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Effects of a high-fat, high-cholesterol diet on brain lipid profiles in apolipoprotein E ε3 and ε4 knock-in mice

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ABSTRACT

Apolipoprotein E (ApoE) is important in facilitating the transport of lipids (cholesterol, phospholipids, and sulfatides) and plays a fundamental role in normal lipid metabolism. High cholesterol levels increases the risk of developing Alzheimer's disease. In this study, we investigated the effects of a high-fat high cholesterol (HFHC) diet on brain lipid profiles in 95 young and aged APOE ε3 and ε4 knock-in mice to determine whether diet leads to altered brain levels of a number of glycerophospholipids, sphingolipids, cholesterol precursors, cholesterol, cholesterol oxidation products, and cholesterol esters. The results in this study revealed significant changes in lipid levels. The HFHC-enriched diet influenced the levels of cholesterol esters. A sharp increase in cholesterol ester levels, particularly in the aged APOE ε4 diet-enriched group, might be suggestive of abnormal acyl-coenzyme A:cholesterol acyltransferase 1 (ACAT) activity and/or levels. Age exerts appreciable effects on the brain lipidome, especially with regard to polar lipid species.

1. Introduction

Alzheimer's disease (AD) is a multifactorial neurodegenerative disease, characterized by a progressive decline in cognitive function and loss of memory (Strittmatter and Roses, 1996; Holtzman, 2002). Better comprehension of AD is urgently required, as dementia in elderly individuals will reach epidemic proportions in the very near future. Many risk factors had been postulated to be associated to the pathogenesis of AD including but not limited to: age, apolipoprotein E (APOE) ε4 alleles and high cholesterol levels. High cholesterol levels are associated mid-life with AD risk.

Apolipoprotein E (ApoE) is a protein involved in the transport of lipids such as cholesterol (Mahley, 1988). ApoE also plays a role in mediating phospholipid and extracellular cholesterol transport by means of lipoprotein particles (DeKroon et al., 2006). The human APOE gene exists as three major isoforms, namely ε2, ε3, and ε4 (Zannis et al., 1982), with allele frequencies in the Australian (Martins et al., 1995) and other white populations (Roses, 1996; Bales et al., 2002) of 7% to 8%, 78%, and 14% to 15%, respectively. APOE ε4 is strongly associated with familial and sporadic late-onset AD (LOAD) (Strittmatter and Roses, 1996). Possession of an APOE ε4 allele increases both the risk of cognitive impairment and the likelihood of developing AD. There is also a gene–dosage effect, such that individuals with two copies of APOE ε4 alleles have the earliest age of onset (Corder et al., 1993; Poirier and Davignon, 1993). Although the molecular mechanisms underlying how ApoE exerts risk of AD is not understood, there is some evidence to indicate that it plays a role in altering lipid metabolism in the brain. Thus, ApoE isoforms have been demonstrated to modulate and influence sulfatide content in brain tissues (Han et al., 2003). Both APOE ε4 (Bentley et al., 2002; Brendza

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et al., 2002; Deane et al., 2008; Hartmann, 2001; Holtzman, 2002; Hone et al., 2003; Manelli et al., 2004; Raber et al., 2004; Wisniewski et al., 1993) and high cholesterol levels (George et al., 2004; Poirier, 2003; Refolo et al., 2000; Shie et al., 2002; Sparks et al., 1994) have been linked to amyloid-β-peptide (Aβ) metabolism, a major hallmark of AD.

Previous studies by our laboratory have demonstrated subtle differences in the levels of lipids such as phosphatidylinositol, phosphatidylethanolamine, phosphatidylcholine, ceramides, and sphingomyelin between APOE ε4 and APOE ε3 KI mice, based solely on age (2 months vs. 12 months of age) and when fed a standard rodent diet (Sharman et al., 2010).

In this study, we investigated the effects of a high-fat, high-cholesterol (HFHC) diet on brain lipid profiles in young and aged APOE ε3 and ε4 knock-in mice to determine whether an HFHC diet would lead to altered brain levels of major lipid species including the following: phosphatidylethanolamine (PE), phosphatidylglycerol (PG), sphingomyelin (SM), phosphatidylinositol (PI), phosphatidic acid (PA), phosphatidylserine (PS), sulfatides (SL), phosphotidylcholine (PC), cholesterol, 24-hydroxylcholesterol, 7-hydroxylcholesterol, 7-ketocholesterol, cholesterol esters, and 14-demethyllanosterols.

2. Method

2.1. Animals

This study was conducted in accordance with the Australian code of practice for the care and use of animals for scientific purposes (National Health and Medical Research Council [NHMRC] 2004), and the experimental protocols were approved by the University of Western Australia Animal Ethics Committee. Male APOE knock-in mice, homozygous for human APOE ε3, and ε4 were originally obtained from Taconic (Germantown, NY) and a colony derived and maintained at the Animal Resources Centre (ARC, Perth, Western Australia). These mice have been described previously (Knouff et al., 1999; Piedrahita et al., 1992; Sullivan et al., 1997; Sullivan et al., 1998). The mice were weaned at 6 weeks of age onto their respective diets: normal rodent diet: SF08-020 AIN93M with extra added vitamins), or HFHC diet (SF08-033 modified AIN93M: 10% fat, 0.75% cholesterol, 0.3% cholate with additional vitamins from Glen Forrest Stockfeeders, WA, Australia) upon arrival at the Biomedical Research Facility (BRF, Shenton Park) until the completion of experiments, either at 18 to 19 weeks or 44 to 45 weeks of age. Food and water were accessible ad libitum. The animals were housed with littermates, and the maximum number housed in a cage was kept at 6 mice.

2.2. Assays performed on plasma samples

Whole-blood samples obtained via cardiac puncture were transferred into EDTA tubes and placed on ice. The tubes were then centrifuged at 2000 rpm for 10 minutes. The plasma layers were carefully removed, transferred into new Eppendorf tubes, and stored at −80 °C until further analysis. Cholesterol levels were measured using the cholesterol assay kit from the Cayman Chemical Company (Ann Arbor, MI), which was based on a fluorometric method of quantifying cholesterol. The levels of 5α−dihydrotestosterone (DHT) were measured using the Alpco diagnostics (Salem, NH) DHT kit.

2.3. Extraction of lipids from brain homogenates

One hemisphere of the mouse brain was homogenized in an equivalent of 10 times the hemispheric weight of PBS. Lipids were extracted using a modified Bligh and Dryer method (Bligh and Dyer, 1959). Briefly, a 900-μL aliquot of ice-cold chloroform−methanol (1:2) was added to 10 mg of homogenized brain tissue sample and vortexed for 15 seconds. These samples were then placed onto a thermomixer and incubated for l hour at 4 °C and agitated at 1200 rpm. After incubation, 350 μL of ice-cold water was added to break phase followed by 300 μL of ice-cold chloroform. The sample was vortexed for 15 seconds and incubated on ice for 1 minute, and this step was then repeated. The sample was then centrifuged at 9000 rpm for 2 minutes at 4 °C. The lower organic phase was transferred into a clean Eppendorf tube. An additional 500 μL of chloroform was added to the sample and vortexed for 15 seconds. These samples were placed onto the thermomixer for a second round of extraction. The samples were incubated for 30 minutes at 4 °C with 1200 rpm agitation and then centrifuged at 9000 rpm for 2 minutes at 4 °C. The lower organic phase obtained from the second round of extraction was pooled together with those obtained from the first round for each respective sample. The collected samples were split into 2 equal aliquots, dried using Speed-Valco (Thermo Savant, Milford, CT) and kept at −80 °C until needed for liquid chromatography−mass spectrometry analysis.

2.3.1. Brain lipid analysis using mass spectrometry

One aliquot of dried samples prepared as described above was re-suspended in 150 μL of chloroform−methanol (1:1), vortexed, and spun down at 12,000 rpm for 10 min at 4 °C. An equal amount of internal standard cocktail was added to the samples. The samples were analyzed on a 3200 QTRAP mass spectrometer (Applied Biosystems) with an atmospheric pressure chemical ionization probe using an Agilent Eclipse XDB-C18 column for the analysis of brain cholesterol, cholesterol esters, and oxysterols (Shui et al., 2011a). The internal standard cocktail used for this analysis included D6-cholesterol, D6-cholesterol ester, D6-14-demethyllanosterol, D7-7-keto-hydroxycholesterol, D7-7β-hydroxycholesterol, and D6-24S-hydroxycholesterol diluted with an appropriate amount of chloroform-methanol (1:1).

The other dried aliquot of samples was re-suspended in 300 μL of chloroform-methanol (1:1), vortexed for 15 seconds, and centrifuged at 12,000 rpm for 10 minutes at 4 °C for the analysis of phospholipids, sulfatides, and sphingomyelin. An equal amount of internal standard mixture was added to the samples. The samples were analysed on a 4000 QTRAP mass spectrometer (Applied Biosystems) via direct flow injection with an electrospray ionisation source. Multiple reaction monitoring transitions were set up for quantitative analysis of various polar lipids (Shui et al., 2011b). Individual lipid species were quantified by referencing to corresponding internal standards. The internal standard cocktail used for this analysis included 1,2-dimyristoyl-glycero-3-phosphoethanolamine (DMPE), dimyristoyl phosphatidylglycerol (DMPG), dimyristoyl phosphatidylserine (DMPS), 1,2-dimyristoyl-sn-glycero-3-phosphate (14:0 PA), dioctanoylphosphatidylinositol (diC8PI), dimyristoyl phosphatidylcholine (DMPC), sphingomyelin 12:0 (SM12), and C12 mono-sulfo galactosyl(β) ceramide (d18:1/12:0) (C12 SL) diluted with appropriate amount of chloroform-methanol (1:1).

3. Statistical analysis

Means and standard errors of the means were calculated using Excel 2008. The Student t-test was performed in SPSS (SPSS Inc, Cary, NC) was performed to calculate the p values of significance for all of the bar charts, and a p value <0.05 was considered statistically significant.