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High-fat diet-induced deregulation of hippocampal insulin signaling and mitochondrial homeostasis deficiences contribute to Alzheimer disease pathology in rodents

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ABSTRACT

Global obesity is a pandemic status, estimated to affect over 2 billion people, that has resulted in an enormous strain on healthcare systems worldwide. The situation is compounded by the fact that apart from the direct costs associated with overweight pathology, obesity presents itself with a number of comorbidities, including an increased risk for the development of neurodegenerative disorders. Alzheimer disease (AD), the main cause of senile dementia, is no exception. Spectacular failure of the pharmaceutical industry to come up with effective AD treatment strategies is forcing the broader scientific community to rethink the underlying molecular mechanisms leading to cognitive decline. To this end, the emphasis is once again placed on the experimental animal models of the disease. In the current study, we have focused on the effects of a high-fat diet (HFD) on hippocampal-dependent memory in C57/Bl6 Wild-type (WT) and APPswe/PS1dE9 (APP/PS1) mice, a well-established mouse model of familial AD. Our results indicate that the continuous HFD administration starting at the time of weaning is sufficient to produce β-amyloid-independent, hippocampal-dependent memory deficits measured by a 2-object novel-object recognition test (NOR) in mice as early as 6 months of age. Furthermore, the resulting metabolic syndrome appears to have direct effects on brain insulin regulation and mitochondrial function. We have observed pathological changes related to both the proximal and distal insulin signaling pathway in the brains of HFD-fed WT and APP/PS1 mice. These changes are accompanied by a significantly reduced OXPHOS metabolism, suggesting that mitochondria play an important role in hippocampus-dependent memory formation and retention in both the HFD-treated and AD-like rodents at a relatively young age.

1. Introduction

Over the last couple of decades a global nutrition transition from undernourishment to overconsumption has taken place. Replacement of traditional diets with cheap and easily available processed foods rich in refined carbohydrates, animal fats and edible oils resulted in a global obesity pandemic. While usually considered the plight of the developed world, obesity is also an emerging public health concern among the growing middle classes in poorer countries [1]. Overweight and moderate obesity (defined as Body Mass Index (BMI) of between 25 and 35) may not have a major impact on life expectancy *per se* [2], however, excessive weight significantly increases the risks of developing a number of pathological conditions. These include metabolic syndrome, diabetes, non-alcoholic steatohepatitis, coronary heart disease, stroke, gallbladder disease, osteoarthritis, some types of cancers [3], cognitive decline and Alzheimer disease (AD) [4–7].

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AD is the most common cause of senile dementia, accounting for between 60 and 80% of all dementias. According to recent estimates, the number of cases of AD worldwide is projected to rise from approximately 30 million in 2010 to 40 million by 2020 and to 100 million by 2050. Apart from the genetic component and old age, seven primary preventable environmental risk factors contributing to AD have been identified: diabetes mellitus, midlife hypertension, midlife obesity, depression, physical inactivity, smoking and cognitive inactivity [8,9]. Thus, it is becoming increasingly evident that most of the prognostic preventable AD risk factors may also be linked to obesity and resulting comorbidities, including metabolic syndrome and diabetes. Even though the epidemiological data suggest an existing relationship between AD and energy metabolism, molecular mechanisms behind this relationship are poorly understood. Because AD is a multifactorial disorder with complex etiology which takes decades to fully develop, it is especially challenging to identify the precise disease mechanisms. For example, in a patient with dementia it is not always easy to tell if the underlying pathology is that of a specific brain disease or whether it is also associated with vascular components, metabolic alterations or additional factors (ie. traumatism). Such difficulties notwithstanding, recent years saw a number of breakthroughs in AD research field which contribute to a greater understanding of the molecular dynamics of this devastating condition.

A classical, but currently hotly debated "amyloid cascade" hypothesis [10,11] states that cognitive decline and memory loss in AD are caused by the formation of large, insoluble beta amyloid plaques in the brain, which result in neuronal death and produce characteristic disease symptoms. However, it is necessary to differentiate between the insoluble plaques and soluble amyloid molecules. Recently emerged alternative theories suggest that the β-amyloid monomers, fibrils, or oligomers, and not the plaques, may in fact be the primary neurotoxic species in the brain, responsible for AD development and progression [12]. Apart from amyloid beta itself, mounting evidence suggests that impaired glucose and insulin signaling and metabolism in the brain play a key role in AD. The discovery of brain-specific insulin signaling deficiencies in the very early stages of AD pathogenesis has led some authors to propose that AD may be termed "type 3 diabetes" [13–15]. This hypothesis is further strengthened by a recent study of diabetes-related genes in the brains of post-mortem AD patients and in a mouse model of AD [16]. Microarray analysis has demonstrated significant alterations in the mRNA expression profiles of genes related to insulin signaling, obesity and diabetes in the frontal cortex, temporal cortex and hippocampus in both species. Interestingly, the biggest differences were observed in the hippocampus, a key area related to memory.

Deficiencies in Tau processing may provide yet another link between diabetes and AD. Hyperphosporylated Tau protein is a principle constituent of neurofibrillary tangles (NFT) [17] which, alongside amyloid beta plaques, have long been considered key histopathological hallmarks of AD. Abnormalities in Tau phosphorylation have been detected in cortex and the hippocampi of both type 1 (streptozotocin-induced) and type 2 (*db*/*db*) mouse models of diabetes [18,19].

Prior research has established a clear relationship between obesity, insulin resistance, diabetes and dementia (reviewed in [20]). Results from published research indicate that there is a close link between insulin deficient diabetes and cerebral amyloidosis in the pathogenesis of AD [21–24]. Epidemiological, clinical, and basic studies have shown a relationship between AD and Type 2 Diabetes Mellitus (T2DM), and that the main physiological link between both conditions is peripheral and central insulin signalling impairment [25,26]. In fact, results from the so called "Hysayama Study" indicate that altered expression of genes related to diabetes mellitus in AD brains is a result of AD pathology, which may thereby be exacerbated by peripheral insulin resistance or diabetes mellitus [16]. These cognitive deficits associated to T2DM have been argued to be due in large part to an impaired central insulin modulation in the hippocampus, which is a critical region for memory processing [27]. Furthermore, a number of recent pilot clinical trials have demonstrated an improvement in AD symptoms in patients upon administration of both the intranasal insulin and Glucagon-like peptide-1 (GLP1) analogues. It has been suggested that these compounds may affect synaptogenesis, neurogenesis, cell repair and inflammation processes, and may additionally help to reduce cerebral β-amyloid load (reviewed in [28]).

As it is especially difficult to study long-term effects of hypercaloric diet in human subjects, we have chosen a mouse model in order to further investigate the underlying molecular events linking brain energy metabolism to AD. A well-established experimental approach to induce insulin resistance in peripheral organs of rodents consists of a high-fat diet (HFD) treatment, which results in obesity [29–31]. We have characterized the neuropathological effects of a HFD in 6-months-old male APPswe/PS1dE9 (APP/PS1) mice in comparison to the nontransgenic C57BL/6 (non-Tg; WT) control animals.

2. Materials and methods

2.1. Animals

Male APPswe/PS1dE9 and C57BL/6 mice were used in this study. APP/PS1 animals co-express a Swedish (K594M/N595L) mutation of a chimeric mouse/human APP (Mo/HuAPP695swe), together with the human exon-9-deleted variant of PS1 (PS1-dE9), allowing these mice to secrete elevated amounts of human Aβ peptide. Both mutations are associated with AD, are under control of the mouse prion protein promoter, directing both mutated proteins mainly to the CNS neurons, and result in age-dependent amyloid plaque depositions in mouse brain. The APPswe-mutated APP is a favorable substrate for β-secretase, whereas the PS1dE9 mutation alters β-secretase cleavage, thereby promoting overproduction of Aβ42. The mice were fed for 5 months with a high-fat diet consisting of 25% fat (45 kcal %), mainly from hydrogenated coconut oil, 21% protein (16 kcal %), and 49% carbohydrate (39 kcal %); Cat# D08061110 (Research Diets Inc, New Brunswick, USA). Body weight was recorded weekly. The animals were kept under controlled temperature, humidity and light conditions with food and water provided *ad libitum*. Mice were treated in accordance with the European Community Council Directive 86/609/EEC and the procedures established by the Department d'Agricultura, Ramaderia i Pesca of the Generalitat de Catalunya. Every effort was made to minimize animal suffering and to reduce the number of animals used. Fifty animals, divided into four groups, were used for the present study, with at least 10 wild-type and 10 6-month-old APP/PS1 transgenic mice, per group. Following *in vivo* testing, the animals were sacrificed at the age of 6 months and at least 6 mice in each group were used for RNA and protein extract isolation, with an additional 4 mice used for immunofluorescence.

2.2. Total blood cholesterol and triglycerides measurements

Total blood cholesterol and triglyceride levels were measured following 4-hour-long fast at the point of sacrifice with Accutrend Plus meter (Roche Diagnostics, Switzerland).

2.3. Glucose and insulin tolerance tests

Intraperitoneal glucose tolerance tests (IP-GTT) and insulin tolerance tests (ITT) were performed in accordance with the previously published guidelines [32]. For IP-GTT, mice were fasted overnight for 16 h. The test was performed in a quiet room, preheated to +30 °C. The tip of the tail was cut with the heparin-soaked (Heparina Rovi, 5000 IU/ml; Rovi S.A.;Madrid, Spain) scissors, 30 min prior to 1 g/kg intraperitoneal glucose injection (diluted in H2O). Blood glucose levels in the tail vein were measured at −30, 0, 5, 15, 30, 60 and 120 min after the glucose injection with the Ascensia ELITE blood glucose

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