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Research Article

White Matter Lipids as a Ketogenic Fuel Supply in Aging Female Brain: Implications for Alzheimer's Disease

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ABSTRACT

White matter degeneration is a pathological hallmark of neurodegenerative diseases including Alzheimer's. Age remains the greatest risk factor for Alzheimer's and the prevalence of age-related late onset Alzheimer's is greatest in females. We investigated mechanisms underlying white matter degeneration in an animal model consistent with the sex at greatest Alzheimer's risk. Results of these analyses demonstrated decline in mitochondrial respiration, increased mitochondrial hydrogen peroxide production and cytosolic-phospholipase-A_2 sphingomyelinase pathway activation during female brain aging. Electron microscopic and lipidomic analyses confirmed myelin degeneration. An increase in fatty acids and mitochondrial fatty acid metabolism machinery was coincident with a rise in brain ketone bodies and decline in plasma ketone bodies. This mechanistic pathway and its chronologically phased activation, links mitochondrial dysfunction early in aging with later age development of white matter degeneration. The catabolism of myelin lipids to generate ketone bodies can be viewed as a systems level adaptive response to address brain fuel and energy demand. Elucidation of the initiating factors and the mechanistic pathway leading to white matter catabolism in the aging female brain provides potential therapeutic targets to prevent and treat demyelinating diseases such as Alzheimer's and multiple sclerosis. Targeting stages of disease and associated mechanisms will be critical.

1. Introduction

Age-related myelin breakdown occurs during normal aging and in major neurodegenerative diseases including Alzheimer's (Bartzokis, 2004; DeCarli et al., 1995; Erten-Lyons et al., 2013; Ge et al., 2002; Lu et al., 2013; Lebel et al., 2012; Zhang et al., 2007; Tang et al., 1997). White matter degeneration in Alzheimer's disease (AD) worsens with progression of disease and is predictive of cognitive decline (Bartzokis, 2004; DeCarli et al., 1995; Erten-Lyons et al., 2013; Ge et al., 2002; Lu et al., 2013; Lebel et al., 2012; Zhang et al., 2007; Tang et al., 1997). Brain regions that myelinate late in brain development and which are populated by small, thinly myelinated are most vulnerable to breakdown and degeneration in AD. Late-myelinating regions include cortical association areas such as fronto-parietal tracts, the genu of the corpus callosum (CC), the uncinate fasciculus, and the superior longitudinal fasciculus (Brickman et al., 2012; Marner et al., 2003). Further, the afferent targets of these fiber systems, which include the hippocampus and subiculum, also show white matter (WM) hyperintensities (Di Paola et al., 2010; Brickman et al., 2012; Marner et al., 2003). Many of these vulnerable fiber tracts, such as the superior longitudinal fasciculus, the CC, internal capsule, corona radiata, and parahippocampal WM also have a high degree of heritability (Sprooten et al., 2014; Jahanshad et al., 2013). Myelin breakdown manifests earlier in apolipoprotein ε4

Abbreviations: WM, white matter; AD, Alzheimer's disease; MTL, medial temporal lobe; H_2O_2, hydrogen peroxide; $cPLA_2$, cytosolic phospholipase A_2; CC, corpus callosum; APO-ε4, apolipoprotein ε4; FDG-PET, 2-[^{18}F]fluoro-2-deoxy-D-glucose; CMRglu, cerebral glucose metabolic rate; PCC, posterior cingulate; PDH, pyruvate dehydrogenase; COX, complex IV cytochrome c oxidase; ABAD, Aβ-binding-alcohol-dehydrogenase; BBB, blood brain barrier; MCT1, monocarboxylate transporter 1; WT, wild type; MIB, mitochondrial isolation buffer; OCR, oxygen consumption rate; RCR, respiratory control ratio; GFAP, glial fibrillary acidic protein; PEI, polyethyleneimine; TLDA, TaqMan low density array; PCR, polymerase chain reaction; MBP, myelin basic protein; HADHA, hydroxyacyl-CoA dehydrogenase; ABAD, Aβ-binding alcohol dehydrogenase; CPT1, carnitine palmitoyltransferase 1; PB, phosphate buffer; LC MS, liquid chromatography mass spectrometer; Cyp2j6, arachidonic acid epoxygenase; ACER3, alkaline ceramidase; MAG, myelin associated glycoprotein; MOG, myelin oligodendrocyte glycoprotein; Erbb3, Erb-B2 receptor tyrosine kinase 3; Cldn11, claudin 11; Olig2, oligodendrocyte transcription factor; NEFA, nonesterified fatty acids; S1P, sphingosine; DHA, docosahexaesnoic acid; ROS, reactive oxygen species; APP, amyloid precursor protein; BACE1, beta-secretase 1; HK, hexokinase.

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(APO-ε4) carriers, a major genetic risk factor for AD (Bartzokis et al., 2006). Regions most vulnerable to WM degeneration map onto regions preferentially affected in the pathological trajectory of AD (Bartzokis, 2004), suggesting a possible link between mechanistic pathways affected early in AD progression, and late stage WM degeneration and cognitive deficits.

The bioenergetic system of the brain is compromised early in the progression of AD and is evident during the prodromal (preclinical) stage of the disease (Mosconi, 2005; Mosconi et al., 2006, 2009b; Reiman et al., 1996, 2004; Yao et al., 2009, 2011b; Brinton et al., 2015; Moreira et al., 2006; Lin and Beal, 2006). Glucose hypometabolism (Mosconi, 2005; Mosconi et al., 2006, 2009b; Reiman et al., 1996, 2004) and a compensatory shift to an alternative fuel substrate, ketone bodies, have been established as a metabolic phenotype characteristic of the AD brain in both clinical and preclinical studies (Yao et al., 2009; Ding et al., 2013; Brinton et al., 2015). Reduction in cerebral metabolic rate of glucose utilization, particularly in the entorhinal cortex and hippocampus, correlate with cognitive deficits over time and most accurately predict future cognitive decline in normal individuals as well as conversion to mild cognitive impairment (de Leon et al., 2001; Herholz, 2010; Jagust et al., 2007; Mosconi, 2005). Multiple clinical studies have identified cerebral glucose hypometabolism in persons with AD as well as in those at increased risk for AD, such as APO-ε4 carriers and women (Mosconi, 2005; Mosconi et al., 2005, 2006, 2008a,b,c, 2009a,b, 2010, 2011; Reiman et al., 1996, 2004; Silverman et al., 2011; Herholz, 2010; Vlassenko et al., 2010; Brinton et al., 2015). Further, persons with incipient AD exhibit a utilization ratio of 2:1 glucose to alternative fuel, whereas comparably aged controls exhibit a ratio of 29:1, whereas young controls exclusively use glucose as with a ratio of 100:0 (Hoyer et al., 1991).

Loss of estrogenic control of glucose metabolism in brain during menopause can lead to decreased glucose utilization, diminished aerobic glycolysis and altered oxidative phosphorylation, which together generate a hypometabolic phenotype (Yao et al., 2009, 2010, 2012; Ding et al., 2013; Nilsen et al., 2007; Yin et al., 2015). Clinical positron emission tomography with 2-[^{18}F]fluoro-2-deoxy-D-glucose (FDG-PET) analyses indicate a significant decline in cerebral glucose metabolic rate (CMRglu) in the posterior cingulate (PCC) in postmenopausal women (Rasgon et al., 2005) and a decline in cognition during the menopausal transition (Weber et al., 2014; Weber et al., 2013). These clinical findings are recapitulated in studies from animal models of female endocrine aging and AD, which demonstrate a decline in mitochondrial bioenergetics and generalized shift from glycolytic energy production toward use of an alternative fuel, ketone bodies, during the transition to reproductive senescence and early in AD pathology progression (Yao et al., 2009, 2010, 2012; Nilsen et al., 2007). Development of Alzheimer's pathology is accompanied by a decrease in expression and activity of enzymes involved in mitochondrial bioenergetics and glucose metabolism, including pyruvate dehydrogenase (PDH) and complex IV cytochrome c oxidase (COX) (Blass et al., 2000; Federico et al., 2012; Gibson et al., 1998, 2005; Gibson and Huang, 2005; Yao et al., 2009, 2010, 2012; Nilsen et al., 2007). Mitochondrial dysfunction leads to decreased mitochondrial respiration, increased oxidative stress, and increased mitochondrial Aβ load and Aβ-binding-alcohol-dehydrogenase (ABAD) expression (Chou et al., 2011; Du et al., 2010; Yao et al., 2009). Basic science discovery analyses indicate that glucose hypometabolism and decline in bioenergetic capacity in brain is associated with generation of hallmark pathologies of AD (Yao et al., 2009, 2011b; Ding et al., 2013; Kadish et al., 2009; Blalock et al., 2003).

Under conditions of diminished glucose availability, the brain will progressively utilize circulating fatty acids as a ketogenic energy source (Morris, 2005; Guzman and Blazquez, 2004). Utilization of liver-derived ketone bodies by brain is well established under two conditions: during breast feeding of high lipid diet and during periods of starvation (Morris, 2005). During these states, the ketone bodies acetoacetate and β-hydroxybutyrate supply up to 60% of the human brain's energy requirements (Veech et al., 2001; Cahill, 2006). Ketone bodies derived from liver metabolism of lipids cross the blood brain barrier (BBB) through the monocarboxylic acid transporter (MCT1) (Ding et al., 2013; Morris, 2005). However, as with glucose transporters, MCT1 expression can decline with age and AD (Ding et al., 2013). A potential compensatory response to decline in peripherally derived ketone bodies is the utilization of brain-derived sources of fatty acids to generate ketone bodies (Yao et al., 2011b). Herein, we provide evidence from the aging female brain indicating that endogenous brain lipids can serve as a source of ketone bodies. Specifically, we provide a mechanistic pathway for myelin catabolism initiated by mitochondrial H_2O_2 activation of the cPLA$_2$-acid sphingomyelinase pathway that leads to loss of myelin integrity, lipid droplet accumulation, fatty acid metabolism, and ketone body generation.

2. Materials and Methods

2.1. Animals

Animal studies were performed following National Institutes of Health guidelines on use of laboratory animals; protocols were approved by the University of Southern California Institutional Animal Care and Use Committee. Five cohorts of female aged (3, 6, 9, 12, 15 and 18 months) wild type (WT) mice were used to investigate the H_2O_2 induced PLA$_2$-sphingomyelinase pathway for the catabolism of the myelin sheath. Female mice were used to interrogate the effects of the menopausal transition on WM degeneration in brain due to the translational comparability of the reproductively aging female rodent to the reproductively aging female human. Age changes in reproductive cycles begin relatively early in the lifespan of all mammals because of ovarian senescence (Finch et al., 1984). In lab rodents, cycle regularity and fertility decline begin after 6 months, the age of 'retired breeders'. The human perimenopause is characterized by the menopausal transition as a 'regularly irregular' process, with marked cycle-to-cycle variability (Prior, 1998; Santoro, 2005; Burger et al., 2007; Burger et al., 2008). This feature of the perimenopause is closely matched in laboratory rodents. Increasing cycle irregularity is characteristic of laboratory rodents of most genotypes after 8 months and presents a convenient model for the irregular cycles of human perimenopause (Yin et al. 2015). By age 12 months, some mice have fewer than the minimum 100 follicles required to maintain cyclicity (Gosden et al., 1983). Therefore four groups in varying stages of reproductive functionality were defined for this study: 3–6 month old mice were designated reproductively competent, 9 months reproductively irregular, 12 months reproductively incompetent and 15–18 months mice were termed aged.

The number of animals included in mitochondrial, genomic, electron microscopy and lipidomics analyses was determined based on the 95% chance to detect changes in 30–50% of animals. Variance in the number of animals per group was due to three variables: 1.) the number of mice available per age group in the mouse colony, 2.) technical mishap e.g. tubes cracking during ultra-centrifugation, and 3.) limitation in assay format e.g. number of wells per plate. Animal randomization was not possible because age was the variable determining group assignment. Mice within each age group were assigned a randomization number to ensure that analyses were conducted blind to age group.

2.2. Replicates

This program of research was designed to sequentially investigate activation of the mechanistic pathway underlying mitochondrial dysfunction to WM degeneration. Key aspects of the hypothesized mechanistic pathway leading to WM degeneration were confirmed i.e. replicated through multiple analytic strategies. For example, cPLA$_2$ activity was determined by enzyme activity assay, immunohistochemical detection and by arachidonic acid production.