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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

meter (Bayer Diagnostics Europe Ltd.; Dublin, Ireland). ITT was performed in similar conditions with the 0.25 IU/kg of human insulin, diluted in saline (Humulina Regular, 100 IU/ml/Lilly, S.A.;Madrid, Spain), except that the mice underwent a 4-5 hour-long morning fast. Blood glucose levels were measured at −30, 0, 15, 30, 45 and 60 min after the insulin administration. If during this time blood glucose levels dropped to below 20 mg/dl, 1 g/kg glucose was administered to counteract the effects of insulin, in order to reduce animal suffering.

2.4. 2-Object novel object recognition test (NOR)

The test was conducted as previously described by us and others [33]. In brief, a 90°, 25 cm long, and 20 cm high L maze was used. The light intensity in the middle of the field was 30 lx. The objects to be discriminated were plastic figures (object A: 5.25 cm high, object B: 4.75 cm high). First, mice were individually habituated to the apparatus for 10 min a day, for two days. On the third day, they were submitted to a 10 min acquisition trial (first trial) during which they were placed in the maze in the presence of two identical novel objects (A + A, or B + B) placed at the end of each arm. A 10 min retention trial, with the objects (A + B) (second trial) occurred 2 h later. The amount of exploration time each animal spent on objects A and B during the acquisition trial varied between 5 and 20 s, depending on the individual mouse. Total exploration time between the 2 objects when calculated for each individual animal indicated the absence of the object preference bias (Fig. 1C) (n = 5–9 per group). During the retention trial, the times that the animal took to explore the new object (tn) and the old object (to) were recorded. A discrimination index (DI) was defined as (tn − to) / (tn + to). In order to avoid further object preference bias, objects A and B were counterbalanced so that half of the animals in each experimental group were first exposed to object A and then to object B, whereas the other half were exposed to object B first, and then to object A. The maze, the surface, and the objects were cleaned with 96° ethanol between animals, so as to eliminate olfactory cues.

2.5. RNA extraction and quantification

Total RNA was isolated from the hippocampi of wild-type and APP/PS1 transgenic mice utilizing Trizol-based extraction (Life Technologies Corporation; Carlsbad, Ca, USA), as described previously [34]. Briefly, the tissue was homogenized in the presence of Trizol reagent (Life Technologies Corporation; Carlsbad, CA, USA). Chloroform was added and the RNA was precipitated from the aqueous phase with isopropanol at 4 °C. RNA pellet was reconstituted in RNAse-free water, with the RNA integrity determined by Agilent 2100 Bioanalyzer (Agilent Technologies; Santa Clara, CA, USA).

2.6. Real-time-PCR

First-strand cDNA was reverse transcribed from 2 μg of total RNA using the High Capacity cDNA Reverse Transcription kit, according to manufacturer's protocol (Applied Biosystems). Each sample was analyzed in duplicate for each target. TaqMan probes (Applied Biosystems), as detailed in Table 2, were used to determine transcription levels of individual genes. Reaction was performed on the StepOnePlus Real Time PCR system (Applied Biosystems; Carlsbad, CA, USA) and the values were normalized to *gapdh* and *tbp*.

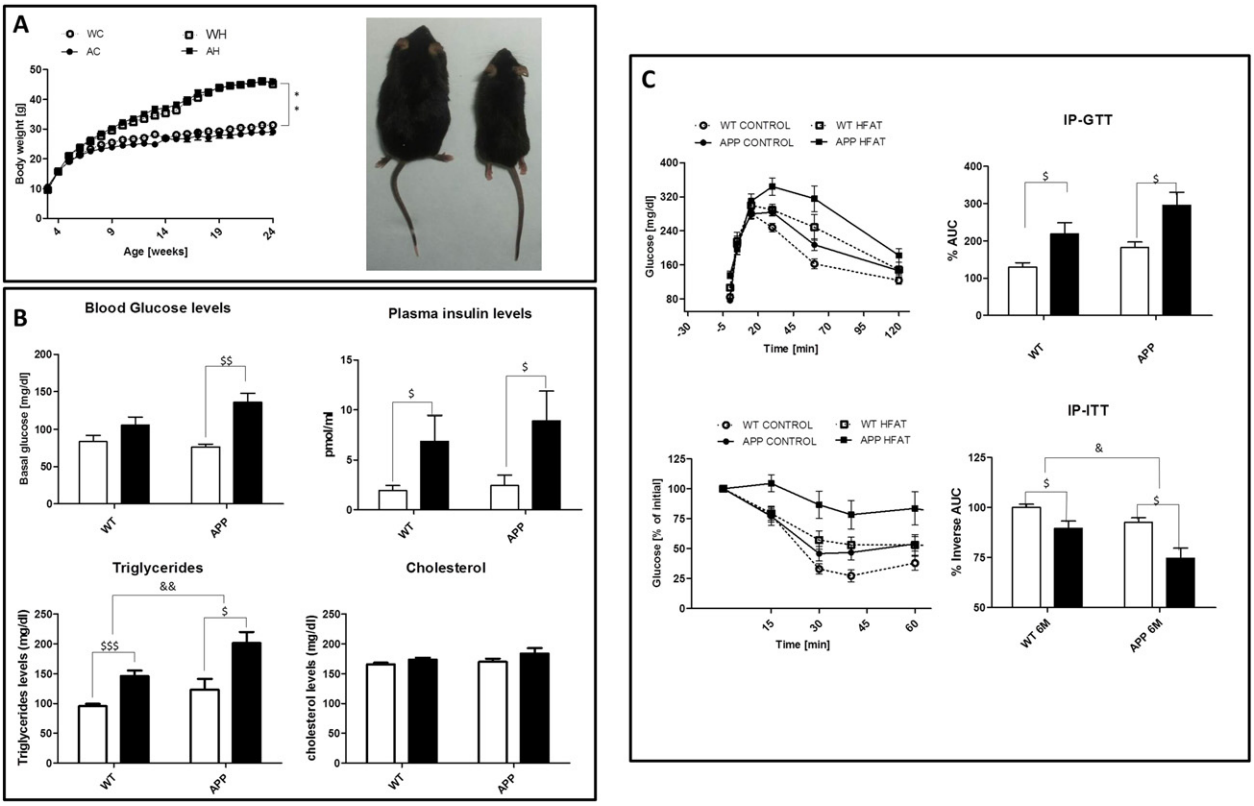

Fig. 1. Peripheral metabolic parameters in HFD-fed WT and APP/PS1 mice. (A) Body weight, (B) fasting blood glucose , fasting serum insulin levels ELISA, total blood triglycerides and cholesterol in 6-month-old animals (n = 5–12 independent samples per group). (C) Intraperitoneal glucose (16 hour fast) and insulin (4 hour fast) tolerance tests in 6-month-old mice (n = 5-12 independent samples per group). For the ITT and the IP-GTT, AUC data were calculated from the timepoint 0 till the end of the experiment. (Statistical analysis was performed with the student's t-test, where $ denotes $p < 0.05$, $$ denotes $p < 0.01$, $$$ denotes $p < 0.001$; regular 2-way ANOVA, where & denotes $p < 0.05$, && denotes $p < 0.01$) *WC*: Wild-type control diet, *AC*: APP/PS1 control diet, *WH*: Wild-type high-fat diet, *AH*: APP/PS1 high-fat diet; open bar: chow, closed bar: high-fat diet.

Table 1
A list of antibodies used for immunoblotting and immunofluorescence.

Protein	Antibody
Akt	#9272 (Cell signaling)
pAkt (S473)	#4060P (Cell signaling)
CDK5	Sc-173 (Santa Cruz biotech)
pCDK5 (Y15)	ab63550 (Abcam)
ERK1/2	#9102 (Cell signaling)
pERK1/2 (T202/Y204)	#9101 (Cell signaling)
GSK3B	#9315(Cell signaling)
pGSK3B (Y216)	Ab74754 (Abcam)
IDE	Ab32216 (Abcam)
IRβ	Sc-20739 (Santa Cruz biotech)
pIRβ (Y1150/1151)	Sc-81500 (Santa Cruz biotech)
IRS1	#2382 (Cell signaling)
pIRS1 (S612)	#2386S (Cel signaling)
IRS2	Sc-1555 (Santa Cruz biotech)
pIRS2 (S723)	Ab 3690 (Abcam)
JNK	#9252 (Cell signaling)
pJNK (Y183/T185)	#9251 (Cell signaling)
Neprelysin	Ab951 (Abcam)
NRF1	Sc-28379 (Santa Cruz biotech)
OXPHOS	MS604 (MitoSciences)
p35	#2680(Cell signaling)
PGC1A	101707 (Cayman chemical)
PPARa	Ab8934 (Abcam)
PPARg	#2430 (Cell signaling)
TAU	AHB0042 (Biosource)
pTAU (S404)	44748G (Life Technologies)
TFAM	DR1071b(Calbiochem)
GAPDH	MAB374 (Millipore)
Thioflavin S	Thioflavin S (Sigma-Aldrich)
2nd –ary Anti-Mouse	170-5047 (Biorad)
2nd –ary Anti-Rabbit	NA934V (GE Healthcare)

2.7. Immunofluorescence, thioflavin S and Hoechst staining

Slides were allowed to defrost at room temperature and then were rehydrated with Phosphate-buffered saline (PBS) for 5 min. Later, the brain sections were incubated with 0.3% Thioflavin S (Sigma-Aldrich; St. Louis, MO, USA) for 20 min at room temperature in the dark. Subsequently, these were submitted to washes in 3-min series: with 80% ethanol (2 washes), 90% ethanol (1 wash), and 3 washes with PBS. Finally, the slides were mounted using Fluoromount (EMS), allowed to dry overnight at room temperature in the dark, and stored at 4C. Image acquisition was performed with an epifluorescence microscope (BX41; Olympus, Germany).For plaque quantification, similar and comparable histological areas were selected, focusing on having the hippocampus and the whole cortical area positioned adjacently [35].

Table 2
A list of Taqman probes used for real-time PCR analysis.

GENE	TaqManProbe
app	Mm01344172_m1
essra	Mm00433143_m1
gapdh	Mm99999915_g1
igf1	Mm01228180_m1
igf2	Mm00439564_m1
igfbp2	Mm00492632_m1
igf1r	Mm00802831_m1
ins1	Mm01950294_s1
insr	Mm01211875_m1
irs1	Mm01278327_m1
irs2	Mm03038438_m1
nrf1	Mm01135606_m1
nrf2 (nfe2l2)	Mm00477784_m1
Ppara	Mm00440939_m1
pparg	Mm01184322_m1
ppargc1a	Mm01208835_m1
prkaa1	Mm01296700_m1
prkaa2	Mm01264789_m1
tbp	Mm00446971_m1
tfam	Mm00447485_m1

2.8. Immunoblot analysis

Aliquots of hippocampal homogenates containing 15 mg of protein per sample were analyzed using the Western blot method. In brief, samples were placed in a sample buffer (0.5 m Tris–HCl, pH 6.8, 10% glycerol, 2%(w/v) SDS,5%(v/v) 2-mercaptoethanol, 0.05% bromophenol blue) and denatured by boiling at 95–100 °C for 5 min. Samples were separated by electrophoresis on 10–15% acrylamide gels. Following this, the proteins were transferred to PVDF sheets using transblot apparatus. Membranes were blocked overnight with 5% non-fat milk dissolved in TBS-T buffer (50 mM Tris; 1.5% NaCl, 0.05% Tween 20, pH 7.5). They were then incubated with primary antibodies, as detailed in Table 1. After O/N incubation, blots were washed thoroughly in TBS-T buffer and incubated for 1 h with a peroxidase-conjugated IgG secondary antibody (1:2000). Immunoreactive protein was detected using a chemiluminescence-based detection kit. Protein levels were determined by densitometry, using Chemidoc XRS + Molecular Imager detection system (Bio-Rad Laboratories Inc.; Hercules, CA, USA)), with ImageLab image analysis software. Measurements are expressed as arbitrary units. All results are normalized to GAPDH, unless stated otherwise.

2.9. Measurement of β-amyloid peptides in cortical tissues by ELISA

Soluble and insoluble β-amyloid (βA) βA_{1-40} and βA_{1-42} were measured in cortical extracts employing the commercially available mouse and human ELISA kits (Cat # KMB3481, KMB3441, KHB3481 and KHB3441; Invitrogen, Camarillo, CA, USA) according to manufacturer´s guidelines. The soluble fraction was separated by centrifuging the samples for 10 minutes at 4000xg. The pellets containing insoluble Aβ peptides were solubilized in a 5 M guanidine HCl/50 mM Tris HCl solution by incubating for 3.5 hours on an orbital shaker at room temperature in order to obtain insoluble fraction. Data obtained from the cortical homogenates are expressed as picograms of Aβ content per milligrams of total protein (pg/mg).

2.10. Data analysis

All data are presented as means ± SEM, and differences are considered significant at $p < 0.05$. Differences between samples/animals were evaluated using student's t-test, and either one-way or 2-way ANOVA, with Tukey's post-hoc test. Both the statistical analysis and the graphs presented here were created with the GraphPad InStat software V5.0 (GraphPad Sofware Inc., San Diego, CA, USA).

3. Results

3.1. HFD treatment increases body-weight gain, insulin and triglycerides levels in blood and provokes impaired glucose and insulin tolerance in both WT and APP/PS1 mice

In order to determine the effects of a high-fat hypercaloric diet on metabolic parameters in a mouse model of AD, male WT and APP/PS1 animals were fed either standard chow or a HFD. Mice were divided into 4 groups (n = at least 10 per group): chow-fed WT control (WC), HFD-fed WT (WH), chow-fed transgenic APP/PS1 (TC) and HFD-fed APP/PS1 (TH). Treatment commenced at the time of weaning (21 days) and lasted until the animals reached 6 months of age. This specific time point was chosen as at six months-old APP/PS1 mice present with AD-like neuropathology, including readily detectable βA plaques and memory loss [33] (Fig. 2). As expected, HFD treatment produced progressive diet-induced obesity, with body weight at completion of the experiment reaching 144% in WH vs. WC ($P < 0.0001$),

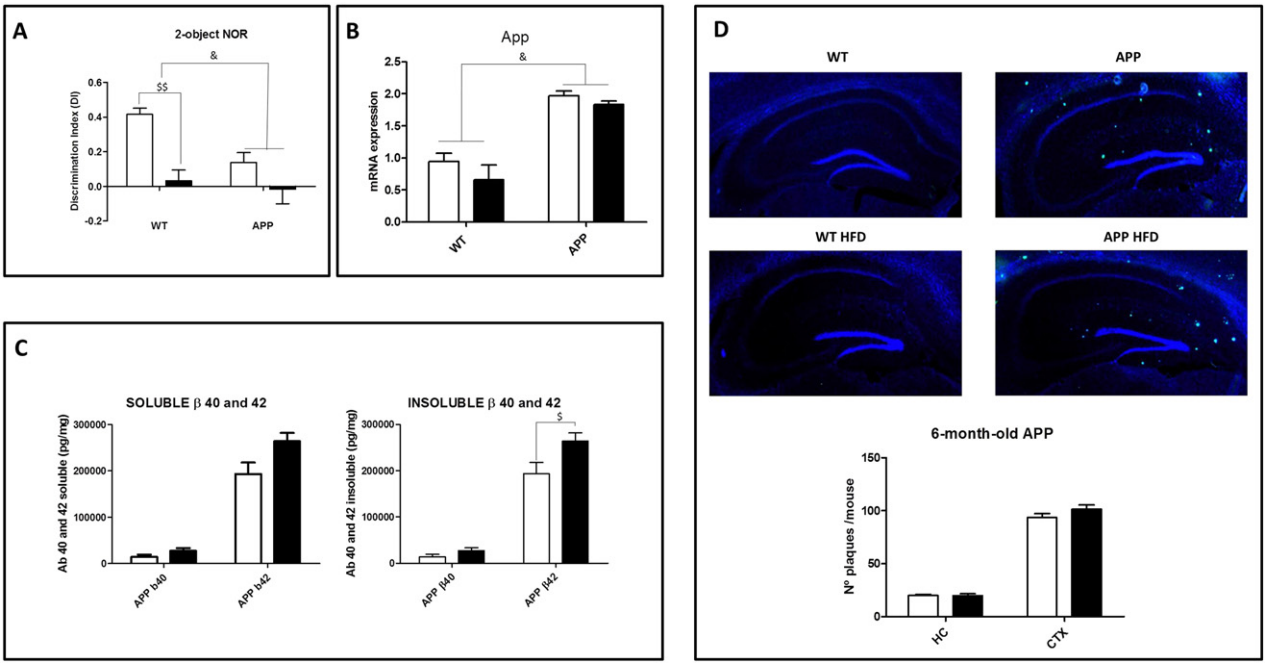

Fig. 2. (A) The results of the 2-object Novel Object Recognition test (NOR), demonstrating significant memory loss as a result of a HFD treatment in 6 month-old wild-type, as well as in chow-fed and HFD-fed APP/PS1 animals, compared to chow-fed wild-type mice (n = 7–12 independent samples per group). (B) Hippocampal mRNA expression of the *app* with the probe recognizing both mouse and human forms of *app* in 6-months-old mice (n = 10-12 independent samples per group, with 3 technical replicates per sample). (C) Concentrations of the soluble and insoluble human βA_{1-40} and βA_{1-42} peptides in the cortical extracts in chow- and HFD-fed 6-month-old APP/PS1 mice, expressed as pg/mg of total protein as determined by ELISA (n = 5–8 independent samples per group, with 3 technical replicates per sample). (D) Immunofluorescence analysis of β-amyloid plaque numbers in the brains of 6-month-old WT and APP/PS1 mice. No plaques were observed in WT animals (n = 4–6 independent samples per group, with at least 5 slices analyzed per sample). Hoechst staining in blue / Thioflavin S staining in green (Statistical analysis was performed with the student's t-test, where $ denotes $p < 0.05$, $$ denotes $p < 0.01$; regular 2-way ANOVA, where & denotes $p < 0.05$, && denotes $p < 0.01$); open bar: chow, closed bar: high-fat diet.

and 158% in TH vs. TC ($P < 0.0001$) (Fig. 1A). This weight increase was accompanied by fasting hyperinsulinemia, with plasma insulin concentrations of 6.88 pM/ml in WH (1.92 in WC; $P = 0.0484$) and 8.92 pM/ml in TH (2.44 in TC; $P = 0.0332$) (Fig. 1B). An additional sign pointing to the possible presence of metabolic syndrome in 6-months-old HFD-fed mice was an increase in fasting triglycerides levels with 146 mg/dl in WH (96 in WC; $P < 0.001$) and 202 mg/dl in TH (123 in TC; $P = 0.0119$). Interestingly, a 2-way ANOVA demonstrated significant differences between WT and APP/PS1 groups as a whole, with higher triglycerides concentrations in transgenic animals, suggesting exacerbated phenotype in AD-like mice (Fig. 1B). Fasting blood glucose levels support this observation, as the HFD treatment resulted in a significant increase of this parameter in TH vs. TC group (136 vs 76 mg/dl; $P = 0.0046$), but not in WH vs. WC (106 vs 84 mg/dl; $P = 0.1452$). Fasting blood cholesterol levels were not affected in any of the groups (Fig. 1B).

As our initial screening has not only demonstrated clear alterations in peripheral glucose metabolism in response to HFD, but also significant differences between the WT and APP/PS1 animals, we performed additional glucose and insulin tolerance tests. Predictably, IP-GTT has shown impaired glucose tolerance in 6-months-old WH and AH groups, when compared to their respective controls ($P = 0.02$ and $P = 0.0436$) (Fig. 1C). The results of the ITT were intriguing. While the test results indicated impaired insulin tolerance in both WH and AH ($P = 0.0106$ and $P = 0.0346$) we, once again, detected a more severe phenotype in an APP/PS1 model ($P = 0.0239$, with 2-way ANOVA) (Fig. 1C). Taken together, our data indicate a possible acceleration of a HFD-induced peripheral metabolic phenotype in APP/PS1 animals compared to control mice. In the following steps, we proceeded to study the effects of HFD on CNS and attempted to identify molecular pathways related to insulin metabolism in the brain, with a particular focus on hippocampal metabolic and insulin signaling.

3.2. High-fat diet contributes to increased cerebral β-amyloid levels and memory loss

We have employed a 2-object Novel Object Recognition (NOR) test as a means of evaluating the impact of HFD on cognitive performance. Interestingly, our results demonstrate that HFD treatment has a significant impact on memory function in both the WT and transgenic animals (Fig. 2A). In order to determine if the resulting memory loss is dependent on the increased cerebral βA load, we have measured hippocampal expression of the APP, cortical levels of the βA_{1-40} and βA_{1-42} and assessed the numbers of senile plaques in the brain.

Because APP/PS1 mouse model expresses a human form of the APP and the βA, it is necessary to quantify the combined expression of both endogenous and transgenic protein. At the mRNA level, a probe recognizing both human and mouse versions of *app* was selected for Real-time PCR analysis. We have detected approximately a 2-fold increase in *app* transcripts in the hippocampal extracts of APP/PS1 mice, compared to WT controls (Fig. 2B). Hypercaloric diet did not influence mRNA expression of this target in either group. Elevated levels of soluble and insoluble forms of βA_{1-40} and βA_{1-42} peptides were detected in cortical homogenates of APP/PS1 animals. However, HFD treatment resulted in a significant increase in insoluble βA_{1-42} levels only, and only in TH versus TC group (~266 compared to ~195 ng/mg) (Table 3 and Fig. 2C). Surprisingly, this increase did not have an effect on the total number of plaques in the hippocampal and cortical areas of the brain (Fig. 2D). Furthermore, there appeared to be an increase in the concentrations of the soluble βA_{1-42} in WH animals, but it only affected a subset of this group, rendering the data unsuitable for statistical analysis (Table 3). Thus, our results suggest that alterations in cerebral amyloid levels do not play a critical role in HFD-induced memory loss in 6-month-old mice.

Table 3
Concentrations of the soluble and insoluble mouse and human βA_{1-40} and βA_{1-42} peptides in the cortical extracts in chow- and HFD-fed 6-month-old wild-type and APP/PS1 mice, expressed as pg/mg of total protein as determined by ELISA (n = 5–8 independent samples per group, with 3 technical replicates per sample). (Statistical analysis was performed with the Student's t-test; ± is S.E.M.). *WC*: Wild-type control diet, *AC*: APP/PS1 control diet, *WH*: Wild-type high-fat diet, *AH*: APP/PS1 high-fat diet, *m*: mouse, *h*: human, *t*: total (mouse + human), *p*: p value (t-test) *N/A*: not applicable, *N/D*: not detected.

Sol. (pg/mg)	m βA 40	h βA 40	t βA 40	m βA 42	h βA 42	t βA 42
WC	N/D	N/A	N/A	10 ± 10	N/A	10 ± 10
WH	N/D	N/A	N/A	1132 ± 632	N/A	1132 ± 632
AC	N/D	649 ± 262	649 ± 262	1120 ± 706	1088 ± 168	2208 ± 863
AH	N/D	879 ± 260	879 ± 260	1976 ± 1121	1244 ± 266	3220 ± 1346
WC vs WH (p)	N/A	N/A	N/A	0.1431	N/A	0.1431
AC vs AH (p)	N/A	0.558	0.558	0.5215	0.588	0.5305
WC	460 ± 158	N/A	460 ± 158	6 ± 6	N/A	6 ± 6
WH	331 ± 17	N/A	331 ± 17	270 ± 270	N/A	270 ± 270
AC	1276 ± 320	14075 ± 5195	15352 ± 4231	1722 ± 655	193310 ± 24590	194688 ± 24396
AH	1350 ± 190	27338 ± 5954	28688 ± 6138	2502 ± 633	263797 ± 18173	265882 ± 18194
WC vs WH (p)	0.3878	N/A	0.3878	0.3024	N/A	0.3024
AC vs AH (p)	0.8467	0.1476	0.1114	0.4245	0.043*	0.041*

3.3. HFD affects expression of genes involved in insulin signaling in the hippocampus

We evaluated mRNA expression profiles of preproinsulin 1 (*Ins1*), insulin receptor (*Insr*), insulin receptor substrates 1 (*Irs1*) and 2 (*Irs2*), insulin-like growth factor I (*Igf1*), and IGF receptor (*Igfr*) in the hippocampus of 6 months-old mice (Fig. 3A). A modest increase in *ins1* transcripts was accompanied by a small but significant reduction in *insr* and *igfr* levels in the TH vs. TC group, while HFD treatment did not have an effect on these molecules in WT brains. Conversely, *igf1* was upregulated in WH vs. WC group, but remained unchanged in transgenic animals. We did not detect significant differences in total IRS1 and IRS2 expression in any of the groups, both at the mRNA and protein levels (Fig. 3A, B). As post-translational modifications and especially ligand-mediated tyrosine$^{1150/1151}$ autophosphorylation of the signal-transducing catalytic β-subunit of IR play a major role in receptor activation, we have measured protein levels of both total IRβ and pTyr$^{1150\text{-}1151}$-IRβ. Immunoblotting analysis revealed no changes in the ratios between the phosphorylated and total IRβ protein in the hippocampal extracts at 6 months of age (Fig. 3B), suggesting that receptor functionality is unaffected. Functional IR is necessary for downstream signaling which is controlled in large part by IRS1 and IRS2 adaptor molecules. Autologous (insulin-mediated) and insulin independent Ser/Thr phosphorylations of IRS may both potentiate and attenuate IR signaling. Mouse pSer^{612}-IRS1 and pSer^{723}-IRS2 (corresponding to human pSer^{616}-IRS1 and pSer^{731}-IRS2) are amongst the better known negative regulators of the IR-IRS pathway. Thus, an insulin-dependent increase in the phosphorylation state of these residues may lead to

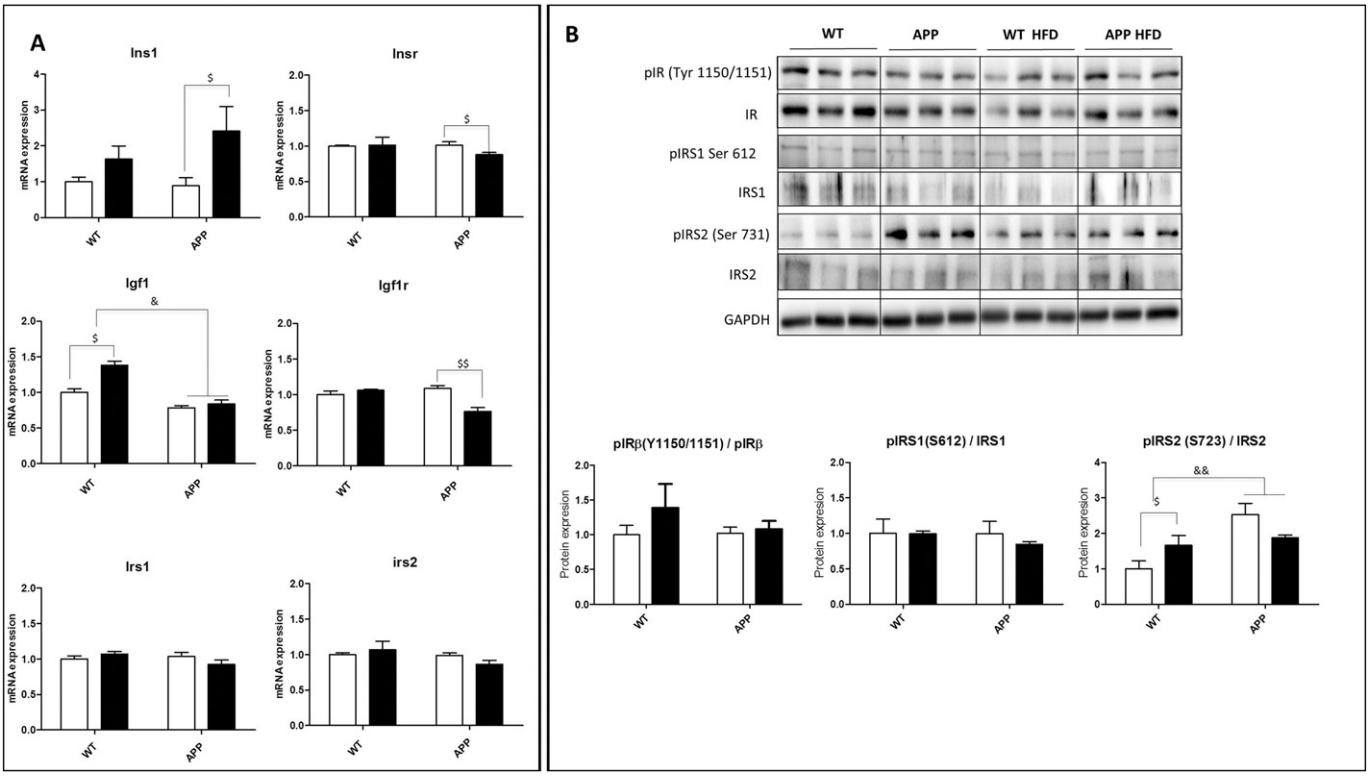

Fig. 3. (A) mRNA expression profile (n = 4–6 independent samples per group, with 3 technical replicates per sample) and (B) representative GAPDH-normalized immunoblot images and quantification (n = 4–6 independent samples per group) of molecules related to proximal insulin signaling in the hippocampal extracts of chow- and HFD-fed 6-month-old wild-type and APP/PS1 mice. Mouse pSer^{612}-IRS1 and pSer^{723}-IRS2 correspond to human pSer^{616}-IRS1 and pSer^{731}-IRS2 (Statistical analysis was performed with the student's t-test, where $ denotes $p < 0.05$; $$ denotes $p < 0.01$; regular 2-way ANOVA, where & denotes $p < 0.05$, && denotes $p < 0.01$); open bar: chow, closed bar: high-fat diet.

desensitization of proximal insulin signaling. We did not detect differences in IRS1 phosphorylation, however, our data demonstrated a significant increase in pSer^{723}-IRS2 in WH vs. WC ($P = 0.039$) and in APP/PS1 vs. WT (2-way ANOVA, $P = 0.0078$) mice (Fig. 3b), indicating a potential role of IRS2 in response to both the hypercaloric diet treatment and in AD-like phenotype.

3.4. Effects of HFD on signaling kinases and tau phosphorylation

Having determined that the proximal insulin signaling is likely perturbed in the hippocampi of HFD-exposed and APP/PS1 transgenic animals, we turned our attention to the signaling kinases implicated both in insulin signaling and senile plaque formation. Apart from their diverse cellular roles, these kinases (except for CDK5) have also been shown to phosphorylate IRS proteins directly, thus modulating the activity of these adaptor molecules. We measured protein levels of pSer^{473}-Akt, pTyr^{216}-Gsk3β, pThr^{183}/pTyr^{185}-JNK1, pThr^{202}/pTyr^{204}-ERK1/2, pTyr^{15}-CDK5 and its activator molecule p35 in the hippocampal extracts of 6-month-old animals. All of the above mentioned phosphorylations result in the activation of the respective kinases. Interestingly, we have observed significant differences in the activation state in all of the kinases tested when compared to at least one of the groups (Fig. 4). For example, ERK1/2 and CDK5/p35 were overactivated in WH vs. WC group only, while JNK1 activity was enhanced in WH vs. WC and TH vs. TC hippocampi. Basal GSK3β activation was higher in the TC vs. WC groups, with the HFD resulting in additional activation in WH group only. In contrast, Akt activity was inhibited in all of the groups when compared to WC (WH vs. WC; TC vs WC; TH vs. TC, as well as in APP/PS1 vs. WT – 2-way ANOVA, $P = 0.0085$). As kinase-mediated Tau hyperphosporylation is one of the principal diagnostic criteria of AD, we have also measured the phosphorylation state of the Tau protein at Ser^{404}. HFD treatment resulted in a significant increase in pSer^{404}-Tau in the WH vs. WC group, an increase which was comparable to the levels observed in the hippocampus of the APP/PS1 animals (Fig. 4). Collectively, our data suggest that the HFD treatment shares some of the features of the distal insulin signaling abnormalities observed in AD-like model.

3.5. Amyloid degrading enzymes (ADE) and HFD

Peripheral hyperinsulinemia and insulin resistance disrupts insulin transport into the CNS, resulting in the reduction of brain insulin levels. Under the normal circumstances, excess insulin is removed by Insulin Degrading Enzyme (IDE), increased expression of which forms a part of a negative feedback loop triggered by insulin itself. Thus, low local insulin levels will result in the reduced expression of IDE. As expected, we have observed a significant reduction in the protein levels of IDE in the hippocampi of both WH and TH mice (Fig. 5). However, IDE may also participate in β-amyloid clearance, and the reduction in its levels may potentially exacerbate APP/PS1 phenotype. Neprilysin is another well-known ADE that had been implicated in Aβ degradation. Interestingly, we detected a significant upregulation of neprilysin levels in TH vs. TC group (Fig. 5), suggesting a possible compensatory mechanism to counteract elevated amyloid load in HFD-treated APP/PS1 mice.

3.6. Mitochondrial metabolism is altered in the hippocampi of HFD-fed WT and APP/PS1 mice

So far, we have mainly focused on the insulin route and have not discussed the implications of altered insulin signaling on mitochondrial homeostasis. As mitochondria are the principal organelles involved in cell metabolism, we measured hippocampal mRNA and protein expression levels of a number of molecules associated with mitochondrial energy status and biogenesis. We did not detect changes in the mRNA expression of α- catalytic subunits of one of the major kinases activated in response to ATP depletion – AMPK (*Prkaa1* and *Prkaa2* levels were not affected in any of the groups). However, we observed a marginal but significant reduction in response to HFD in a key transcriptional

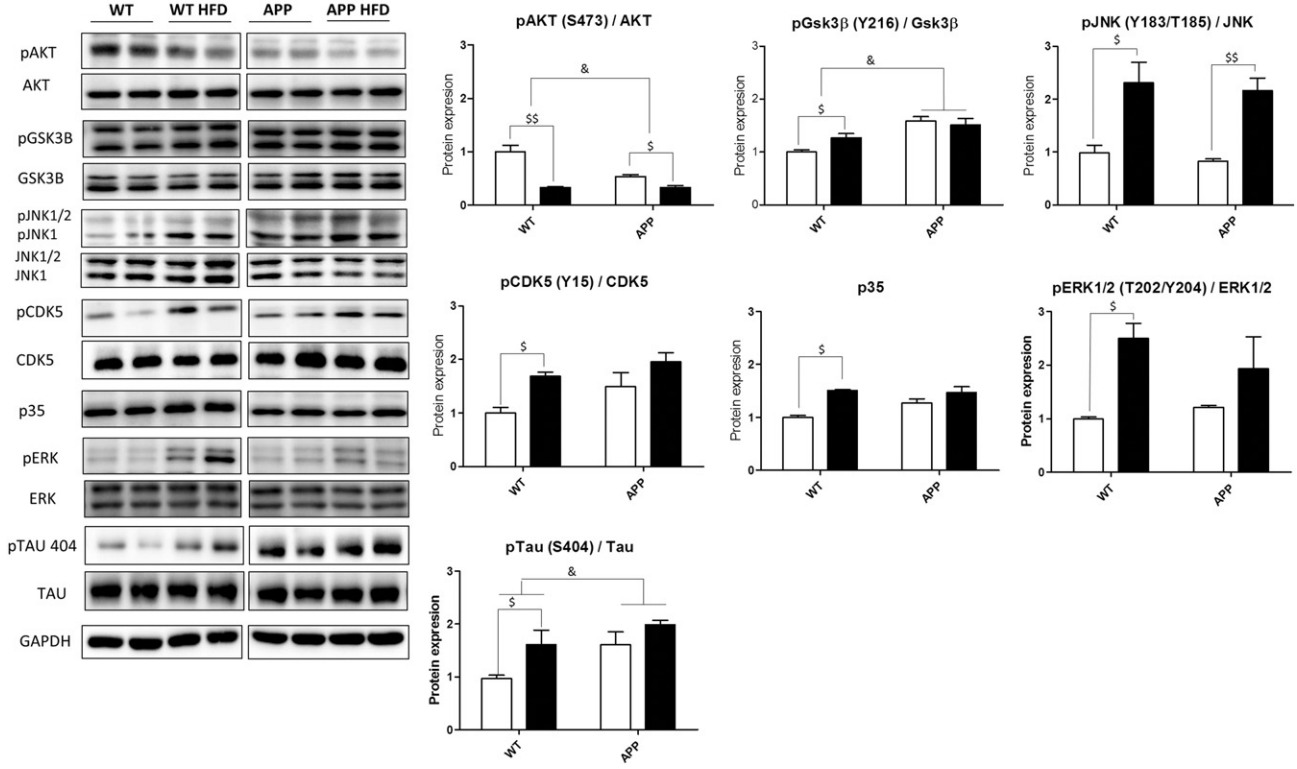

Fig. 4. Representative GAPDH-normalized immunoblot images and quantification (n = 4–6 independent samples per group) of molecules related to distal insulin signaling in the hippocampal extracts of chow- and HFD-fed 6-month-old wild-type and APP/PS1 mice. (Statistical analysis was performed with the student's t-test, where $ denotes $p < 0.05$; $$ denotes $p < 0.01$; regular 2-way ANOVA, where & denotes $p < 0.05$); open bar: chow, closed bar: high-fat diet.

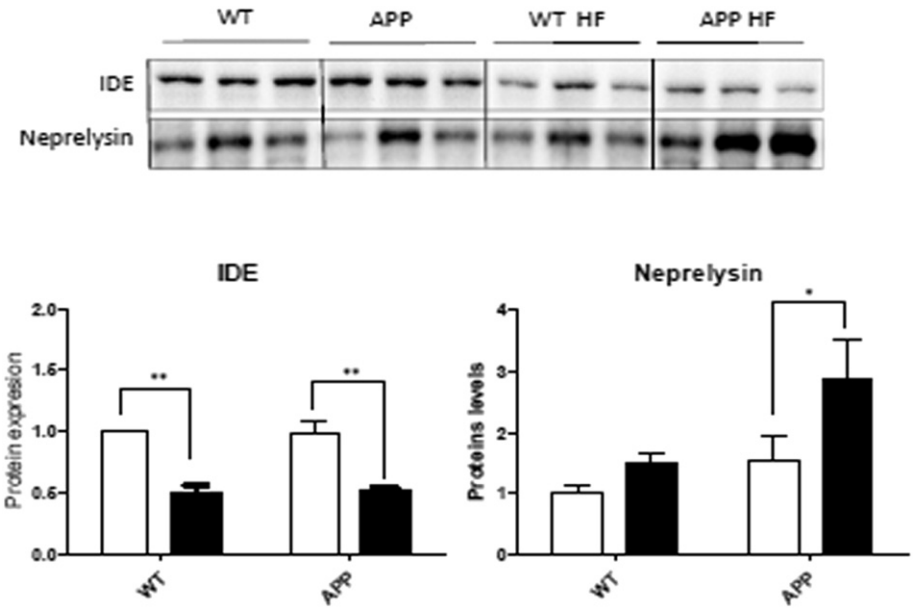

Fig. 5. Representative GAPDH-normalized immunoblot images and quantification (n = 4–6 independent samples per group) of enzymes implicated in β-amyloid degradation in the hippocampal extracts of chow- and HFD-fed 6-month-old wild-type and APP/PS1 mice. (Statistical analysis was performed with one-way ANOVA with Tukey's post-hoc test, where * denotes $p < 0.05$, ** denotes $p < 0.01$); open bar: chow, closed bar: high-fat diet.

co-regulator of mitochondrial biogenesis PGC-1α, which is partly regulated by AMPK (*ppargc1a* was reduced in APP/PS1 vs. WT groups; 2-way ANOVA, P = 0.0144; PGC-1α was reduced in WH vs. WC and TH vs. TC, P = 0.0044 and P = 0.0022, respectively) (Fig. 6A, B).

We have also determined the expression levels of PGC-1α co-regulated transcription factors, including peroxisome proliferator-activated receptors α (PPAR-α) and γ (PPAR-γ), mRNA and protein levels of which remained unchanged (Fig. 6A, B). Differences in the mRNA expression of the nuclear respiratory factors 1 (*nrf1*) and 2 (*nrf2*) between the groups were very small, even though statistically significant, and were comparable to the changes observed in estrogen-related receptor α (*esrra*) and mitochondrial transcription factor A (*tfam*) transcripts (Fig. 6A). At the protein level, we detected a significant reduction of TFAM in WH vs. WC and TH vs. TC groups (Fig. 6B).

In addition, we evaluated mitochondrial function impairment via immunoblotting analysis of OXPHOS complexes. Our results demonstrated significant reduction in OXPHOS I, II, III and IV in the hippocampi of 6-months-old WH vs. WC mice. All of the OXPHOS complexes were down regulated in the basal state in TC vs. WC animals, and were not further reduced in response to HFD treatment (Fig. 7). Taken together, our data indicate significant perturbations in cellular energy metabolism in the brains of HFD-treated and APP/PS1 mice.

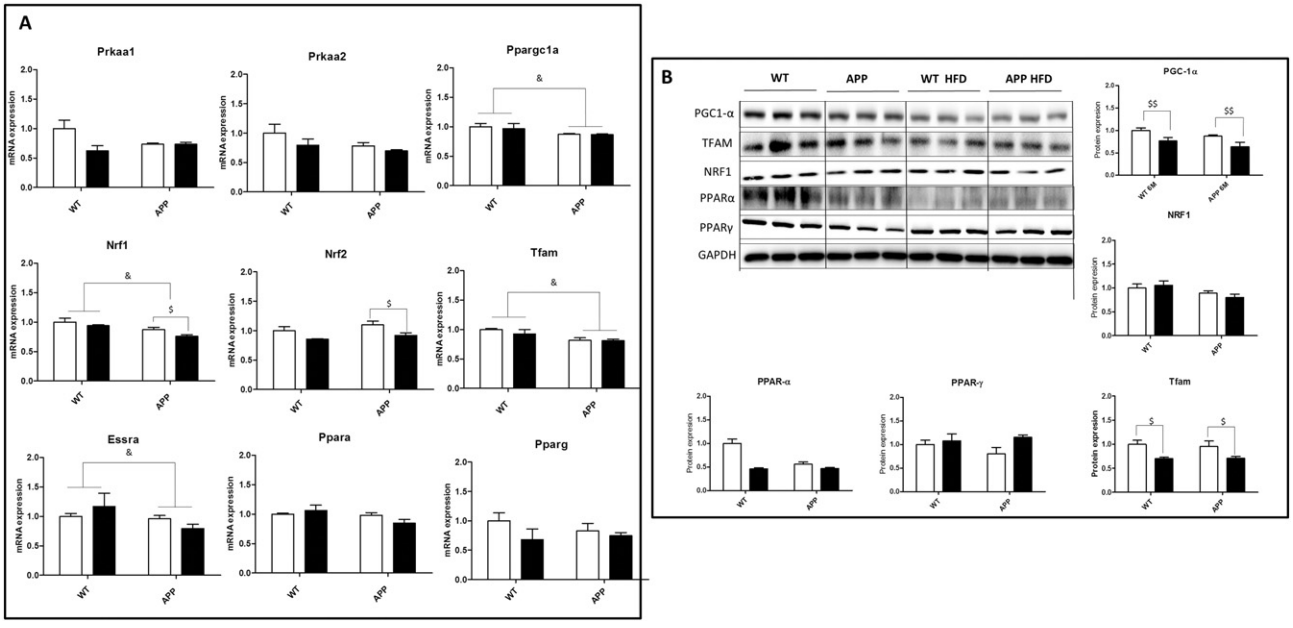

Fig. 6. (A) mRNA expression profile (n = 4–6 independent samples per group, with 3 technical replicates per sample) and (B) representative GAPDH-normalized immunoblot images and quantification (n = 4–6 independent samples per group) of molecules related to distal insulin signaling and mitochondrial homeostasis in the hippocampal extracts of chow- and HFD-fed 6-month-old wild-type and APP/PS1 mice. (Statistical analysis was performed with the student's t-test, where $ denotes $p < 0.05$; regular 2-way ANOVA, where & denotes $p < 0.05$); open bar: chow, closed bar: high-fat diet.

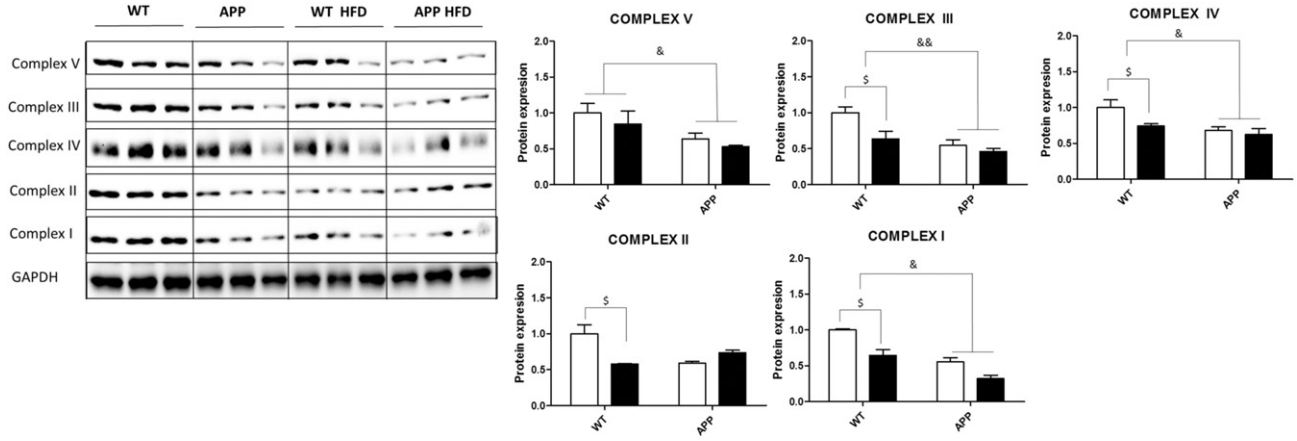

Fig. 7. Representative GAPDH-normalized immunoblot images and quantification (n = 4–6 independent samples per group) of mitochondrial OXPHOS complexes in the hippocampal extracts of chow- and HFD-fed 6-month-old wild-type and APP/PS1 mice. (Statistical analysis was performed with the student's t-test, where $ denotes $p < 0.05$, $$ denotes $p < 0.01$; regular 2-way ANOVA, where & denotes $p < 0.05$, && denotes $p < 0.01$); open bar: chow, closed bar: high-fat diet.

4. Discussion

The original "amyloid cascade" hypothesis postulated over 20 years ago in a seminal *Science* paper by Hardy and Higgins [10] has undoubtedly led to major breakthroughs in understanding the pathophysiology of AD. At its core, the hypothesis defined the cascade as a sequence of events starting from abnormal β-amyloid peptide processing leading to β-amyloid deposition, tau phosphorylation and neurofibrillary tangle formation, all of which ultimately result in cellular dysfunction and neuronal death [11]. Since then, significant research efforts on the part of academic labs and the pharmaceutical industry have largely focused on the amyloid cascade as the principal target for AD treatment. Unfortunately, none of the molecules that reached Phase II and III clinical trials targeting this pathway have proven to be more effective than placebo at treating AD (reviewed in [36]). Mounting criticism of the amyloid cascade hypothesis has recently prompted John Hardy himself to address the issues plaguing AD drug development [37]. The general failure of the amyloidocentric approach in pharmaceutical development strongly suggests that the β-amyloid is unlikely to be solely responsible for AD progression.

It has been long suspected that mitochondria play a significant role in neurodegenerative diseases [38–40]. By 2004, Swerdlow & Khan [41] proposed a comprehensive model which takes into account mitochondrial alterations occurring during the course of AD. The "mitochondrial cascade" hypothesis suggests that the cause and effect relationship in AD are reversed when pitted against an "amyloid cascade" hypothesis. The theory implies that the damage to the mitochondria occurs prior to the β-amyloid accumulation. This view has recently been expanded [42–44] and is supported by a number of studies (reviewed in [45]) which classify AD as a primarily metabolic disorder. According to this view, dementia develops as a result of the diminished ability of the brain to efficiently utilize the available glucose, leading to reduced neuronal plasticity which affects cerebral capacity to form and retain memories.

In the present paper, we present results that reinforce the hypothesis that AD may be viewed as a metabolic disorder, with the disease neuropathology at least partially related to insulin signaling failure and energy depletion in hippocampal neurons. Our data notwithstanding, before we continue the discussion regarding the implications of insulin signaling and mitochondrial regulation on hippocampal memory, we would like to address some of the discrepancies in peripheral metabolic parameters in our results compared to previously published studies. Ramos-Rodriguez and colleagues recently reported that a similar HFD treatment did not result in an increase in basal fasting blood glucose levels, nor in plasma triglycerides, but instead increased plasma cholesterol in 6 months-old APP/PS1 animals [46]. Our data clearly demonstrate elevated basal blood glucose in TH group, an increase in total blood triglyceride levels in WH vs. WC and in TH vs. TC groups, and no changes in cholesterol levels. Regarding blood glucose measurements, our methodology is very similar with 16 hours-long fast applied in both studies. However, the glucose values approximating 60 mg/dl detected by Ramos-Rodriguez *et al*, suggest that the measurements were taken at room temperature, which may explain the observed differences in the basal glucose levels in WC animals (~80 mg/dl in our study), as our tests were conducted at 28-29C [32]. As for the differences in cholesterol and triglycerides levels, these can be easily explained by the different diets used (60% Kcal obtained mainly from animal fat (lard) in Ramos-Rodriguez *et al*, vs. 45% Kcal mainly from hydrogenated coconut oil (vegetable fat) in our study). Interestingly, increases in plasma insulin levels in mice treated with either diet were comparable. Taken together, our data indicate that metabolic syndrome is indeed present in our model.

The connection between AD, obesity and type-2 diabetes has been known by epidemiologists for some time now. In fact, as early as 2004, in a study carried out at the Mayo clinic, investigators reported either impaired fasting glucose or diabetes in 81% of human subjects suffering from AD [47]. However, it is only recently that we are beginning to understand the pathophysiological processes linking peripheral metabolic abnormalities and neuronal dysfunction. Defective insulin signaling and mitochondrial redox imbalances appear to play a major role in the development of both the metabolic syndrome and AD. Crucially, it has been suggested that the insulin-dependent signaling in the brain may potentially be regulated via the pathways independent of its glucoregulatory functions in the periphery [48–50]. In the present study, we compared molecular pathways involved in memory loss in mice as a result of a HFD treatment, with animals which lose memory as a consequence of an APP/PS1 genotype. It was reported previously that the continuous exposure of juvenile (3-weeks-old) mice to a HFD causes relational memory loss (hippocampus-dependent) in a significant number of animals by 5 months of age [51]. We have chosen a similar experimental approach, but have focused on 6-months-old animals, with our data indicating that virtually all HFD-fed WT mice demonstrate pronounced memory loss at this age, as measured by NOR test. Importantly, 6-months-old APP/PS1 control animals present with similar memory deficits when compared to WH group. This has permitted us to make direct comparisons between the treated and untreated WT and APP/PS1 animals. One of the intriguing findings in our study is that the HFD-induced memory loss appears to be independent of the levels of cerebral β-amyloid peptide in mice not predisposed to abnormal βA processing (even though we detected a significant increase in insoluble βA_{1-42} levels in cortical homogenates in TH vs. TC group).

In our experiments, HFD treatment produces metabolic syndrome which includes peripheral hyperinsulinemia, peripheral insulin resistance, peripheral and central hyperglycemia and dyslipidemia among others. So, what are the components of metabolic syndrome that result in memory loss in HFD-fed WT animals? Assuming that the insulin is involved, we need to consider that one of the key differences in brain insulin signaling between AD and diet-induced obesity is the availability/quantity of insulin hormone in the brain. Diet-induced obesity results in the disruption of the Blood-Brain-Barrier (BBB), which limits receptor-mediated insulin transport to the CNS. Therefore, somewhat counterintuitively, peripheral hyperinsulinemia actually provokes insulin deficiency in the brain [52–56]. On the other hand, in AD, cerebral insulin resistance has been widely implicated in disease pathogenesis (reviewed in [48]). While the distinction between insulin deficiency and insulin resistance may seem trivial to some authors in the neuroscience field (to the point that a large number of studies use both terms interchangeably), it may have a significant impact on potential treatment strategies. For example, in an insulin-resistant (at the CNS level) non-obese AD patient, it may actually be beneficial to choose an oral anti-diabetic drug treatment as a means for improving AD symptoms. Conversely, the same treatment in an obese patient may not have the desired effects due to the inability of the extra insulin to cross BBB, in which case the intranasal administration may provide better outcomes. Regardless of the root cause, if we consider insulin signaling perturbations to be one of the contributors to memory loss and AD-like symptoms in our model, then both the proximal and distal insulin signaling pathways should be altered in the hippocampal region. Proximal insulin signaling consists of an insulin-IR/IGF-1R-IRS axis, whereby the binding of the insulin to the extracellular alpha subunit of the IR or IGF-1R initiates a series of Tyr autophosphorylations in the beta subunit, disinhibiting intracellular Tyr kinase activity towards IRS, thereby allowing these adaptor molecules to interact with a large number of targets. Once activated, IRS1 and 2 are further regulated via a highly complex mechanism involving multiple Ser/Thr kinases, which can phosphorylate the tail regions of IRS molecules at over 50 Ser/Thr residues. Unlike Tyr phosphorylation by the IR, which activates IRS, modifications at Ser/Thr residues are capable of both promoting and inhibiting downstream IRS-mediated signaling [57]. As previously mentioned, cerebral insulin resistance has been implicated in AD pathogenesis. Such a resistance may stem from defects in IR itself [58], or may be mediated via negative regulation of the IRS. Our data suggest that the IR is functional in the hippocampi of 6 months-old HFD-treated WT and APP/PS1 mice. Furthermore, we have also detected a significant increase in *igf1* transcripts in WH vs. WC animals, possibly in response to cerebral insulin deficiency. We then considered the possibility that the downregulation of the IRS pathway, via inhibitory Ser/Thr phosphorylation, may attenuate downstream insulin signaling. As it is nearly impossible to determine the phosphorylation state of all of the 50 + Ser/Thr residues of the IRS molecules within the scope of a single paper, we have performed immunoblotting analysis of mouse pSer^{612}-IRS1 and pSer^{723}-IRS2 (corresponding to human pSer^{616}-IRS1 and pSer^{731}-IRS2). Elevated levels of pSer^{616}-IRS1 were previously detected in neurons of AD patients and were associated with insulin resistance [59], and an increase in pSer^{723}-IRS2 protein levels was observed in dorsal root ganglia neurons of diabetic mice [60]. As indicated in our findings, there were no differences in pSer^{612}-IRS1 for any groups. However, we detected a significant increase in hippocampal pSer^{723}-IRS2 in WH, TC and TH mice. While our analysis is by no means exhaustive, it does support the hypothesis that the hippocampus-dependent memory loss is, at least in part, dependent on insulin signaling deficiencies.

Ser/Thr phosphorylation of the IRS molecules is mediated by both insulin-dependent (autologous) and insulin-independent (heterologous) kinases. Autologous regulation is thought to be the predominant form of downstream insulin-mediated signaling under physiological conditions. In a disease state, however, preferential activation of the heterologous pathway may contribute to the underlying pathology. Cellular stress and/or proinflammatory phenotype may cause inappropriate Ser/Thr modifications of IRS, which may result in the "hijacking" of the normal physiological route. In the current study, we measured hippocampal expression of IRS-regulating Ser/Thr kinases, which are also known to play a role in neurodegenerative diseases. These include autologous (Akt, ERK 1/2), heterologous capable of phosphorylating IRS in the basal cellular state (AMPK, GSK3β), as well as heterologous kinases activated in response to cellular stress and sympathetic activation (JNK1) [57].

4.1. Insulin-IRS-Akt-GSK-3β pathway

Apart from the involvement of insulin, insulin-related molecules and their receptors in the cognitive loss observed in HFD-fed animals, we would like to further discuss key pathways and molecules related to insulin signaling. This pathway is one of the major regulators of distal insulin signaling. Activated IRS recruits PI3K, thus activating downstream signaling cascade involving 3-phosphoinositide-dependent protein kinase-1 (PDK1), Akt and GSK-3β. In the context of glucose regulation, GSK-3β promotes glycogen synthesis, but it is also one of the principal kinases responsible for Tau phosphorylation [61]. Successful activation of Akt requires not only phosphorylation at Thr308 by PDK1, but also additional "priming" at Ser473 residue by mammalian target of rapamycin (mTOR) complex [62]. The activity of GSK-3β is, in turn, negatively regulated by phosphorylation at Ser9 residue by activated Akt, as well as positively regulated by autophosphorylation at Tyr216 [63]. Our data demonstrate both HFD- and transgene-dependent decrease in hippocampal pSer^{473}-Akt levels, accompanied by an increase in activated pTyr^{216}-GSK-3β protein levels in 6-months-old mice. Furthermore, we have also observed an upregulation in pSer^{404}-Tau, which suggests abnormalities in IRS-Akt-GSK-3β signaling. These results are in line with previously published postmortem examinations of the brains of human patients suffering from AD and diabetes [64].

4.2. AMPK, PPAR and ERK

ERK has been implicated as a key molecule involved in hippocampal memory consolidation. While directly stimulated by insulin, it may also be partially activated via IRS-independent mechanisms [57], thus offering a potentially attractive modulatory target for the pharmaceutical intervention. ERK acts via ERK/CREB/CBP pathway, activation of which ultimately results in the transcription of genes required for neuronal plasticity and long-term potentiation (LTP), in particular. In the brain, ERK was shown to be positively regulated by AMPK [65], a key energy sensor in the peripheral tissues, the role of which in the CNS remains controversial [66]. In addition, activated ERK was shown to be recruited (thus activating) to PPARγ, a nuclear receptor and a transcription factor which activates a number of genes related to insulin sensitivity and cognition [67]. We did not detect any changes in the α subunit of the AMPK, nor in PPARα and γ levels, but we did observe an increase in pThr^{202}/pTyr^{204}-ERK1/2 in response to HFD treatment. Considering that ERK expression is positively correlated with memory consolidation our results may seem contradictory, however this is not the case. Feld *et al* [68] have recently demonstrated that a minimum threshold of ERK expression is required to maintain memory function, and that the aberrant overexpression of ERK protein leads to memory impairment in a similar manner as ERK deficiency.

4.3. JNK1

This stress activated pro-inflammatory and pro-apoptotic MAPK has been previously linked to neurodegeneration and to AD, albeit the existing evidence for its importance is rather strenuous (reviewed in [69]). There is much more support for the involvement of JNK1 in diet-induced obesity and the peripheral insulin resistance (reviewed

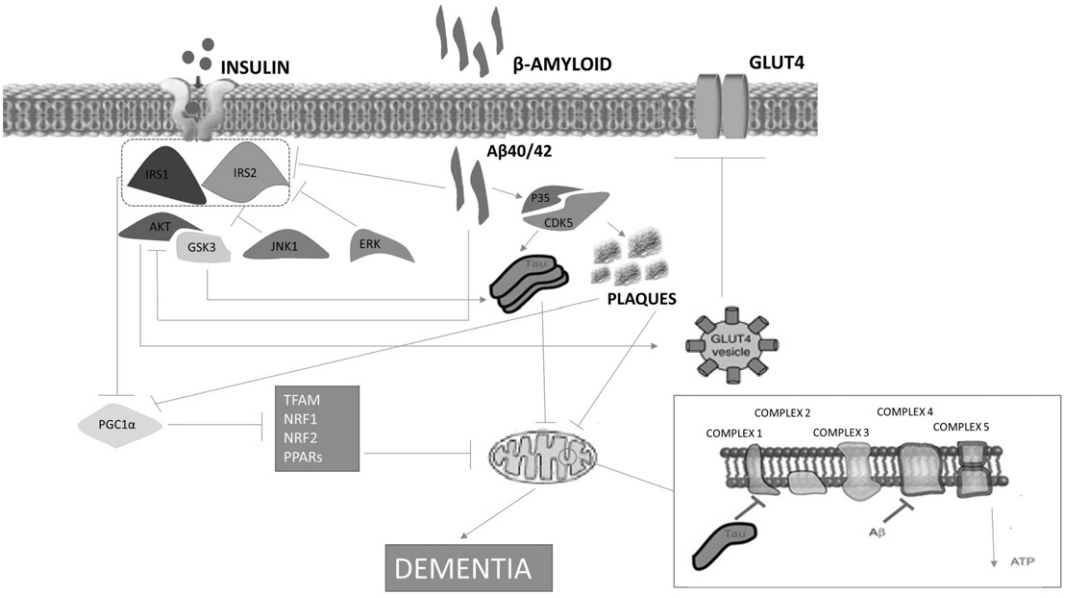

Fig. 8. A proposed mechanism whereby insulin signaling and mitochondrial dysregulation contribute to hippocampal phenotype in HFD-fed and AD-like mice. Increased circulating triglyceride levels in response to HFD treatment initially provoke cerebral insulin deficiency, which results in the downregulation of the canonical insulin signaling pathway in the hippocampus. At the same time, the free fatty acid-mediated increased metabolic stress subverts autologous IR-IRS-Ser/Thr kinase axis signaling, thus favoring heterologous regulation. This route, once activated, initiates a series of self-propagating events which ultimately lead to insulin resistance in a manner similar to that observed in response to elevated β-amyloid levels. As functional IR signaling in the hippocampus is, at least partially, regulating neuronal glucose entry [74], the inhibition of this pathway may result in the reduced supply of readily available energy to the mitochondria, affecting neuroplasticity. Mitochondrial OXPHOS metabolism deficiencies may thus be explained by two possible mechanisms: (a) as a direct result of reduced glucose availability, and (b) as a consequence of decreased mitochondrial biogenesis (PGC1) and/or disruptions to mitochondrial function due to hyperphosphorylated Tau protein and increased β-amyloid levels.

in [70]). The fact that we detected a significant increase in pThr^{183}/pTyr^{185}-JNK1 in the hippocampi of HFD-fed mice, irrespective of the β-amyloid load, is curious for two reasons. One: it suggests that hippocampal insulin signaling may in fact be strikingly similar to the peripheral phenotype. Two: very high levels of β amyloid, as observed in 6-months-old APP/PS1 control animals, are not sufficient to trigger elevated JNK1 expression.

4.4. CDK5

Cyclin-dependent kinase 5 (co-activated by p35) does not phosphorylate IRS directly, however increased activity of this kinase (and GSK-3β) contributes to Tau hyperphoshorylation, resulting in neurofibrillary tangle formation (reviewed in [71]). We have detected a significant upregulation of both the CDK5 and GSK-3β kinases in the hippocampi of HFD-fed WT mice. These data correlate well with the observed increase in pSer^{404}-Tau.

4.5. PGC-1α

Our data demonstrate a significant decrease in insulin signaling and impaired glucose homeostasis in the hippocampus. As hippocampal neurons require copious amounts of energy in order to be able to form and retain memories, such deficiencies will likely have an effect on mitochondrial energy metabolism. Therefore, in the latter part of this study, we have focused on the pathways related to mitochondrial biogenesis and OXPHOS pathway.

The peroxisome proliferator-activated receptor-γ coactivator-1α (PGC-1α) is a transcriptional co-activator which, apart from other functions, plays a critical role in mitochondrial biogenesis. PGC-1α was previously shown to regulate the expression, either directly or indirectly, of the nuclear respiratory factors 1 (NRF1) and 2 (NRF 2), estrogen-related receptor α (ESRRa) and mitochondrial transcription factor A (TFAM) (reviewed in [72]). While we have observed a significant reduction in PGC-1α protein levels in response to HFD treatment, and various statistically significant changes in the above mentioned molecules, most of the differences were rather modest in numerical values, and may or may not represent the behavior of individual targets under similar experimental conditions. Taken together, however, our data suggest clear impairments in mitochondrial function. Regarding the expression of OXPHOS proteins, we detected significant decreases in mitochondrial OXPHOS metabolism in both the HFD-fed WT mice and in APP/PS1 animals. This finding is especially significant as OXPHOS deregulation has been previously linked to both the βA and Tau pathologies in AD brains (reviewed by [73]).

In conclusion, we have demonstrated some parallels between the hippocampus-dependent HFD-induced memory loss vs. the memory loss occurring in a mouse model of Alzheimer disease. Our results indicate that the brain β-amyloid levels seem not to be the primary cause of the HFD-induced memory perturbations. It appears that the reductions in brain insulin signaling and the resulting mitochondrial dysfunction are among the key culprits leading to cognitive decline in early-stage AD-like rodent models. A summarized view of our hypothesis is provided in Fig. 8.

Disclosure statement

The authors declare no competing financial interests.

Conflict of interest

All authors don't have any actual or potential conflicts of interest including any financial, personal or other relationships with other people or organizations. All authors have reviewed the contents of the manuscript being submitted, approve of its contents and validate the accuracy of the data.

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