learning task at 12 weeks (88) and displayed behavioural patterns associated with hippocampal dysfunction (89). Additionally, hyperactivity was observed in female transgenic S100β mice suggesting possible sex-specific differences (89). On the other hand, mutant S100β knockout mice developed normally with no detectable abnormalities in the brain (90). These mice displayed enhanced spatial memory, greater associative emotional memory and strengthened synaptic plasticity by way of enhanced hippocampal long-term potentiation. These animal findings suggest a role for over-expression of S100β in behavioural AD symptomology and possible cognitive enhancements when S100β is not present.

Transgenic (Tg2576) mice overexpressing human S100β crossed with an AD mouse model displayed increased brain parenchymal and cerebral vascular Aβ deposits and greater Aβ overall (83). Additionally, over-expressing S100β mice exhibited hyperphosphorylated tau structures (91) and at one year of age, have a significant loss of dendrites when compared with controls, suggesting the presence of neurofibrillary tangles and cytoskeletal collapse (88). S100β is involved in the development of serotonin terminals, but overexpression can lead to decreased serotonin innervation and a loss of terminals in the hippocampus (91). Mice also presented neuroinflammatory changes that are observed in AD including reduced quantity of mature, stable astroglial cells, greater activated microglial cells and increased microglial expression of RAGE receptors (91). Additionally, mice overexpressing S100β also reported increased reactive astrocytosis and microgliosis and exacerbated pro-inflammatory cytokines TNF-α, IL-1β and IL-6 prior to Aβ deposition (83). Furthermore, it was suggested that S100β overexpressing mice undergo synaptic remodelling by inhibiting phosphorylation of growth associated protein-43 (92), as well as experiencing reduced hippocampal cell integrity (92). One study has found that chow containing Vitamin E (1000IU/kg) led to an increase in microglial activation and upregulation of RAGE expression suggesting that neuroinflammatory processes are affected by reduced oxidation activity when S100β levels are elevated (93).
<table>
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<tr>
<td>Winocur et al., 2001 (87)</td>
<td>Assess changes in behaviour and cognition in transgenic S100β mice model.</td>
<td>CD-1 mice (6-8mth) with 70 copies of the human S100β gene were assigned to the RAM, SNMTS and NSNMTS behavioural tasks and compared with controls.</td>
<td>Transgenic S100β mice made more errors (p&lt;0.00001), responded incorrectly (p&lt;0.00001) and showed clear deficit (p&lt;0.0001) compared with controls, in the RAM, SNMTS and NSNMTS, respectively.</td>
<td>Mice exhibited general learning or memory impairment on all tasks typical of both hippocampal and non-hippocampal dysfunction.</td>
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<tr>
<td>Whitaker-Azmita et al., 1997 (88)</td>
<td>Determine the impact of S100β on the neuronal cytoskeleton by examination of the MAP-2 protein in transgenic S100β mice.</td>
<td>CD-1 mice with 70 copies of the human S100β gene were selected at either 5w or 1y. Mice completed a behavioural water task for 5d and were analysed.</td>
<td>Young control mice outperformed both young and old transgenic S100β mice (p&lt;0.01). Transgenic mice displayed greater loss of dendrites and cytoskeletal collapse with age.</td>
<td>Over-expression of S100β may result in aberrant cytoskeletal morphology and loss of dendrites during ageing.</td>
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<tr>
<td>Gerlai et al., 1995 (89)</td>
<td>Analyse behavioural changes and presence of brain dysfunction in transgenic S100β mice.</td>
<td>CD-1 mice with 110, 70, 10 and 8 copies of the S100β gene were age-matched with controls, and all undertook exploratory behavioural testing.</td>
<td>Transgenic S100β mice displayed hyperactivity (females only, p&lt;0.001), lack of habituation to novelty (p&lt;0.0001) and reduced T-maze spontaneous alternation rate (p&lt;0.005).</td>
<td>Over-expression of S100β may result in abnormalities in exploratory behaviour in novel situations as commonly observed in hippocampal dysfunction.</td>
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<td>Nishiyama et al., 2001 (90)</td>
<td>Determine the effect of S100β as a glial modulator of neuronal synaptic plasticity.</td>
<td>C57BL/6J S100β-Null mice were generated, and males undertook behavioural testing at 3-6 mth of age in a blinded manner.</td>
<td>No anatomical abnormalities were observed in S100β-Null mice up to 18mth of age. S100β-Null mice were enhanced in synaptic plasticity, long-term potentiation, spatial memory, and fear memory compared to controls (All p&lt;0.05).</td>
<td>The absence of S100β did not prevent mice from developing normally, and lower S100β may enhance brain processing through modulation of glial-neuronal interactions.</td>
</tr>
<tr>
<td>Mori et al., 2010 (83)</td>
<td>Evaluate the impact of S100β over-expression on AD-like pathology.</td>
<td>AD model of Tg2576 mice was crossbred with Tghu S100β mice to over-express human S100β and analysed compared to CD-1 controls.</td>
<td>Double transgenic mice had: increased Aβ burden and Aβ deposits, augmented reactive astrocitosis and microgliosis, increased S100β expression and increased pro-inflammatory cytokines (All p&lt;0.001).</td>
<td>Over-expression of S100β acts to accelerate AD-like pathology and may promote amyloidogenic APP processing in addition to supporting brain inflammation processes.</td>
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<tr>
<td>Reference</td>
<td>Study Description</td>
<td>Findings</td>
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<td>Shapiro et al., 2010 (91)</td>
<td>Examine the role of S100β in the development and plasticity of the serotonergic neurotransmitter system. CD-1 transgenic S100β mice (male) were analysed at 10w and 28w and compared to CD-1 controls. Transgenic S100β mice present with increased serotonergic fibres in the hippocampus at 10w but an accelerated loss at 28w (p&lt;0.05) but no alterations in the raphe nucleus, similar observations were noted in humans with AD. As transgenic S100β mice age, there is increased numbers of activated microglial cells and RAGE expression plus the eventual appearance of hyperphosphorylated tau structures.</td>
<td>Over-expression of S100β may result in a loss of serotonin neuroplasticity in the hippocampus as well as neuroinflammatory changes commonly observed in AD.</td>
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<tr>
<td>Shapiro et al., 2004 (92)</td>
<td>Examine effects of S100β over-expression on the neuronal cytoskeleton. CD-1 transgenic S100β mice were analysed at 70d or 200d and compared to CD-1 controls. Young transgenic S100β mice exhibited greater MAP-2-immunoreactivity in numerous regions of the hippocampus compared to control mice in the hilus (p=0.025), infrapyramidal blade (p=0.030), supra pyramidal blade (p=0.033) and CA1 stratum radiatum (p=0.010). Older transgenic S100β mice had greater GAP-43 staining than controls in the supra pyramidal blade (p=0.050) and CA1 stratum oriens (p=0.034).</td>
<td>Chronic over-expression to S100β negatively impacts the hippocampal cellular integrity and suggests regulation of synaptic remodelling but may have a supportive effect earlier in life.</td>
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<tr>
<td>Bialowas-McGoey et al., 2008 (93)</td>
<td>Determine the effect of vitamin E on the oxidation rate of S100β. CD-1 mice (male) were assigned to control, transgenic S100β control, CD-1 + Vitamin E and transgenic S100β + Vitamin E groups for 4w. In transgenic S100β mice, Vitamin E rich diet increased RAGE expression (p&lt;0.015) and resulted in large increases in microglial activation (p&lt;0.02).</td>
<td>Vitamin E increased microglial activation and RAGE expression in over-expressing S100β mice. It is hypothesised that antioxidant activity may interrupt the neurotrophic properties of elevated S100β.</td>
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Key: RAM, radial arm maze; SNMTS, spatial non-matching-to-sample; NSNMTS, non-spatial non-matching-to-sample; MAP-2, microtubule-associated protein 2; AD, Alzheimer’s disease; Aβ, β-Amyloid; APP, amyloid precursor protein; RAGE, receptor for advanced glycation end products; GAP-43, growth associated protein 43
S100β as a biomarker of Blood-Brain Barrier (BBB) permeability

The BBB is a selectively permeable barrier that limits plasma components, red blood cells, and leukocytes from entering the brain (94). Its responsibilities include the delivery of essential nutrients, removal of surplus substances from the brain and the prevention of neurotoxin entry to the brain (37). The integrity of the BBB is compromised with the increase in permeability during numerous conditions including ischemic injury, head trauma and AD (94). The S100β has been shown to be raised during periods of increased BBB permeability leading to its use as a marker of BBB dysfunction (42, 95, 96). A 2002 study by Kapural et al. (42), used mannitol to produce a transient opening of the BBB in patients with lymphoma without inducing neuronal damage. Subjects underwent iatrogenic BBB disruption by intra-arterial mannitol infusion before infusion treatment of methotrexate. This led to increased serum S100β after both mannitol and methotrexate infusion which remained elevated during recovery. Elevations in CSF S100 proteins have long been considered a marker of active cell injury in the CNS (97), yet the interpretation of serum S100β levels is not clearly defined. For example, a study by Marchi et al. (96) in 2004, using mannitol to disrupt the BBB reported that when the BBB was intact, increased CSF S100β did not result in changes in serum S100β levels. Furthermore, a study in 2010 by Kleindienst et al. (98) compared S100β fluctuation from the CSF across the BBB with another recognised marker of BBB permeability known as the albumin quotient (albumin$_{\text{CSF}}$/albumin$_{\text{serum}}$ quotient; Q$_{\Lambda}$) in patients with TBI and subarachnoid haemorrhage. Although high CSF S100β was associated with injury outcome, this was not consistent with serum S100β levels even with high BBB permeability as determined by Q$_{\Lambda}$, possibly due to the congregation of S100β at the area of insult. Also in 2010, Pham et al. (99) used induced intra-arterial mannitol BBB disruption in 200 subjects and western blot tissue analysis to conclude extracranial sources of S100β are not robust enough to affect serum levels. It has also been suggested that S100β should be collected at regular time intervals to account for individual variance and that there is a relative delay in the peak of serum S100β compared to CSF S100β (100). Mathematical modelling has been proposed to interpret S100β results to assess the likelihood of future neuronal damage (96). Overall, there is no doubt that there is a fluctuation of S100β across the BBB but to what extent, is still a matter of some debate and there is not yet a consensus regarding the validity of serum S100β as a biomarker of BBB disruption.

Investigating the link between BBB permeability and AD is challenging due to the time it takes for the pathophysiological features to become apparent. Relatively recently, BBB leakage was associated with cognitive decline and dementia in patients with early AD based on magnetic resonance imaging (MRI) and local plasma volume (37). Patients with AD had higher BBB leakage rates compared with controls as well as decreasing MMSE scores with increasing leakage in the deep grey matter and cortex. Damage to the BBB is associated with AD pathophysiology including accumulation of Aβ, in part due to reduced clearance from the brain (101), and BBB dysfunction has been observed in MRI analysis of hippocampus in individuals with mild cognitive impairment (MCI) (102). Animal models have also investigated S100β as a marker of BBB in the presence of different dietary fat intake, finding an increase in S100β and Aβ deposition with a high saturated fat experimental diet (95). This study proposed that a dietary fat-induced increase in S100β may
influence AD risk by exacerbating peripheral delivery of Aβ to the brain due to greater BBB permeability. Despite this evidence, a recent multi-pronged investigation conducted in 2015, found a lack of BBB permeability in multiple preclinical mouse models of AD, as well as an absence of an association between AD and BBB impairment from brain infarcts in post-mortem human brains (103). Also, no differences were measured in the BBB permeability of IgG in both Apolipoprotein E ε4 (APOE4) mice and Apolipoprotein E (APOE) knockout mice (103). APOE4 is acknowledged a major genetic risk factor associated with AD (104) and other animal models have reported that APOE4 negatively affects BBB function (105, 106).

**APOE genotype as a modifier of S100β**

APOE is present in three isoforms (epsilon 2, 3 and 4), differing by the amino acids present in positions 112 and 158 and has numerous roles in the brain including lipid transport and support of the neuronal function (107, 108). However, its effects differ in an isoform-dependent manner with the APOE4 allele recognised as an established risk factor for sporadic AD and lower the age of onset (109). The presence of one APOE4 allele is associated with 3.5-fold increase risk of AD while two copies are associated with approximately 10-fold increased risk (3). APOE4 is the least efficient isoform at clearing Aβ from the brain and contributes to increased deposition of Aβ plaque (110). APOE4 is also implicated in AD through processes involving synaptic plasticity, cholesterol metabolism and neuroinflammation (110, 111). Serum, plasma, and brain concentrations of APOE are lower in AD patients and significantly lower in APOE4 carriers (112). Lower plasma APOE levels are associated with smaller hippocampal size in AD patients, especially in APOE4 carriers (113). It has been hypothesised that increasing APOE concentration may offer protection against AD (111) and this is currently an area of focus for pharmaceutical development of APOE mimetics (114). APOE4 is also a risk factor for coronary heart disease (107, 115) and is a genetic indicator of reduced longevity (116).

There is very limited research linking APOE genotype and the effect of S100β in humans. A small prospective consecutive case study in patients with severe TBI found higher S100β in APOE4 carriers compared with non-carriers (117). The clearance of elevated serum S100β towards normal levels was slower in the APOE4 group and a non-significant trend towards worse clinical outcomes after three months in APOE4 carriers. Higher S100β levels and lower APOE concentrations have been observed in CSF following TBI (118), and APOE4 carriers have higher S100β levels than non-carriers following cardiac surgery (119). However, this association was not significant in patients undergoing non-cardiac surgery (56). Although this evidence is limited, it warrants strong consideration for testing of the APOE genotype when interpreting S100β levels, especially as it pertains to AD.

**S100β, diet and physical activity**

**S100β and dietary fat**

In observational studies, dietary fats, particularly polyunsaturated trans-fatty acids (TFA) and saturated fatty acids (SFA) have been linked to an increased risk of dementia and AD (120, 121). This risk may be
enhanced in APOE4 carriers due to poorer CVD risk markers and an association of higher low-density cholesterol with SFA intake in e4 carriers (122). However, to our knowledge, there is no evidence of associations between S100β, AD and dietary fats in humans. Nevertheless, in animal models, elevated S100β levels have been observed in mice consuming a high SFA diet. The study by Takechi et al. (95) assigned mice to groups consuming 40% of digestible energy from either polyunsaturated fatty acids (PUFA), monounsaturated fatty acids (MUFA), or SFA for three months. The PUFA-enriched diet included docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA) and oleic acid, while the SFA-enriched diet contained high palmitic and stearic acid. Serum S100β levels were increased by 80% in the SFA-fed mice compared with low-fat controls (p<0.01) while no significant increase was observed in the mice fed the PUFA and MUFA diets. The BBB leakage allowed for a large increase in delivery of plasma proteins to the brain including ApoB lipoproteins enriched with Aβ. These findings suggest that SFA may enhance peripheral delivery to the brain of circulating lipoprotein-Aβ thus contributing to the accumulation of Aβ. A study by Pallebage-Gamarallage et al. (123) tested a DHA-enriched PUFA diet in mice to determine if BBB integrity could be restored following three months of the SFA-enriched diet. The DHA-enriched PUFA diet was found to increase S100β levels, amplify parenchymal ApoB retention and increase total plasma cholesterol in comparison with the low-fat control. S100β concentrations in the low-fat control also increased, indicating considerable neuroinflammation and BBB damage that had already manifested beyond repair. Although these studies have isolated detrimental effects of a diet high in SFA compared with PUFA and MUFA, the experimental diets were also high in sucrose and casein protein, therefore, an interaction between SFA and other dietary components resulting in increased S100β and BBB dysfunction cannot be excluded. Several large reviews and meta-analyses have suggested that SFA has a neutral effect on human health (124-127) and substituting refined carbohydrate for SFA may increase the risk of cardiovascular disease (127). Western dietary patterns in humans are typically high in both SFA, and simple carbohydrate and associations with AD, BBB disruption, hippocampal dysfunction and cognitive impairment have been reported (29, 128, 129).

High fat, low carbohydrate ketogenic diet’s (KD) have been shown to affect S100β levels in mice. The KD results in the production of ketone bodies (KB) in the liver during fatty acid oxidation, with the predominant forms being beta-hydroxy-butyrate (BHB), acetoacetate and acetone. When undertaking a KD, KB becomes the primary source of energy for the brain replacing glucose as unlike triacylglycerides; they can cross the BBB and convert to acetyl-CoA, generating ATP. KB are capable of being metabolised by neurons, astrocytes, and oligodendrocytes as precursors for lipid synthesis crucial for myelination (130, 131). KB have been well described with respect to their therapeutic benefits in childhood epilepsy (132), yet the underlying mechanisms have not been completely elucidated. In vitro, the addition of BHB to astrocyte cultures resulted in an initial transient increase in S100β after 1 hour and a decrease after 24 hours (133). In rats, a six-week KD lead to a decline in S100β levels in the CSF of rats as well as a reduction in severity of experimentally induced seizures (134). The positive effects have been proposed to be due to a decrease in neuronal excitability (135) and the modulation of neurotransmitters and biogenic monoamines (136).
Similarly, an eight-week KD provided to mice, as well as an omega-3 enriched KD; both led to approximately a 1.0ng/mL reduction in CSF S100β compared with controls and no change in S100β concentration in the hippocampus and striatum (137). Both studies (134, 137) used experimental diets consisting of high amounts of lard, which is rich in SFA. However, a study analysing the hippocampal slices of rats fed a ketogenic diet found no significant difference in S100β after one or six weeks when compared with controls (138). The KD studies suggest there may be a mechanism by which the KD can potentially decrease or maintain S100β levels, while the study by Tachechi et al. (95) implicated the high fat, SFA-enriched diet in AD pathology and increased S100β. More research is warranted to investigate the dietary implications of these findings in AD models.

**Role of S100β in insulin resistance?**

Research on AD and insulin resistance, particularly the role of brain insulin resistance which has been notionally defined as “type 3 diabetes”, has recently been received with considerable interest (139-142). Insulin resistance results in impaired glucose metabolism and insulin signalling dysfunction in the brain which may precede or accompany cognitive decline associated with AD (7). Post-mortem analysis of brains from individuals clinically diagnosed with AD by Talbot et al. (143) suggested that brain insulin resistance is an early and common feature of AD as demonstrated by reduced insulin and insulin-like growth factor-1 (IGF-1) in the hippocampus. Markers of insulin resistance were also found to be increased from normal cases to cognitively impaired AD brains irrespective of diabetes or APOE4 status.

Two recent studies have investigated the action of S100β in vitro in relation to glucose and insulin. A study in 2016 by Wartchow et al. (66), suggested a relationship between S100β levels and the ability of the brain to metabolise glucose. At physiological levels, S100β decreased glucose uptake in C6 glioma cells and acute hippocampal slices via RAGE and mitogen-activated protein kinase (MAPK) activity. Administration of insulin resulted in increased secretion of S100β via PI3K signalling, thus further impairing glucose utilisation. Further in vitro investigations in rat muscle cells by Hosokawa et al. (144) in 2017, reported that S100β treatment impaired glycolysis and suppressed glucose utilisation, even in the absence of insulin. This may be a feature of disrupted glycolysis, and impaired brain glucose transport observed in AD brain hypometabolism (145). In addition, S100β secretion has been shown to be reduced in a high glucose medium, potentially affecting neuronal survival and activity (146). The link between fasting and S100β has also been explored in rats undertaking a 48 hour fast with a two-fold increased serum S100β (147). As insulin levels drop during fasting, it is suggested that S100β is released from adipocytes in the absence of food. However, no change was observed in the CSF S100β. Taken together, S100β may be involved in impaired glucose metabolism both in the brain and peripherally with implications for both brain function and obesity. These mechanisms pose interesting questions on the consequences of impaired brain insulin signalling and impaired brain energy metabolism as they pertain to chronic disruption of intra-cranial S100β levels.
Two human studies have investigated serum S100β and glucose metabolism. Healthy participants undertaking an oral glucose tolerance test had a serum S100β reduction of 20% after one-hour post glucose ingestion compared to baseline (148). An inverse relationship with serum insulin was also found suggesting that S100β decreases peripherally when insulin is high. This may be related to an inhibitory effect of insulin on S100β content in the adipocytes, and reduced S100β release by adipocytes of insulin injected rats compared with enhanced release in diabetic and starved rats (149). In another human study, serum S100β levels were investigated to determine if there is a link between BBB dysfunction and clinical diabetes. It was found that serum S100β levels were not different between type 1 diabetes and non-diabetic controls (150). However, T2D sufferers had significantly lower serum S100β levels compared with both non-diabetic controls and type 1 diabetics, possibly due to a link between the dysfunctional insulin action in T2D and S100β. Overall, it appears that there is preliminary evidence to suggest that there is a close relationship between S100β and energy metabolism. Future studies should consider analysis S100β (both in the CSF and serum) in individuals with AD, diabetes or both conditions to clarify this relationship.

Effect of botanicals on S100β levels

Numerous botanicals have been found to elicit effects on S100β levels in in vitro and animal models. The most abundant green tea constituent epigallocatechin gallate (EGCG) (151) sparked interest due to its neuroprotective effect on cognition and memory (152). In a streptozotocin (STZ) induced model of dementia, EGCG (10mg/kg/d for 4w) prevented an increase in hippocampal S100β levels in rats, as well improved cognitive performance in the Morris’ water maze (153). Similar studies using rutin (citrus bioflavonoid) and saffron (plant high in carotenoids) have revealed neuroprotective effects of these plant-derived bio-actives. Rutin (25 and 50mg/kg) protected neonatal mice from an increase in S100β levels following anaesthesia administration (154). Saffron administration (200mg/kg) also reduced S100β levels in rats exposed to the insecticide diazinon (155). The bitter melon extract has been shown to improve obesity-associated oxidative stress, and neuroinflammation in mice fed a high-fat experimental diet consisting of 58% of total energy from fatty acids (156). S100β levels were found to be lower in the high-fat diet with bitter melon compared with both controls and the high-fat diet alone. In another STZ model, silymarin (estimated 150mg/kg) has been shown to inhibit an increase in S100β levels and reduce the formation of advanced glycation end products (AGE) while improving markers of oxidative stress and inflammation (157).

In two studies using kainic acid (natural marine acid from seaweed) induced rat models of epilepsy, Cat’s claw (Uncaria rhynchophylla) (1g/kg) was found to attenuate increases in S100β compared with controls and reduced seizure frequency by increasing glial cell proliferation (158, 159). Resveratrol (15mg/kg), a non-flavanoid polyphenol (160), had a similar effect in a pentyleneetrazol-induced seizure model of epilepsy, including reduced CSF S100β compared with controls and activated SIRT1 (161). Incubation of rat astrocyte cells and C6 glioma cultures with resveratrol was shown to increase extracellular S100β in a concentration-dependent manner (162). In models of peripheral nerve injury, S100β is
upregulated, especially at the point of damage. The flavonol Quercetin (25µmol/kg), increased tissue S100β levels at 12 hours and decreased serum S100β after 24 hours in rats with thoracic spinal cord compression injury (163). Use of natto (fermented soybean) increased tissue S100β levels and suppressed inflammatory markers in rats with sciatic nerve injury (164). In in vitro assays, quercetin and catechin were shown to inhibit S100β mediated inflammatory expression of MCP-1 mRNA in human THP-1 monocytic cells by regulating MAPK signaling (165). In vitro, cinnamon polyphenols attenuated oxidative stress in C6 glioma rat cells (166) and enhanced the expression of SIRT1. SIRT1 has been rigorously studied for its neuroprotective effects in brain injury and AD. The findings of this review suggest that certain botanicals are associated with positive effects on S100β levels. However, how this research translates to human studies is still unknown.

**The impact of physical activity on S100β levels**

Participation in physical activity has been identified as a modifiable risk factor of AD risk (31) and is an effective strategy to improve cognitive function in older adults (167). Exercise has been shown to be beneficial relative to S100β levels in a murine model of chemically induced neurodegeneration (168). The mice completed four weeks of treadmill exercise with a reduction in S100β levels compared to sedentary mice. In humans, increased S100β levels have been extensively studied as a marker of BBB disruption following exercise. In a 2014 systematic review by Koh et al. (169), S100β levels increased in 15 of 23 included studies from pre- to post-exercise across a variety of physical activities. The authors attributed the rise in S100β levels to an increase in BBB permeability resulting from exercise-related trauma to the head. While several of the included studies apply to sports involving physical contact (170-173), and as such do not apply to older individuals at risk of AD, other studies have found evidence of increased S100β in activities that may be undertaken by older adults. S100β levels were found to increase following running possibly due to repeated striking of the ground causing repeated subtle head trauma (173). Increased S100β levels have been observed following swimming (174), and higher levels correlate with reduced cognitive performance during high altitude physical activity (175). These studies indicate that intense exercise in younger individuals can increase S100β, although supervised exercise at moderate intensities may be best in older individuals to improve cognitive function (167).

Exercise, together with body weight may also influence S100β levels. In untrained obese individuals, serum S100β levels are higher after 20 min of continuous submaximal aerobic exercise compared to before exercise, as well as both before and after compared with healthy controls (176). This study indicates that the increase of S100β during exercise is possibly related to its release from adipocytes, This may be due to an individual’s body mass index (BMI), however, there is conflicting evidence surrounding whether bodyweight and extra-cranial S100β are related (99, 177-180). Further animal studies and research in older and elderly individuals may identify whether S100β holds trophic or neurotoxic properties dependent on exercise intensity and whether this impacts cognitive function.
Discussion

The current review has identified considerable evidence surrounding the interaction between S100β levels and AD in animal models and in vitro. The research in humans has determined an association between elevated CSF S100β and AD severity. However, more studies are required to investigate the usefulness of serum S100β as a less invasive biomarker of AD status. The impact of extracranial sources of S100β on CSF and serum levels is becoming better understood, yet the fluctuations across the BBB relative to dietary intake and physical activity have not been tested in humans to our knowledge. We have identified altered S100β levels in animal models dependent on the fatty acid content in the diet that provide the basis for hypothesis testing of the MD and KD to examine their effects on AD pathology including BBB permeability. In addition, the current research suggests positive implications of these diets pertaining to S100β levels and insulin signalling that may support the use of these dietary strategies.

Mediterranean diet

The MD has been established as of benefit to AD sufferers (17, 19, 24, 181) and this review has identified possible links involving S100β expression. In 2010, Takechi et al. (95) described mechanisms that may implicate SFA in AD in their animal trial. No association between MUFA and PUFA consumption was reported. Hence, dietary patterns higher in MUFA and PUFA such as the MD may offer neuroprotection. An increase in the consumption of omega 3 PUFA, particularly DHA and EPA, is associated with reduced mortality risk (182) and is considered an important component of the MD and traditional Japanese diet. However, in 2012, Pallebage-Gamarallage et al., (123) demonstrated that DHA worsened BBB disruption following the high SFA diet in mice, suggesting that DHA may oxidise in the presence of neuroinflammation. Further, in 2013, Vizuete et al. (137), identified no additional benefit of both DHA and EPA omega 3 PUFA during a KD in mice. The effects of omega 3 PUFA in reducing AD risk as a preventative measure have become more evident (183, 184); however, findings on its effect on S100β levels are still inconclusive.

The current review has identified numerous botanicals, which are shown to have a positive influence on S100β levels in vitro and in animal models. Freshly grown plant foods are an integral part of the MD and other healthy dietary patterns (25), but the exact mechanisms by which they exert their benefits are not completely understood, especially as it pertains to brain health. The MD is known to possess potent antioxidant properties (185). However, in mice overexpressing S100β, vitamin E has been shown to selectively increase microglial activation and RAGE expression (93). Vitamin E supplementation has been tested in a three-year trial of individuals with probable or possible AD with no benefit compared to placebo (186). Emphasis on the overall dietary pattern when assessing S100β levels could prove valuable, yet determining the efficacy of specific novel botanicals is still warranted. For example, it has been recently found that the plant bioactive curcumin elevates enzymes in the liver that are responsible for the synthesis of DHA, possibly leading to elevated levels in the brain (187). Future research should consider monitoring
changes in S100β and BBB permeability during investigations of the beneficial effects of botanicals in the context of neurodegenerative conditions such as AD.

**Ketogenic diet**

Brain insulin resistance is a common feature in AD and may result in increased secretion of S100β from glial cells in response to dysfunctional insulin signalling and glucose metabolism (66). As CSF S100β may be increased in AD, and the KD appears to reduce CSF S100β in mice, it is possible that a ketogenic diet (KD) or low carbohydrate diet may promote healthy brain insulin signalling by means of limiting chronic elevation of S100β. Several reviews have discussed the potential application of the KD and KB in the treatment and prevention of neurodegenerative conditions including AD, through the management of dysfunctional insulin signalling and glucose metabolism (140, 188-191). The reduced S100β levels observed in mice consuming a KD (134, 137) is partly attributed to the production of BHB that can cross the BBB and provide energy to the brain as a safe alternative in the presence of impaired glucose metabolism (192). In addition, a KD in mice has also been shown to lower Aβ levels (193). Infusion of BHB in vitro supports a beneficial role in AD pathophysiology including improvements in neuronal cell survival, neurite growth (194) and reduced S100β in astrocyte cultures (133). It must be considered that the benefits of a KD may be due to the absence of high glycaemic carbohydrate foods that are commonly consumed as part of a Western dietary pattern (195). The increased S100β and AD pathology in the study by Takechi et al. (95) may be related to an interaction between SFA and the carbohydrate in the experimental diet. As the KD diet appears to reduce S100β levels in mice (134, 137), despite also containing SFA (from lard), an interaction between SFA and the carbohydrate used in the experimental diet may be inferred. Interestingly, an animal study reported improved physical and cognitive performance in mice consuming a 30% ketone enriched diet and 39% carbohydrate for five days when compared to mice consuming isocaloric high carbohydrate and western diets (196). Ketone salt and ester supplementation is a novel area of research, and short-term studies have reported positive effects on cognition in humans and mice possibly due to improved insulin sensitivity (145, 196-200). Additional research in ketogenic protocols for the treatment of chronic and a neurodegenerative disease is warranted to explore its application as a preventative measure for AD. Measurement of S100β levels in such studies may provide insight into the potential benefits of the KD.

**Individualised dietary considerations for APOE4 carriers**

APOE4 genotype has been linked to BBB dysfunction, and the presence of APOE4 may exacerbate the deleterious effects of high S100β levels (106). Greater adherence to a Mediterranean-style diet by APOE4 carriers (n=148, age=68.4±6.1y) has been found with better cognitive performance when compared with a Western Diet over a period of 36 months (201). Adherence to a MD may also prevent cortical thinning in APOE4 carriers (181), and weekly seafood consumption has also been reported with lower AD pathology and slower rates of cognitive decline (202). A 2017 review by Yassine et al. (203) recommends supplementation of DHA as an AD preventative strategy in APOE4 carriers based on current evidence. A 2016 study found a reduction in hippocampal APOE concentration in mice fed a high-fat Western diet, but
normal levels in mice fed a KD (204). In this study plasma APOE was significantly increased in APOE4 mice fed the KD. Reduced APOE concentration is found in AD, and ongoing research is likely to discover APOE mimetics to promote higher levels, particularly for APOE4 carriers. APOE4 carriers may have a genetic disposition to increase fatty acid mobilisation and utilisation when consuming large amounts of dietary fat (205). Cognitive performance has been shown to improve in APOE4 carriers following a high-fat meal compared to a low fat, protein matched meal (206). Despite this, higher intake of overall calories is associated with increased risk of AD in APOE4 carriers (207). Determining the association between S100β and APOE genotype may provide valuable insight into AD risk especially as it applies to BBB disruption and preventative dietary strategies in at-risk AD individuals.

**Future Directions**

Large increases in S100β levels are strongly associated with poorer outcomes in individuals with brain injury, but the significance of slight variations from normal levels remain uncertain, due in part to S100β’s neurotrophic properties. While serum S100β levels appear to be age dependent, with marginal increases in levels occurring in older adults (208), additional research is required to determine the relationship between S100β levels and AD. High levels of S100β in the CSF appear to associated with poorer outcomes in AD (72, 74), yet the still undefined transition of S100β across the BBB as well as the presence of extracraniol sources of S100β, brings the validity of S100β as a blood-based biomarker into question. Relatively normal, serum S100β levels at 0.24µg/L was found to be positively associated with cognitive function in healthy older individuals (81), but a comparison with CSF was not conducted, and a future study investigating this relationship is desirable. It has also been suggested that serum S100β decreases during the early stages of AD (75, 80), perhaps inverse of CSF S100β. The *in vitro* research has implicated the involvement of S100β in AD pathology including increased expression of β-APP and neurotoxic cytokines (82, 84, 85, 88, 91), while animal models have reported impaired cognitive performance with over-expression of S100β (83, 87, 89). Additional research is required to identify the relationships in human studies further before S100β can be considered as a biomarker for AD. Future studies investigating the ability of the MD and KD to manage blood glucose levels and promote normal insulin signalling in AD are warranted. Insulin resistance is associated with AD, independent of APOE genotype (209). High blood glucose is known to be an independent risk factor for the onset and development of dementia (210, 211) and individuals with AD also have lower CSF insulin and higher plasma insulin compared to healthy controls (212). The mechanisms by which high blood glucose levels impart adverse effects in the brain are not entirely known and represent a major area of current research. Treatment with intranasal insulin provides direct access to the CNS that has been shown to be a promising therapeutic modality in AD and MCI (213), which may be moderated by APOE (214). Consideration of the role of S100β in these relationships is warranted as antidiabetic medication has been shown to attenuate increases in S100β in AD (76), linking S100β to impaired glucose metabolism in humans. The production of the insulin-degrading enzyme in the breakdown of Aβ and binding of insulin has been discussed as a key pathway to prevent AD (139), and *in vitro* studies may be designed to further determine a possible role of S100β in this process. Further elucidation of the mechanisms behind the
changes in S100β levels, including how BBB permeability responds to the MD and KD, and why serum S100β differs in type 1 and type 2 diabetes, may provide insight into the prevention of AD. Importantly, the fluctuations of S100β in the prodromal stage of AD is worthy of further investigation.

Conclusion

To our knowledge, this is the first review of the potential interactions between S100β, diet and lifestyle changes in the context of AD. S100β has been proposed as an early detection biomarker for AD, but challenges remain surrounding its utility due to differences in S100β levels in the CSF and serum. This review paper highlights the available evidence in vitro, in animal models and in individuals with AD, thus implicating the involvement of S100β in AD pathophysiology. This evidence has revealed changes in S100β levels dependent on a variety of dietary factors, particularly dietary fatty acid composition and numerous botanicals. S100β has been shown to be involved in brain insulin resistance and studies have shown that S100β is influenced by physical activity. The overall findings support the movement towards individualised dietary recommendations involving fat and carbohydrate intake as a useful systematic and preventative measure for AD, particularly in carriers of the APOE4 allele. This review also describes a potential role for the MD and KD in promoting S100β levels that may encourage brain insulin function. Despite these findings, there are still significant limitations surrounding the interpretation of S100β and several unanswered questions regarding its role in AD. In conclusion, measurement of S100β in future clinical trials involving AD, diet and lifestyle factors is warranted to compliment ongoing in vitro research aimed at understanding the S100β mechanisms of action.
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