High fat diet treatment impairs hippocampal long-term potentiation without alterations of the core neuropathological features of Alzheimer disease

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HIGHLIGHTS:

- high fat diet could not trigger Alzheimer's Disease (AD) pathology in a knock-in mouse model
- short or long term high fat diet exposure did not affect amyloid beta production, Tau phosphorylation, or cognitive performance.
- Long term diet treatment triggers a decrease of N-acetyl aspartate/myo-inositol metabolite ratios in the hippocampus.
- Impaired long term potentiation is seen in hippocampal acute slices after 10 months of high fat diet exposure.
- Opposite to previously published work, no alterations in hippocampal insulin response or insulin downstream signalling is observed in high fat diet-treated mice.

Type 2 diabetes (T2DM) and obesity might increase the risk for AD by 2-fold. Different attempts to model the effect of diet-induced diabetes on AD pathology in transgenic animal models, resulted in opposite conclusions. Here, we used a novel knock-in mouse model for AD, which, differently from other models, does not overexpress any proteins. Long-term high fat diet treatment triggers a reduction in hippocampal N-acetyl-aspartate/myo-inositol metabolites ratio and impairs long term potentiation in hippocampal acute slices. Interestingly, these alterations do not correlate with changes in the core neuropathological features of AD, i.e. amyloidosis and Tau hyperphosphorylation. The data suggest that AD phenotypes associated with high fat diet treatment seen in other models for AD might be exacerbated because of the overexpressing systems used to study the effects of familial AD mutations. Our work supports the increasing insight that knock-in mice might be more relevant models to study the link between metabolic disorders and AD.

INTRODUCTION

Alzheimer disease (AD) is a progressive neurodegenerative disorder characterized by the presence of extracellular amyloid plaques (composed of amyloid-beta (Aß) peptide) and intracellular neurofibrillary tangles (formed by aggregates of hyperphosphorylated Tau) (Hardy and Higgins 1992). These hallmarks are also accompanied by a cascade of pathological events including neuroinflammation, synaptic dysfunction, neuronal loss and brain atrophy which eventually leads to cognitive deficits and memory loss (De Strooper and Karran 2016). Rare forms of the disease, called familial or early onset, are caused by autosomal dominant inherited mutations in amyloid precursor protein (APP) and presenilins (PS) genes, which alter the length and hydrophobicity of the Aß peptide, triggering the formation of amyloid plaques (Chávez-Gutiérrez et al. 2012; Szaruga et al. 2017). However, the vast majority of AD cases (>99%) are called sporadic and their etiology is still not known. There are several risk factors associated with the sporadic forms of the disease. The strongest one is ageing (Swerdlow 2011), but there are also genetic risk factors, such as the presence of the ApoE4 allele (Holtzman, Morris, and Goate 2011).

Another important risk factor for AD is type 2 diabetes *mellitus* (T2DM). Epidemiological studies suggest that T2DM increases the risk for AD by 2 fold (Biessels et al. 2006). Interestingly, T2DM alone has also been associated with cognitive dysfunction independent from AD, with T2DM patients showing cognitive decline and brain atrophy (Biessels and Reijmer 2014). T2DM is a metabolic disorder characterized by blood hyperglycemia, hyperinsulinemia and peripheral insulin resistance (Wu et al. 2014). This disorder is usually accompanied by other comorbidities such as obesity, peripheral inflammation, dyslipidemia or hypertension altogether constituting the metabolic syndrome (Kaur 2014).

Different groups have studied the association between metabolic syndrome and AD by using a diet-induced obesity, which triggers the development of peripheral insulin resistance and T2DM in different transgenic mouse models for AD (Heydemann and Ahlke 2016). However, the effect of the diet-induced T2DM on AD- pathology is still controversial. For instance, some studies reported no alterations on brain amyloidosis even after 13 months of high fat diet exposure (Knight et al. 2014; Studzinski et al. 2009), while others described increased amyloid beta levels already after 5 months of high fat diet (Ho et al. 2004; Petrov et al. 2015).

In this paper, we studied the effect of short and long term high fat diet treatment on a novel *APP* knock-in mouse model carrying a humanized Aß sequence with the Swedish mutation (KM670/671NL) (NL) under the murine endogenous promoter (Saito et al. 2014). This clinical mutation leads to dominantly inherited AD in humans (Mullan et al. 1992). However, although these mice present an overproduction of Aß, they do not form amyloid plaques when bred in control conditions and do not show cognitive impairments even at 18 months of age (Masuda et al. 2016).

Here, we tested the hypothesis of whether diet-induced T2DM is sufficient to trigger the appearance of AD-like pathology in this predisposed background for AD.

MATERIALS AND METHODS

Animals and diets

Homozygous $App^{NL/NL}$ knock-in mice generated by (Saito et al. 2014) were used for this study. Male animals, generated in a C57/Bl6 background, were housed in standard mouse cages (3-5 mice per cage) with wood-shaving bedding. Food and water were available *ad libitum* in temperature and humidity controlled rooms with a 12-hour light-dark cycle. All experiments were approved by the institutional ethical committee of the KU Leuven for use on experimental animals. At 2 months of age, littermate mice were separated into two independent groups and feed with either control diet (ssniff DIO D12450B) containing 20% kcal from protein, 70% kcal from carbohydrates, 10% kcal from fat; or high fat diet (DIO D12492) containing 20% kcal from protein, 20% kcal from carbohydrates, 60% kcal from fat) from (see **Table 1**). At either 6 or 18 months of age, mice were behaviorally tested for 1 month, then they underwent proton magnetic resonance scanners. Finally, mice were sacrificed by cervical dislocation. Fat in the abdominal cavity was measured and brains were snap frozen in liquid nitrogen for further biochemical analysis. For immunostainings, mice were intracardially perfused with PBS and 4% PFA for fixation. Finally, for the electrophysiological recordings, a different cohort was maintained in either control or high fat diet for 10 months, and hippocampal acute slices were prepared.

Ingredients (Kcal)	Control Diet (DIO12450B)	High fat Diet (DIO12492)
Casein, 80 Mesh	800	800
L-Cystine	12	12
Corn Starch	1260	0
Maltodextrin 10	140	500
Sucrose	1400	275
Soybean Oil	225	225
Lard	180	2205
Cholesterol (mg) / kg	51.6	279.6
Vitamin Mix V10001	40	40

Table1: Diets composition

Metabolic measurements

All the metabolic measurements were carried out in starvation conditions (6 hours fasting).

- Serum extraction and insulin detection

Blood was extracted from the aorta after mouse decapitation, and 1/10 dilution of sodium citrate (3.2%) was added to prevent coagulation. Blood samples were spun down for 10 minutes at 5000xg at 4°C and the supernatant (serum) was collected. Insulin levels were measured with the mouse insulin ELISA kit (Mercodia #10-1247-10) following the protocol provided by the manufacturer. 10µl serum was used for detection. Number of animals was: n=8 for control; n=9 for high fat diet treated animals (HFD).

-Glucose tolerance test

This test was performed after 5 weeks of high fat diet exposure. Mice were fasting for 6 hours before receiving an intraperitoneal injection of 2 g per kg of body weight of D-glucose diluted in sterile 0.9% NaCl solution. Blood was collected from the tail vein at 0, 15, 30, 60, 90 and 120 minutes after the injection; and glucose levels were immediately measured with a glucometer (OneTouch Verio). 7 animals per diet group were used for the test.

-Insulin tolerance test

This test was performed after 5 weeks of high fat diet exposure. Mice were fasting for 6 hours before receiving an intraperitoneal injection of insulin (0.35U per kg of body weight diluted

in 0.9% NaCl solution and 0.8% bovine serum albumin. Blood was collected from the tail vein at 0, 15, 30, 60, 90 and 120 minutes after the injection; and glucose levels were immediately measured with a glucometer (OneTouch Verio). 7 animals per diet group were used for the test.

Soluble Aß extraction and ELISA detection

For soluble *Aß* extraction, frozen hippocampi were homogenized in 200 µl of tissue protein extraction reagent (Pierce) supplemented with complete protease inhibitors (Roche) using a 22G syringe. Samples were spun down 5 minutes at 5000 x g and supernatants were centrifuged 1 hour at 100,000 x g at 4°C (Beckman TLA 100.4 rotor). Supernatants (soluble fraction) were recovered and protein concentrations were quantified with the Pierce®BCA Protein Assay kit (Prod #23227). To extract the deposited Aß, pellets were further solubilized with GuHCl (6M GuHCl/50mM Tris-HCl), sonicated and centrifuged 20 minutes at 70000 rpm at 4°C. *Aß* peptides were quantified by ELISA using Meso Scale Discovery (MSD) 96-well plates. MSD plates were coated over night with the human JRF cAb040/28 antibody for Aß40 or JRF Ab042/26 antibody for Aß42 (Janssen Pharmaceutica), all used at 1.5 mg/ml diluted in 1x PBS. Next day, plates were washed 5 times with PBS-0.05% Tween 20 (washing buffer) and blocked with 0.1% casein for 1 hour at room temperature. 25 µl of standard curve (serial dilutions of Aß40 and Aß42 rPeptide) and samples, were loaded with 25 µl of detection antibody (Janssen huAB25-HRPO) diluted in 0.1% casein and were incubated over night at 4°C. After washing, the plate was developed with 2x Read Buffer and measured immediately on MSD Sector Imager 6000 n=5-6 animals per diet group.

Tissue homogenization and western blotting

Hippocampal tissue was homogenized in protein extraction buffer following the same protocol described for the soluble *Aß* extraction. 50 µg of proteins were separated by SDS-PAGE (NuPAGE® Novex 4-12% Bis-Tris gel; Invitrogen), transferred to a 0.2 µm nitrocellulose membrane, and probed with specific primary antibodies listed below (**Table 2**). Immunodetection was done with horseradish peroxidase-coupled secondary antibodies (Bio-Rad, 1/5000) and the chemiluminescent detection reagent Renaissance (PerkinElmer Life Sciences). n=5-6 animals per diet group

Antigen	Host	Dilution	Manufacter
ß-actin	Mouse	1/5000	Sigma
Insulin receptor	Rabbit	1/500	Santa Cruz
IRS-1	Mouse	1/500	BD Bioscience
AKT	Rabbit	1/1000	Cell Signaling
AKT phospho Ser473	Mouse	1/500	Cell Signaling
GSK-3ß	Rabbit	1/1000	Cell Signaling
GSK-3ß phospho Ser9	Rabbit	1/500	Cell Signaling
PHF-Tau clone AT180	Mouse	1/500	Thermo Scientific
PHF-Tau clone AT270	Mouse	1/500	Pharmingen
Iba 1	Rabbit	1/500	Synaptic systems
GFAP	Rabbit	1/1000	Dako
B63	Rabbit	1/200	(Zhou et al. 2011)

Table 2: Primary antibodies used for western blot

RNA extraction and real time semi-quantitative PCR for pro-inflammatory cytokines

For RNA extraction, hippocampi were homogenized in 1ml Trizol (Invitrogen, Life Technologies Corporation) using a syringe needle. After the addition of 200 µl chloroform, samples were centrifuged and the aqueous phase was mixed with 1.25 volumes ethanol 100%. Solution was transferred to miRVana spin columns (Ambion, Life Technologies Corporation) and washing and elution steps were done following the protocol described by the manufacturer. Reverse transcription of 200ng RNA was performed using the Superscript II reverse transcriptase (Invitrogen, Life Technologies Corporation). Real time semi-quantitative PCR was performed using the SensiFast SYBR No-Rox Kit (GC biotech BV). Cp (crossing points) were determined by using the second derivative method, and were normalized to two housekeeping genes (Actin and GAPDH). Fold changes were calculated with the ΔΔCt method (Livak and Schmittgen 2001). The primer sequences used are listed in **Table3.** *n*=5-6 animals per group.

Primers	Sequence
IL_6 forward	5' TCCAGAAACCGCTATGAAGTTC 3'
IL_6 reverse	5' CACCAGCATCAGTCCCAAGA 3'
CCL2 forward	5' TTAAAAACCTGGATCGGAACCAA 3'
CCL2 reverse	5' GCATTAGCTTCAGATTTACGGGT 3'

Actin forward	5' TCCTCCCTGGAGAAGAGCTA 3'	1
Actin reverse	5' GCAATGATCTTGATCTTC 3'	2
GAPDH forward	5' TTGATGGCAACAATCTCCAC 3'	
GAPDH reverse	5' CGTCCCGTAGACAAAATGGT 3'	3

Table 3: Primers sequences used for RT-qPCR

Behavioral tests:

For behavioral experiments, animals were transported to a different animal house where they habituated for 1-2 weeks. The two different cohorts (6 and 18 months of age) were tested independently at different times. Following numbers of animals were used: 6 month-old (*n*= 11 control; *n*=10 HFD); 18 month-old (*n*= 10 control; *n*=11 HFD). Mice were randomized according to the diet treatment. Every animal went through all the behavioral tasks in the following order: 1) Open field; 2) Social preference/social novelty; 3) Elevated plus maze; 4) Contextual fear conditioning.

-Open field

Locomotor activity was measured in a 50 cm × 50 cm arena (transparent Plexiglass), illuminated by 2 bright spots. Mice were first habituated to the dark for 30 minutes and then placed in the illuminated arena for 10 minutes free exploration. Free movement was recorded and analyzed using ANY-maze™ Video Tracking System software (Stoelting Co., IL, USA).To assess locomotion, total distance travelled and speed were quantified.

-Elevated plus maze

The elevated plus maze consisted of two open arms (21 cm × 5 cm) and two arms enclosed by high walls (21 cm × 5 cm). The same type of arms were located opposite to each other and they were elevated 30 cm from the surface. Animals were placed in the center of the maze and left 10 minutes for free exploration. The activity of the mice was monitored by five infrared beams situated in the open and enclosed arms.

-Contextual fear conditioning

Context- and cue-dependent fear conditioning was studied using a protocol adapted from (Paradee et al. 1999) and described earlier (Salas et al. 2017). Briefly, mice were first placed for 5 minutes in the StartFear cage (Panlab, Spain) with a specific context (Context A) for acclimation. The next day, mice were replaced in the same context A and after 2 minutes of exploration, they were exposed twice to a 30s tone (4 kHz, 80 dB) that co-terminated with a mild footshock (2s, 0.3 mA) with an inter-stimulus interval of 60s. 24 hours later, fear memory of context and cue was measured. For contextual memory, animals were placed again in context A for 5 minutes and freezing was recorded. Thereafter, the animal was returned to its home cage and 90min later tested for cued fear memory. For this test, mice were placed in a novel context (distinct from A in visual, tactile and olfactory dimension) for 6 minutes. After 3 minutes of exploration (new context), the tone (cue) was delivered for 3 minutes (Tone test). During each trial, freezing behavior was recorded by a pressure sensitive weight transducer system (Panlab, Spain). The percentage of freezing was calculated per trial as reliable readout for innate and acquired fear in rodents.

-Acoustic test

Mice were placed in a small restrainer inside the StartFear cage (Panlab, Spain) used for the contextual fear conditioning test. After 5 minutes habituation, animals were exposed to 10 tones at different frequencies (3, 4, 7, 10, 15 kHz) for 12 milliseconds. Each tone was separated by a fixed pre-startle time of 12 seconds and an alternated interval between 8 and 12 seconds. Each different frequency was repeated 10 times at random orders. Percentage of startle responses at 4 kHz was calculated by manual quantification of the number of positive startles responses out the 10 exposures to 4 kHz tone.

-Social preference/social novelty

Sociability test was based on a protocol described by (Nadler et al. 2004), and modified and explained in detail by (Naert et al. 2011). A transparent plexiglass box (94 x 28 x 30 cm^3) was divided in three different chambers. Left and right chamber contained cylindrical wire cups (height x diameter: 11 × 12 cm) that could contain a stranger mouse and were connected to the central chamber via manual guillotine doors (w × h: 6 × 8 cm). Mice movement was

recorded by two cameras located 60 cm above the setup and ANY-maze™ Video Tracking System software (Stoelting Co., IL, USA) was used for the analysis. Three consecutive trials were performed. Between each trial, the test mouse remained in the central chamber. During the first, acclimation, trial, the mouse was placed in the central chamber for 5 min with divider doors closed. In the second (Social preference) trial (10 min), a stranger mouse (stranger 1) was placed into a wire cup in either left or right chamber while the other chamber held an empty cup. Guillotine doors were opened and the test mouse could freely explore all three chambers. Finally, during the third (Social novelty) trial (10 min), the empty cup was replaced with one containing a second stranger mouse (stranger 2) while stranger 1 remained in the same place. Doors were lifted again to give access to all three chambers and free exploration to STR1 or STR2. Exploration was defined as time the head of the test mouse was within 3 cm of the wire cup. For data analysis we calculated the discrimination index during the Social preference trial as: [Time stranger 1-Time empty/(Time stranger1 + Time empty)] and during the Social novelty trial as: [Time stranger 2-Time stranger 2/(Time stranger 1+ Time stranger 2)].

Proton magnetic resonance imaging and spectroscopy

Magnetic resonance data was acquired using a small-animal 9.4 Tesla MRI scanner (Biospec 94/20, Bruker Biospin, Ettlingen, Germany). The scanner was equipped with a 20 cm horizontal bore magnet and actively shielded gradients (600 mT m^{-1}, inner diameter 11.7 cm). A linearly polarized resonator (7 cm diameter) was used for transmission, combined with a mouse brain surface coil for reception (both Bruker Biospin, Ettlingen, Germany). Mice were anaesthetized with isoflurane (3-4% for initiation and 1-2% during scanning) in 100% oxygen. During the experiments the respiration was controlled and maintained at 60-100 min^{-1} and body temperature at 37±1 °C. After acquisition of localizer images, 2D multi-slice MR images were acquired for planning of the MR spectroscopy (spin echo sequence, slice thickness 0.5mm, TR=2000ms, TE=35ms). Images were processed using Paravision 5.1 (Bruker Biospin). Single voxel MR spectra were acquired as described before (M I Osorio-Garcia et al. 2011; M. I. Osorio-Garcia et al. 2011)]. ^{1}H-MRS voxel were placed in the hippocampus selecting a volume of interest (VOI) of 1.3 x 2 x 2.2 mm^3. MR spectra were acquired using a PRESS sequence with implemented pre-delay outer volume suppression and water

suppression using the VAPOR method. All spectra were acquired with 256 averages, TE=20ms and TR=1.8s. Shimming of the static field was performed using FASTMAP. Spectra were processed using the jMRUI software (Stefan et al. 2009). Only metabolites with a CRL < 25% were considered for analysis. The following metabolites were quantified with the QUEST algorithm (Ratiney et al. 2004) in jMRUI using a simulated (nmrscopeb) metabolite basis set: Creatine (Cr), Glutamate (Glu), myo-inositol (mIns), N-acetyl aspartate (NAA), Taurine (Tau). Results are reported in reference to the non-suppressed water signal. Statistical significance for differences was tested using multiple T-test with a false discovery rate correction (FDR) correction (GraphPad). The total number of animals used was: *n*=8 control diet (CD), 7 high fat diet (HFD), for the 9 month-old cohort; n=9 CD, 10 HFD for the 18 month-old group. For the mIns and NAA/mIns ratio measurements *n*=6 CD, 5 HFD (9 month-old cohort) and *n*=4 CD, 8HFD (18 month-old cohort) after removal of animals with a CRL < 25%.

Immunofluorescence and confocal microscopy

20 month-old $App^{NL/NL}$ mice were anesthetized with CO_2 and intracardially perfused with phosphate-buffered saline (PBS) followed by 4% paraformaldehyde (PFA) solution. Brains were then removed, post-fixed for 48 hours in 4% PFA and cut into 50µm-thick slices using a Leica VT1000S vibratome. Before immunofluorescence, antigen retrieval was performed by microwave boiling the slides in 10 mM tri-Sodium Citrate buffer pH 6.0. After that, slices were blocked for 1 hour with 3% normal goat serum and 0.3% x-triton in PBS and incubated with primary antibody diluted in blocking buffer: [Rabbit Iba 1 (1/500 Wako # 019-19741), Guinea pig GFAP (1/1000 Synaptic systems # 173004)]. After 1.5 hours at RT, slices were washed with PBS and incubated for 1.5 hours with secondary antibody: [Alexa 568 goat anti rabbit (1/500 Invitrogen #A11011), Alexa 488 Goat Anti-Guinea Pig (1/500 Jackson Immunolabs #106-545-003)]. Nuclei staining was performed incubating the slices with DAPI (SIGMA) before mounting. Images were acquired on a Leica SP8 confocal microscope and quantified with Fiji software. *n*=3 animals per diet group were assessed.

Electrophysiology recordings

Hippocampal acute slices and electrophysiological recordings were done as described previously in (Marciniak et al. 2017). Briefly, mice were sacrificed by cervical dislocation and dissected hippocampi were placed into ice cold artificial cerebrospinal fluid (ACSF) (117mM NaCl, 4.7mM KCl, 2.5mM $CaCl_2$, 1.2mM $MgCl_2$, 1.2mM NaH_2PO_4, 25mM $NaHCO_3$, and 10mM glucose) continuously oxygenated with 95% O_2, 5% CO_2. After that, 400-µm-thick slices were prepared at 4°C with a custom-made tissue chopper and transferred into a submerged-type recording chamber where they were maintained at 32°C, constantly superfused with oxygenated ACSF at a rate of 2.5 ml/min. After an incubation period of at least 1 hour, a tungsten electrode was placed in the stratum radiatum of area CA1 to evoke field excitatory postsynaptic potentials (fEPSPs) which were recorded about 200 µm apart using ACSF-filled glass micropipettes (2–5 MΩ). Signals were recorded and amplified with an A-M Systems 1700 differential amplifier. Long-term potentiation was induced by theta-burst stimulation (10 burst of four 100 Hz stimuli (double pulse width), separated by 200 ms. . LTD was induced by the application of 0.5µM insulin into the ACSF for 30 minutes. All values were expressed relative to the control level (percentage of baseline). n= 6 animals per diet group.

Statistical analysis

All data are shown as mean ± standard error of the mean (SEM). Statistical significance was evaluated using GraphPad Prism 7.01 (La Jolla, California, USA). Differences between mean values were determined using unpaired t-test or 2-way analysis of the variance (ANOVA) with Bonferroni's test for multiple comparisons. To test for inter-group differences in the electrophysiological time-series, glucose and insulin tolerance tests, a repeated measures ANOVA (RM-ANOVA) was used. Significance level was set as $p<0.05$ for all the experiments.

RESULTS

1. **High fat diet treatment induces metabolic syndrome in *App*$^{NL/NL}$ mice**

We first investigated whether our high-fat diet treatment was sufficient to trigger obesity and T2DM in *App*$^{NL/NL}$ mice. To this end, we exposed two month-old mice to either a control or a high fat diet for 4 months and we analyzed different metabolic indices.

Firstly, we assessed body weight every two weeks from the beginning of the diet treatment As shown in **Fig1A**, by week 4, body weight was significantly increased in high-fat diet treated mice ($F_{(8,144)} = 248.3$; $p<0.0001$, Diet x Time, RM-ANOVA). Changes in body weight were accompanied by a 3-fold increase in abdominal fat compared to controls (control: 0.6 ± 0.06 vs high fat: 1.8 ± 0.08 g; $p<0.0001$, unpaired T-test) **(Fig1B).**

Next, we evaluated whether the diet treatment also induced blood hyperglycemia and hyperinsulinemia at 6 months of age. Fasting glucose measurements revealed a 1.7-fold increase in blood glucose in high fat-fed mice levels compared to controls (control: 152.7 ± 9.2 vs high fat: 256.9 ± 6.7 mgr/dL; $p<0.0001$, unpaired T-test) **(Fig1C)**. We also analyzed serum insulin levels by ELISA detection. Mice exposed to high fat diet showed an apparent 2.4-fold increase in serum insulin levels compared to the control group, however, the differences were not significant due to high variability (control: 0.38 ± 0.02 vs high fat: 09 ± 0.26 mgr/dL; $p=0.06$, unpaired T-test) **(Fig1D)**.

Finally, we determined whether the diet treatment affected glucose and insulin responses. The glucose tolerance test showed a clear impairment in blood glucose clearance after the intraperitoneal injection of a highly concentrated glucose solution. Consistently, obese mice did not recover the basal glucose levels in blood even 2 hours after the injection ($F_{(5, 65)} = 3.62$; $p=0.006$, RM-ANOVA, diet x time) **(Fig1E)**. Similarly, the insulin tolerance test revealed no drop in glucose levels in response to an intraperitoneal insulin injection in the high fat diet-treated group, compared to the controls ($F_{(5.60)} = 7.239$; $p<0.0001$, RM-ANOVA, diet x time) **(Fig1F)**, implying a clear peripheral insulin resistance. From these results, we can conclude that four months of high fat diet exposure triggers obesity and T2DM in *App*$^{NL/NL}$ mice.

To assess whether the metabolic alterations were maintained upon longer high fat diet exposure, we analyzed mice after 10 months of diet treatment. We could verify a significant increase in body weight and blood glucose levels **(Supplementary Fig1).** However, the differences in the glucose tolerance test between the two diet groups disappeared after prolonged periods of diet treatments.

2. High fat diet for 4 months does not affect amyloidosis, Tau phosphorylation or cognitive function in 6 month-old *App*$^{NL/NL}$ mice.

We then investigated the consequences of diet-induced T2DM on AD-like pathology in *App*$^{NL/NL}$ brains.

Firstly, we analyzed the production of soluble Aß$_{40}$ and Aß$_{42}$ peptides in the hippocampus from the *App*$^{NL/NL}$ mice at 6 months of age. Differently to what has been previously published in other AD mouse models (Ho et al. 2004; Vandal et al. 2014), high fat diet exposure in this model is not associated with increased soluble Aß levels in hippocampal homogenates (**Fig2A**).

Next, we also performed a guanidine extraction from the deposited pellet (see materials and methods) but we could detect no Aß deposits in the GuHCl soluble fraction (data not shown). Finally, to further study the presence of Aß deposits in the brain, we stained brain slices using the 6E10 antibody against human Aß (BioLegend #803003), but again we could not detect the presence of amyloid aggregates in the brains from these animals (data not shown).

APP ß-cleavage, has been previously suggested to be affected by high fat diet exposure (Maesako et al. 2015). To investigate whether high fat diet treatment affected BACE activity, we measured the levels of APP_C99, released upon BACE cleavage. As shown in **Supplementary Fig2A**, 4 months of high fat diet treatment did not affect the production of APP-C99, implying no alterations in BACE activity.

Then, we evaluated whether high fat diet induced phosphorylation of Tau at disease-linked residues: i.e. at Thr181 (AT 270 epitope) and at Thr231 (AT180 epitope). Western blot analysis from hippocampal homogenates from 6 month-old *App*$^{NL/NL}$ mice revealed no differences in Tau phosphorylation between the two diet groups (**Fig2B**).

We also studied whether diet-induced T2DM has an effect on cognition at 6 months of age, as epidemiological studies from patients suggest (Biessels et al. 2006). We first characterized whether the increase in body weight affected general locomotion and anxiety behavior. As expected, obese mice showed a reduction in average speed movement and distance travelled in the open field test (**Supplementary Fig3A, B**). However, they did not show differences in the anxiety behavior, spending comparable percentage of time in the open arm from the elevated plus maze (**Supplementary Fig3C**).

To avoid that the decreased locomotion observed in obese mice affected the results from the behavioral tasks, we choose two memory tests that do not require excessive locomotor activity: contextual fear conditioning (to measure contextual memory) and social preference/social

novelty test (to measure social memory). Both types of memories were impaired in AD mouse models (Faizi et al. 2012)

In the contextual fear-conditioning test, mice first received an electric shock paired to a tone, in a specific context. During the conditioning phase, high fat diet-treated mice showed a significant decrease in freezing compared to the controls (control: 20.28 ± 1.66 vs high fat: 14.03 ± 2.3 % freezing; p=0.04, unpaired T-test, **Fig2D Conditioning).** However, 24 hours later when animals were placed in the same context (Context test, Fig2C) or exposed to the tone (cued test, Fig2C) both diet groups exhibited a significant increased freezing. Showing no significant differences in contextual (control: 41.15 ± 4.14 vs high fat: 45.54 ± 3.69 % freezing; p>0.05, unpaired T-test) (**Fig2D Context test**) or cued (control: 70.45 ± 4.54 vs high fat: 56.82 ± 5.35 % freezing; p=0.066 unpaired T-test) (**Fig2D cued test**) fear memory.

Then, we evaluated social memory with the social preference/social novelty test. In the first phase of the test (social preference), animals could freely choose to enter a chamber with either an empty cage, or a mouse that was not seen before (stranger 1)). To our surprise, mice exposed to high fat diet exhibited a significant increase in social preference compared to the control-fed group (control: 0.1 ± 0.04 vs high fat: 0.29 ± 0.07, p=0.05 unpaired T-test). In the second part of the test (social novelty), we replaced the empty cage with a second mouse (stranger 2). In this case, both diet groups spent significantly more time exploring the novel versus the familiar mouse, showing a similar discrimination index (control: 0.22 ± 0.08 vs high fat: 0.28 ± 0.09, p>0.05, unpaired T-test) (**Fig2F**). From these results, we can conclude that despite an increased social preference associated with the high fat diet treatment, this group shows no differences in social memory compared to the control diet-treated group.

Finally, we evaluated the effect of high fat diet treatment on neuroinflammation. Other studies have shown changes in hippocampal Iba1 upon high fat diet treatment (Pistell et al. 2010). Here, however, we could not detect significant differences in hippocampal Iba1 or GFAP levels between the control and the high fat diet-treated group (**Fig2G**). To further assess the effect of the diet on brain inflammation we measured the mRNA levels of two different pro-inflammatory cytokines: CCL2 and IL-6, which are upregulated upon high fat diet exposure (Pistell et al. 2010). **Fig2H** shows no significant differences in CCL2 and IL-6 mRNA between the two diet groups, confirming absence of neuroinflammatory signs associated with the diet treatment.

3. High fat diet for 16 months does not trigger Alzheimer's-associated biochemical or behavioral alterations in 18 month-old *App*$^{NL/NL}$ mice

The previous results indicate that high fat diet treatment does not affect AD pathology in 6 month-old *App*$^{NL/NL}$ mice. However, metabolic syndrome and AD are both age-associated disorders, and they mainly develop in old individuals. For this reason, we decided to investigate the effect of a high fat diet treatment in older animals (18months old), exposed to the diet for 16 months.

First, we evaluated the effect of the diet on soluble Aß production in the hippocampus. Similar to what happened in 6 month-old mice exposed to the diet for 4 months, long-term high fat diet did not have an effect on hippocampal amyloidosis, with the two diet groups showing comparable soluble $Aß_{40}$ and $Aß_{42}$ values (**Fig3A**). We also quantified the production of APP-CTF upon ß-cleavage and we did not find significant alterations associated with the diet treatment (**Supplementary Fig2B**). The levels of Tau phosphorylation in the hippocampus were also not changed upon long-term high fat diet exposure. The trends for increased AT180- (control: 1 ± 0.07 vs high fat: 1.4 ± 0.23 normalized values, p=0.12 unpaired T-test) and AT270- stainings (control: 1 ± 0.02 vs high fat: 1.5 ± 0.24 p=0.07 unpaired T-test) in the high fat diet-treated mice, were not statistically significant (**Fig3B**).

We then studied the combined effect of aging and metabolic stress on cognition with the contextual fear conditioning and social preference/ social novelty tests. In the fear conditioning test, high fat-fed animals exhibited a robust decrease of freezing during the habituation phase (controls: 8.29 ± 2.3 vs high fat: 2.02 ± 0.52 % freezing, p=0.01, unpaired T-test) (**Fig3C Habituation).** Consistently, they also showed, albeit not significant, a tendency towards less freezing in the context test (control: 53.1 ± 7.34 vs high fat: 35.7 ± 6.74 p>0.05 unpaired T-test) (**Fig3C Context test**) Moreover, when the mice were placed in a new context for the cued test, the high fat-fed group showed again a significant reduction in freezing during the habituation phase before the tone started (controls: 36.6 ± 5.6 vs high fat: 17.8 ± 3.4 % freezing, p=0.009, unpaired T-test) (**Fig3C New context**). Nevertheless, after exposure to the tone, both groups exhibited comparable extent of freezing (**Fig3D)**. To study possible hearing alterations associated with age, we also tested the auditory capacities and we found no differences between the two diet groups (**supplementary Fig 4**). These data show that long-term high-fat diet only slightly affects cued fear memory in 18 month-old *App*$^{NL/NL}$ mice

Next, we assessed the effect of long-term high fat diet treatment on sociability and social memory. Surprisingly, 18 month-old *App*$^{NL/NL}$ mice fed with the high fat diet showed no differences in social preference compared to the control group (**Fig3E**), in contrast to the increased sociability observed at younger ages. During the social novelty test, obese mice exhibited a trend to a decreased social memory towards the familiar mouse (control: 0.25 ± 0.97 vs high fat: 0.089 ± 0.06 discrimination index, p=0.18, unpaired T-test) (**Fig3F**). Interestingly, while the mean discrimination index from the control diet group was significantly different from 0 (p=0.03, one-sample T-test), which implies a significant preference towards the novel mice; the high fat-fed group was not different. This may suggest mild social memory impairments associated with the long-term high fat diet treatment in older mice.

Finally, we investigated whether longer high fat diet treatment triggered an immune response in the hippocampus of 18 month-old *App*$^{NL/NL}$ mice. **Fig3G** show not changes in the levels of hippocampal Iba1 or GFAP and no alterations in the expression of pro-inflammatory cytokines (**Fig3H**).

Altogether, these data suggest that long-term high fat diet treatment in older animals does not lead to the development of the classical AD hallmarks

4. Proton magnetic resonance spectroscopy revealed a reduction in N-acetyl aspartate/myo-Inositol ratio in long-term high fat diet treated mice.

Previous works suggest that canonical neurodegeneration signs of AD are preceded by subtle biochemical alterations followed by subclinical cellular responses (De Strooper and Karran 2016). In order to assess this possibility, we evaluated the effect of the high fat diet on brain metabolism by proton magnetic resonance spectroscopy (MRS). This noninvasive neuroimaging technique allows the local quantitative measurement of different brain metabolites that are associated with AD (Foy et al. 2011). We performed MRS in the hippocampus from living 9 and 20 month-old *App*$^{NL/NL}$ mice treated with the high fat or control diet for 7 and 18 months respectively. Levels of creatine (Cr), glutamate (Glu), myo-inositol (mIns) N-acetyl-aspartate (NAA) and taurine (Tau) metabolites were not significantly changed after 7 **(Fig4B)** or 18 months of high fat diet exposure **(Fig4E)**. AD progression is associated with a decrease of NAA (marker for neuronal viability) and increased mIns

(marker for gliosis) levels. Thus, a decrease in the NAA/ mIns ratio is commonly used for assessment of the pathology (Wang et al. 2015).When we calculated the NAA/ mIns ratio from the hippocampal region in younger animals, we could not detect significant differences associated with the diet treatment (**Fig4C**). However, in 18 month-old *App*$^{NL/NL}$ animals, the NAA/ mIns ratio was significantly reduced in the high fat diet-treated group compared to the controls (control: 3.6 ± 0.34 versus high fat: 2.4 ± 0.2, p=0.008 unpaired T-test) (**Fig4F**).

5. High fat diet treatment triggers mild impairments in hippocampal long term potentiation

The changes in NAA/mIns ratio observed by MRS in the long-term high fat diet treated mice could reflect either a reduced neuronal viability (reduced NAA) or an increased brain gliosis (increased mIns levels). To directly assess the effect of long-term high fat diet on glia, we stained brain slices with the microglial marker Iba1 and the astrocytic marker GFAP and we quantified the number of Iba1 or GFAP positive cells. As shown in **Supplementary Fig5A, B** we could not find differences in cell number between the control and the high fat diet groups. Because microglia activation is also associated with changes in cell morphology (irregular cell bodies, thickened, partial or complete loss of processes) (Knight et al. 2014), we searched for possible morphological changes in the Iba1-stained sections. The morphology of Iba1 stained cells was not different between high and normal fat fed mice (**Supplementary Fig5C).** These results demonstrate that 18 months of high fat diet exposure are not sufficient to induce microglial activation in the hippocampus despite a reduction in NAA/mIns ratio.

Next, we tested if the reduced NAA/mIns ratio would reflect alterations in neuronal function. To test this possibility, we evaluated synaptic transmission and plasticity of Schaffer collateral-CA1 synapses; functional read-outs that are well documented to be impaired early on in AD mouse models (Rowan et al. 2003). We performed electrophysiological field recordings in acute hippocampal slices from *App*$^{NL/NL}$ mice exposed to a control or long-term high fat diet. First, we investigated potential effects on hippocampal basal synaptic transmission by determining input-output curves, measuring the slope of field excitatory postsynaptic potentials (fEPSP) of CA1 as a function of stimulation intensity applied to the presynaptic Schaffer collaterals fibers from CA3. As shown in **Fig5A,** input-output curves of the two diet groups were virtually identical.

Next, we studied the effect of high fat diet treatment on long-term synaptic plasticity. First, we induced long-term potentiation (LTP) by theta-bust stimulation (TBS) of the presynaptic Schaffer collateral fibers. Interestingly, while the control-diet treated animals exhibited a 50% potentiation of the response four hours after stimulation, the mice exposed to the high fat diet failed to maintain the potentiation already one hour after stimulation, implying an impaired LTP upon high fat diet treatment (50-240 min $F_{(1, 10)}$=29.44, p=0.03, **Fig5B**). Finally, we evaluated whether high fat diet treatment induced defects on insulin-dependent long-term depression (LTD). It has been previously described that 30 minutes of 0.5μM insulin treatment induces depression of fEPSP in acute hippocampal slices (Marciniak et al. 2017). Here, we confirm a decay of fEPSP slopes upon insulin stimulation, when compared to the baseline. However, this insulin-induced LTD was similar between the two diet groups (**Fig5C**).

These results imply two important findings: i) high fat diet treatment impairs hippocampal LTP but not insulin-dependent LTD; and ii) high fat diet treatment does not affect brain insulin responsiveness.

To investigate further whether high fat diet affected brain insulin signaling, we studied the activation of the insulin pathway in the brain under basal conditions. Homogenized cortices from 6 and 18 month-old *App*$^{NL/NL}$ mice, showed no differences in the levels and phosphorylation state of the main proteins involved in insulin signaling pathway between the control or high fat diet-treated groups (**Supplementary Fig6**). Nonetheless, at 6 months of age, the total levels of IRS-1 were significantly upregulated in the high fat-fed mice, compared to the controls (control: 1 ± 0.07 vs high fat: 1.57 ± 0.12 normalized values, p=0.001 unpaired t-test). Interestingly, in old 18 month-old mice, the levels of IRS-1 were no longer altered **(Supplementary Fig6B).**

DISCUSSION

In this study, we have analyzed the effects of high fat diet on brain phenotypes in the *App*$^{NL/NL}$ knock-in mouse model for AD. These mice are homozygous for a causal FAD mutation, which leads to an overproduction of the human Aβ peptide in the brain (Saito et al., 2014). It is unclear why these mice, which are considered "predisposed" to amyloid pathology; do not develop amyloid plaques or other aspects of AD. Given this genetic predisposition, we

hypothesized that an additional trigger could be needed to induce the appearance of the classical neuropathological signs of AD, i.e., Aß deposition and Tau hyperphosphorylation. Nevertheless, despite the induction of obesity and peripheral insulin resistance in the mice, the effects on AD pathology were rather limited. On the other hand, long-term high fat diet exposure promoted a subtle decrease in N-acetyl-aspartate/myo-inositol ratio in the hippocampus and impaired LTP. Further work is required to determine any functional association between these two alterations. Overall, however, the effects of diet on the central nervous system as assessed by behavior, MRS and pathological assessments turned out to be surprisingly mild.

These results are in contrast with previous publications stating a strong effect of high fat diet treatment on brain amyloidosis (see Table 3). Nevertheless, evidence from clinical data suggests that T2DM patients, despite showing an increase risk to develop dementia (Biessels et al. 2006), do not exhibit changes on brain amyloid levels as proven by *in vivo* longitudinal imaging studies with Carbon11–labeled Pittsburgh Compound B (Roberts et al. 2014; Thambisetty et al. 2013). Therefore, our findings showing no effect of metabolic alterations on brain amyloidosis might not be surprising, and, in fact, may provide a better model mimicking the human condition than the previously used overexpression models.

Most of the precedent studies used transgenic mouse models for AD, which overexpress mutant APP and PS genes with exogenous promoters resulting in ectopic expression of the transgenes. To our knowledge, only one group has used previously a knock-in mouse model to address these questions (Studzinski et al. 2009). Interestingly, and similarly to what is reported here, these authors did not find an increased amyloid production associated with the high fat diet treatment. It is thus tempting to speculate that the exacerbated amyloid deposition upon high fat diet treatment found in transgenic mouse models for AD might at least partially result from the excessive and ectopic overexpression of APP and PS. This goes in line with other observations that have previously described phenotypes in overexpression models which need to be interpreted with caution (Saito et al. 2016). In the latter paper authors claimed "60% of the phenotypes observed in Alzheimer's model mice overexpressing mutant amyloid precursor protein (APP) or APP and presenilin are artifacts".

Interestingly, AD transgenic models have also been suggested to develop metabolic disturbances with aging (Macklin et al. 2017). Therefore, it might be that high fat diet

treatment amplifies the metabolic alterations triggered by the transgene expression, leading to more detrimental effects than in the present study.

Another possibility is that metabolic stress driven by the high fat diet may only exacerbate AD-related pathology once the pathology is already present due to more aggressive mutations. This could explain why the high fat diet cannot trigger AD pathology in our phenotypically "normal" knock-in model.

Other important issue that should be taken into account and can explain the high variability of the results is the composition of the diet and potential genetic variability in different mouse strains. Most of the studies used slightly different high fat diet composition which might have an important effect on the phenotype (Heydemann and Ahlke 2016). For example, it has been shown that particularly palmitic acid might affect AD pathology by affecting Aß production and Tau phosphorylation in primary neurons (Patil, Melrose, and Chan 2007). Furthermore, the metabolic responses to high fat diet can be very heterogeneous even within the same mouse strain (Burcelin et al. 2002). In this study we used a commercial high fat diet containing 60% Kcal from lard fat (40% saturated, 60% unsaturated). The same diet has already been shown to induce T2DM and AD-like pathology in a transgenic model for AD (Ho et al. 2004). Nonetheless, we have to acknowledge the limitations of the control diet used here, as it contains high levels of sucrose, which have also been linked to a variety of adverse effects in the brain (Rodrigues at al. 2017). We also have to keep in mind that all the experiments presented were done in male mice, and we cannot exclude an effect of the diet in female animals. Although it has been previously reported that male mice are more vulnerable than females to the high fat diet treatments (Hwang et al. 2010).

Surprisingly, and opposite to what has been published before (Jeon et al. 2012) our data suggest no major effects of the high fat diet treatment on hippocampal neuroinflammation. However, further work is needed to assess potential neuroinflammatory effects of the diet in a more robust manner. In addition, although this has never been suggested before, we cannot rule out the possibility of a potential anti-inflammatory effect of the APP Swedish mutation which might prevent the appearance of diet-induced inflammation in the brain.

We also investigated the effect of high fat diet treatment on cognition. Besides the studies done on transgenic AD models (see Table 3), other groups have shown cognitive deficits using genetic models for T2DM (ob/ob, db/db or Zucker rats) (Winocur et al. 2005) or high fat diet

treatments on wild type mice (Arnold et al. 2014; Pistell et al. 2010), which in this case are not the consequence of overexpression artifacts. Here, we first evaluated the effect of short (4 months) or long (16 months) term high fat diet exposure on contextual fear conditioning. The high fat diet-treated group showed an overall general reduction in freezing, which may interfere with the interpretation of the results. However, given that we could not find significant differences in the freezing responses during the contextual or cued-tests between the two diet groups, we may conclude that high fat diet treatment does not induce major defects in contextual fear memory. Heyward et al. also reported normal contextual fear memory after 5 months of high fat diet treatment in C57Bl/6 animals (Heyward et al. 2012). However, another study showed impaired contextual fear responses in C57BL/6J mice maintained on a high fat diet for 9-12 months (Hwang et al. 2010).

Intriguingly, here we show that short term high fat diet treatment induced an increase in social preference. Another study has also reported an increase in social interaction after 5 weeks of high fat diet treatment (Takase et al. 2016). Authors speculated that a possible explanation for the alterations in sociability could be diet-induced epigenetic changes in the hypothalamus (Takase et al. 2016). Another potential interpretation for the increased sociability, together with the reduced freezing responses shown in the contextual fear conditioning test, is an increased disinhibition as a result of the high fat diet feeding. Nevertheless, the results from the elevated plus maze do not suggest alterations in anxiety.

Despite the diet-induced changes in social preference, high fat diet exposure did not alter social memory, with both diet groups showing a comparable discrimination for the novel mouse. Interestingly, Parashar et al. also reported no differences in social memory in T2DM rats injected with streptozotocin when the memory was assessed immediately after social exposure (similarly to what was done in our study). However, when the memory was tested 24 hours later, they could find a significant reduction in social memory in T2DM rats (Parashar et al. 2017). From these observations, we could speculate that the memory tasks that we used here were not difficult enough to tease out potential mild memory defects triggered by the high fat diet treatment.

Alterations in brain insulin signaling have also been suggested to be partially responsible for the cognitive deficits found in T2DM patients and animals models (Biessels and Reagan 2015). Here, we present strong electrophysiological and biochemical evidence showing no

defects in the insulin response in the hippocampus of *App*$^{NL/NL}$ mice upon high fat diet treatment, opposite to what has been previously published (Ho et al. 2004). The normal brain insulin response found in our mouse model might therefore explain the absence of cognitive deficits presented here.

On the other hand, the long-term diet treatment triggered a reduction in hippocampal N-acetyl-aspartate/myo-inositol (NAA/mIns) ratio. Murray and colleagues have shown that changes in brain metabolites detected by ante-mortem proton magnetic resonance spectroscopy are sensitive biomarkers for early neurodegenerative processes associated with AD progression (Murray et al. 2014). The authors found a correlation between decreased NAA/mIns ratios in the posterior cingulate gyrus and reduced synaptic immunoreactivity. In addition, NAA/mIns ratios negatively correlated with phosphorylated Tau and Aß burden. This suggests that the altered NAA/mIns hippocampal ratio induced by the diet treatment could be associated with an early synaptic dysfunction. In fact, early NAA depletion is a marker of neuronal dysfunction which precedes neurodegeneration induced by a mitochondrial toxin (Dautry et al. 2000). Interestingly, here we also showed an impaired LTP in *App*$^{NL/NL}$ mice treated with high fat diet compared to the control group. Hwang et al. have previously described impairments in hippocampal long term plasticity in obese C57Bl/6 mice, with no alterations in the presynaptic releasing machinery or basal synaptic transmission, similarly to what we show here (Hwang et al. 2010). In addition, it has also been reported that 6 months of high fat diet impairs LTP recorded *in vivo* in rats (Karimi et al. 2013).

To conclude, in this paper we have shown that high fat diet treatment, despite inducing obesity and a T2DM peripheral phenotype, failed to trigger AD pathology in *App*$^{NL/NL}$ mice, a genetically predisposed mouse model for AD. However, the diet treatment impaired hippocampal LTP which correlated with alterations in NAA/mIns ratio.

These results provide a good model mimicking the clinical data shown for T2DM patients (Biessels and Reagan 2015) and call for caution when interpreting the data in the literature (table 3). Further work is clearly needed to understand how obesity and high fat diet exposure affect brain function and whether and how it influences the risk for AD.

Reference	Animal model	Type of Diet	Duration /age	Phenotype
(Knight et al. 2014)	3xTgAD mouse. $PS1_{M146V}$, APP_{Swe}, Tau_{P301L}	60% vs 12% energy from fat	From 2 MO until 4-7-11-15MO	- Impaired Y-maze and MWM from 12MO. - No effect on A-beta or tau. - Increased microgliosis
(Studzinski et al. 2009)	APP/PS1 ΔNL and P264L.	40% versus 10% fat	Starts 1MO until 2 MO.	-Elevated protein oxidation and lipid peroxidation -Altered adipokine levels. - No changes in A-beta levels.
(Vandal et al. 2014)	3xTg-AD (APP_{swe}, PSI_{M146V}, tau_{P301L})	60 vs 12% Kcal from fat	Starts at 6 MO until 15 MO	-Impaired cognition (object recognition and Barnes maze). -Increase in soluble Aß40 and Aß42 in the cerebral cortex (after 9 but not 4 months diet treatment).
(Fitz et al. 2010)	APP23 Tg mice. Swedish mutation	41% Kcal from fat.	Starts at 9 MO until 12-14MO	-Impaired cognition (MWM). -Increased amyloid plaques. -Increased soluble A-beta in the cortex. -Increased ApoE, TNFα levels.
(Graham et al. 2016)	APPswe,PSEN1dE9 WT	16.4% vs 4.6% total fat.	Starts at 2MO until 10MO	-Increased GFAP and IbaI staining in hippocampus and entorhinal cortex -Decrease in NeuN+ in the hippocampus. -Increased Soluble $Aß_{42}$ and amyloid plaques in the hippocampus.
(Julien et al. 2010)	3xTg-AD (APPswe, PS1M146V, Tau P301L)	5% vs 35% fat (w/w) with low o normal n-3:n-6 PUFA ratio	Start at 4 months	-Changes in brain FA profile. - Increased insoluble Abeta. -Increased p-Tau in the cortex. -Decreased postsynaptic debrin -Increased GFAP
(Ho et al. 2004a)	Tg2576 female mice	60 vs 10% fat diet	5 months exposure, 9 MO.	-9MO: increased soluble Aß peptides and Aß plaques. -Impaired MWM. -Altered cortical insulin signaling. - Decreased pAKT pathway -Decreased IDE levels/activity
(Petrov et al. 2015)	APPSwe/PS1dE9	45 kcal % fat	Start at 1MO until 6 MO.	-Impaired NOR in WT and Tg mice - Increased hippocampal insoluble $Aß_{42.}$ -Impaired insulin signaling in the hippocampus. -Increased p-Tau (s404) in Wt and Tg -Decresed IDE levels
(Hooijmans et al. 2007)	APPswe/PS1dE9	1% cholesterol or 0.5% docosahexaenoic acid	Starts at 6MO maintain until 12MO	-Increased hippocampal plaque burden. - Increased relative cerebral blood volume.
(Herculano et al. 2013)	APPswe/PSEN1dE9	32% fat	Starts at 4 MO for 8 weeks.	-Decline in the contextual memory. -No changes in hippocampal amyloidosis. -Increased protein glycosylation (RAGE staining) -Increased activated microglia.

(Nam et al. 2017)	APP23 hemizygous	40 vs 16% calories from fat	Starts at 9 MO for 3 months.	-Increased amyloid plaques -Worsen cognitive performance -Increased expression of immune-related genes. -Downregulation of neuronal and synaptic genes.

Table 3: Studies of high fat diet treatment on Alzheimer disease mouse models

Abbreviations: MWM (Morris water maze), FA (fatty acids), MO (month-old).

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FIGURE LEGENDS

Fig 1: High fat diet treatment induces metabolic syndrome in *App*$^{NL/NL}$ mice

Two month-old *App*$^{NL/NL}$ mice were exposed to either high fat or control diet, and animals were assessed for different metabolic indices at 6 months of age.

(A) Body weight was measured every 2 weeks showing a robust increase in the group exposed to the high fat diet compared to the control diet. **(B)** Wet abdominal fat was removed and weighted just after mice sacrifice (n=10). **(C)** Blood was extracted from the tail vein to measure glucose levels after 6 hours of fasting. High fat diet-fed group exhibited a robust hyperglycemia compared to controls (n=7). **(D)** ELISA detection of serum insulin levels after 6 hours of fasting (n=8-9). **(E)** Intraperitoneal glucose tolerance response: blood glucose was monitored over time following an i.p. injection of glucose (2 g/Kg BW) after 6 hours of fasting (n=7). **(F)** Intraperitoneal insulin tolerance response: blood glucose was monitored over time following an i.p. injection of insulin (0.35U/Kg BW) after 6 hours of fasting (n=7). Values represent mean (±S.E.M). Statistical significance (*p<0.05; **p<0.01; ***p<0.005) was evaluated with RM-ANOVA (A, E and F) or unpaired T-test (B, C and D).

Fig 2: Four months of high fat diet exposure does not affect amyloid load, Tau phosphorylation or cognitive function

Hippocampi from 6 month-old *App*$^{NL/NL}$ mice were analyzed for Alzheimer disease-like pathology after 4 months exposure to high fat diet.

(A)ELISA detection of $A\beta_{40}$ and $A\beta_{42}$ shows no differences in the levels of hippocampal soluble $A\beta$ between the high fat (black dots, n=5 mice) and the control diet (white dots, n=6 mice) treated groups. **(B)** Levels of Tau phosphorylation in the hippocampus were not changed with the high fat diet treatment. Left: representative blots from AT180, AT270 and total Tau (CD: control diet, n=6); HFD: high fat diet, n=5); right: graph shows relative protein levels normalized to total Tau and to the control diet-group. **(C)** Explanatory diagram for the contextual and cued-fear conditioning protocol followed. **(D)** Graph compares mean (±S.E.M) percentage of freezing during each phase of the task. Both diet groups learned to associate the context and the tone with the shocks showing a significant increase % of freezing in the context and the cue test compared to the habituation.

n=10-11 mice per group. **(E)** Social preference test: Graph shows the mean (±S.E.M) social preference discrimination ratio: (time with stranger 1-time in empty/total time exploring). High fat diet-treated group exhibits a significant increased social preference compared to the control-fed group. (*p<0.05 unpaired T-test). **(F)** Social novelty test: Histogram represents the mean (±S.E.M) social novelty discrimination ratio: (time in stranger 2-time in stranger 1/total time exploring). Both diet groups show a similar preference for the novel mouse versus the familiar one (*n*=10-11 mice per group). **(G)** Top panel: Representative blots from Iba1 and GFAP in the brain. Bottom: Graph show relative protein levels normalized to actin and the control diet group (*n*=5 mice per diet group). **(H)** Hippocampal mRNA levels from the pro-inflammatory cytokines CCl2 and IL-6. Graphs represent relative values normalized to GAPDH and actin, and to control-diet group. (n= 5 mice per diet group). Statistical significance (*p<0.05, ****p<0.0001) was assessed using unpaired T-tests.

Fig 3: Longer term diet treatment does not affect amyloidosis, Tau phosphorylation or cognition in 18 month-old $App^{NL/NL}$ mice.

$App^{NL/NL}$ mice were treated with a high fat or control diet for 16 to 18 months and Alzheimer-like pathology and cognitive function were assessed at 18 to 20 months of age.

(A)ELISA detection of soluble $A\beta_{40}$ and $A\beta_{42}$ peptides shows no differences between the high fat (black dots, *n*=5) and control diet (white dots, *n*=5) treated groups. **(B)** Levels of Tau phosphorylation in the hippocampus at 20 months of age. Left: representative blots from AT180, AT270 and total Tau (CD: control diet, *n*=5; HFD: high fat diet, *n*=5). Right: graphs showing relative protein levels normalized to total Tau and to the control diet-group. **(C)** Explanatory diagram for the contextual and cued-fear conditioning protocol followed. **(D)** Histogram shows mean (±S.E.M) percentage of freezing during each phase of the task **(E)** Social preference test: graph shows the mean discrimination ratio: (Time STR1-Time empty)/ (Time STR1+Time empty). **(F)** Social novelty test: graph shows the mean discrimination ratio: (Time STR1-Time STR2)/ (Time STR1+Time STR2). *n*=10-11 mice per group. **(G)** Top panel: Representative blots from Iba1 and GFAP in the brain. Bottom: Graph show relative protein levels normalized to actin and the control diet group (*n*=6 mice per diet group). **(H)** Hippocampal mRNA levels from the pro-inflammatory cytokines CCl2 and IL-6. Graphs represent relative values normalized to GAPDH and actin, and to control-diet group. (n= 5

mice per diet group).Statistical significance (*p<0.05, **<0.001, ****p<0.0001) was assessed using unpaired T-tests

Fig 4: Proton magnetic resonance in the hippocampus from *APP*$^{NL/NL}$ mice treated with high fat diet

(A) *In vivo* proton magnetic resonance spectroscopy (MRS) spectra acquired in the hippocampus of 9 month-old *APP*$^{NL/NL}$ mice treated with control (green line) or high fat diet (black line). Right image shows the voxel localization in the hippocampus (1.3 x 2 x 2.2 mm^3). **(B)** Neurochemical profile from the hippocampus at 9 months of age (n=8 CD, 7 HFD). **(C)** N-acetyl-aspartate/ myo-inositol (NAA/mIns) ratio in the hippocampus from *APP*$^{NL/NL}$ mice at 9 months of age (n=6 CD, 5 HFD). **(D)** MRS spectra from the hippocampus of 18 month-old *APP*$^{NL/NL}$ mice treated with control (green line) or high fat diet (black line). **(E)** Neurochemical profile from the hippocampus at 18 months of age (n=9 CD, 10 HFD). **(F)** NAA/mINS (N-acetyl-aspartate/myo-inositol) ratio in the hippocampus from *APP*$^{NL/NL}$ mice at 18 months of age is decreased upon long term high fat diet exposure (n=4 CD, 8 HFD). Bars represent mean (±S.E.M). Statistical significance was evaluated using multiple T-test with FRD correction for panels B and E and unpaired T-tests for panel C and F.

Fig 5: High fat diet treatment triggered an impaired LTP in the CA3-CA1 hippocampal synapses

(A) Input-output relations (curves) between increasing stimulus intensities applied to the CA1 Schaffer collateral fibers and the slope of field excitatory postsynaptic potentials (fEPSPs) do not reveal any effect of high fat diet treatment on basal synaptic transmission. **(B)** When LTP was induced by theta-burst stimulation (TBS), *App*$^{NL/NL}$ mice fed a high fat diet for 10 months expressed only a decremental potentiation while animals receiving a control diet developed robust LTP. This resulted in a significant effect of high fat diet treatment (50-240 min $F_{(1, 10)}$=29.44, p=0.03, RM-ANOVA) . **(C)** LTD induced by the application of 0.5 µM insulin into the ACSF for 30 minutes did not differ between groups. n=6 mice per group. Bars represent mean (±S.E.M).

FIGURES

Fig 1: High fat diet treatment induces metabolic syndrome in *App*$^{NL/NL}$ mice

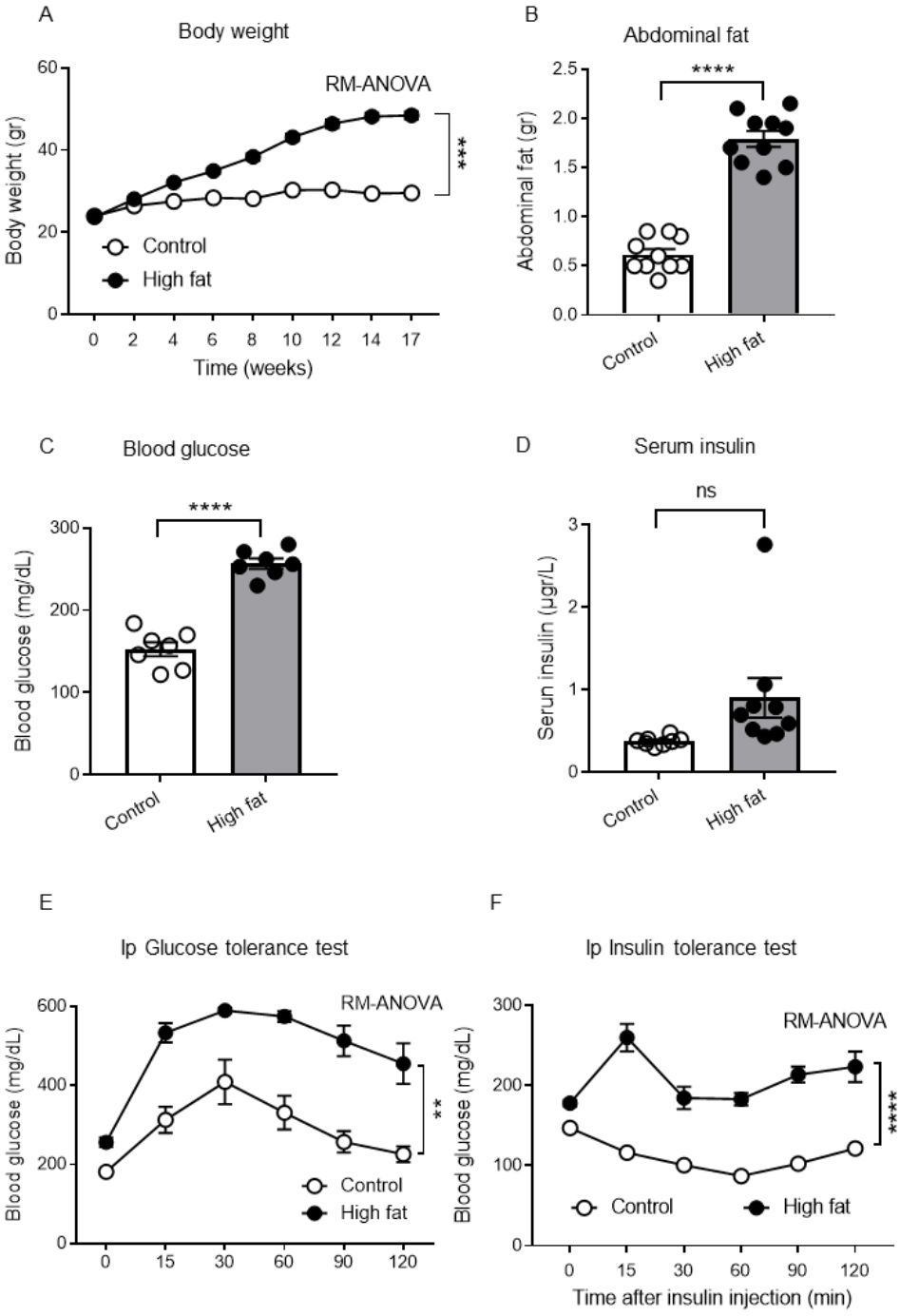

Fig 2: Four months of high fat diet exposure does not affect amyloid load, Tau phosphorylation or cognitive function

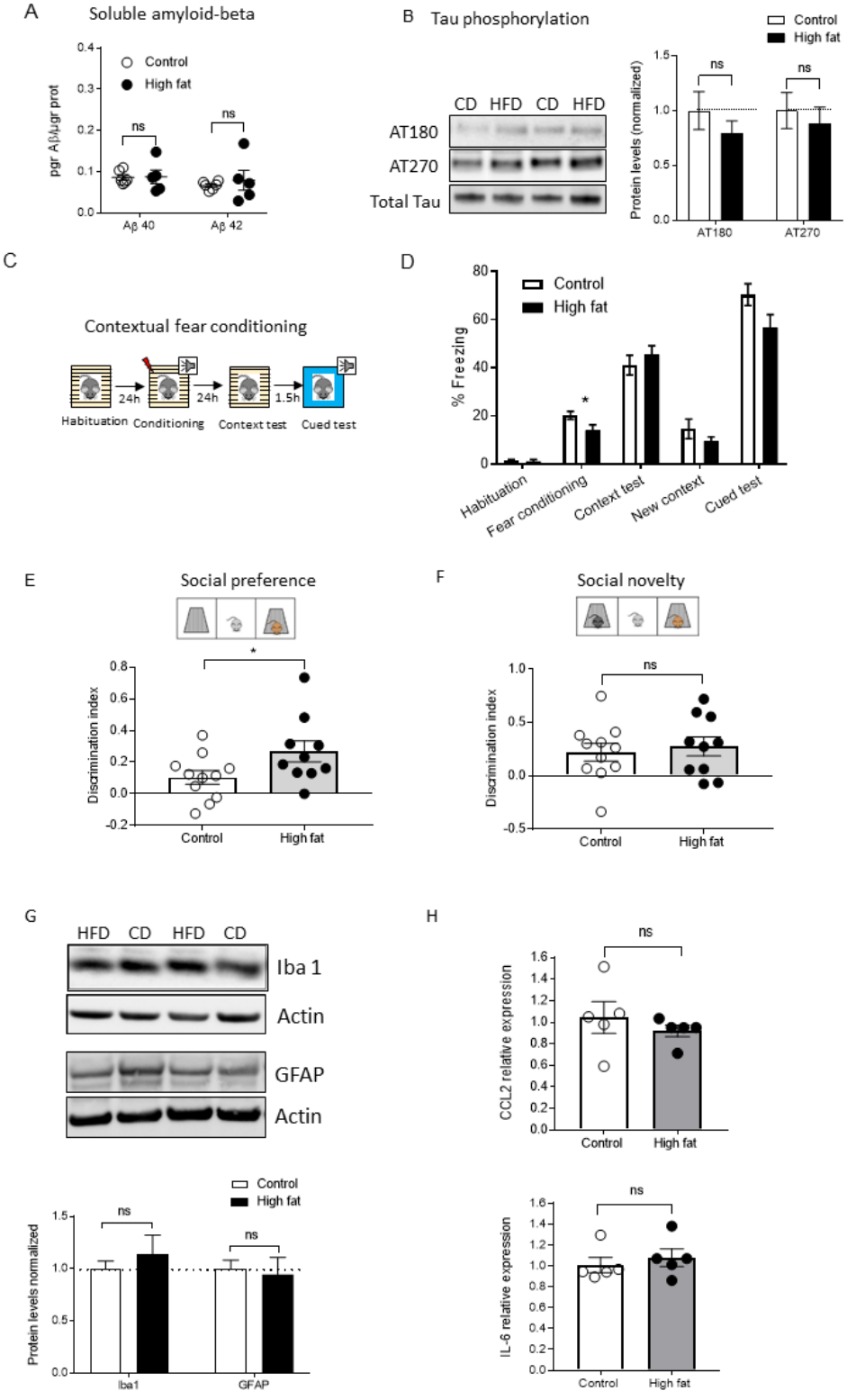

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Fig 3: Longer term diet treatment does not affect amyloidosis, Tau phosphorylation or cognition in 18 month-old *App*$^{NL/NL}$ mice.

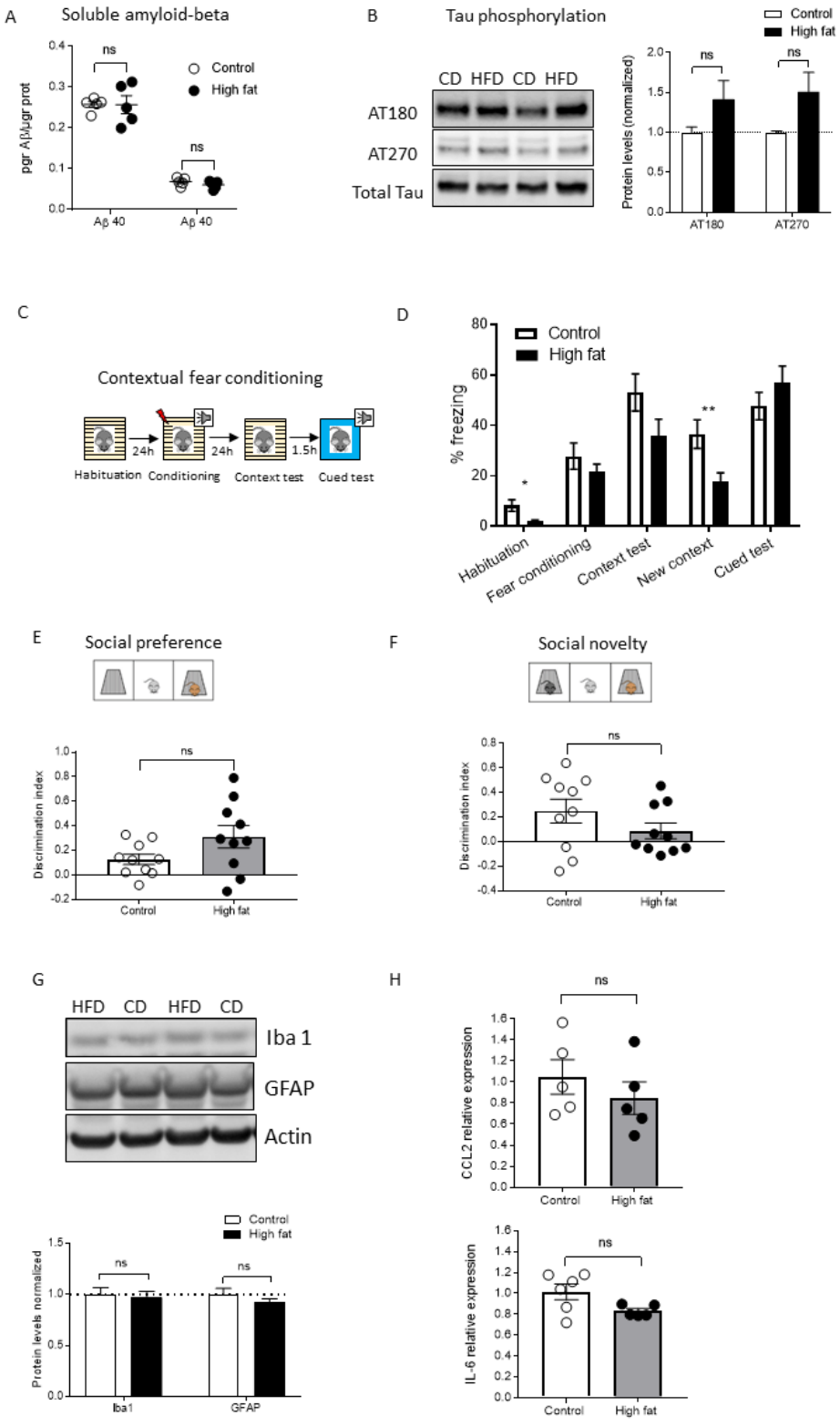

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Fig 4: Proton magnetic resonance in the hippocampus from *APP*$^{NL/NL}$ mice treated with high fat diet

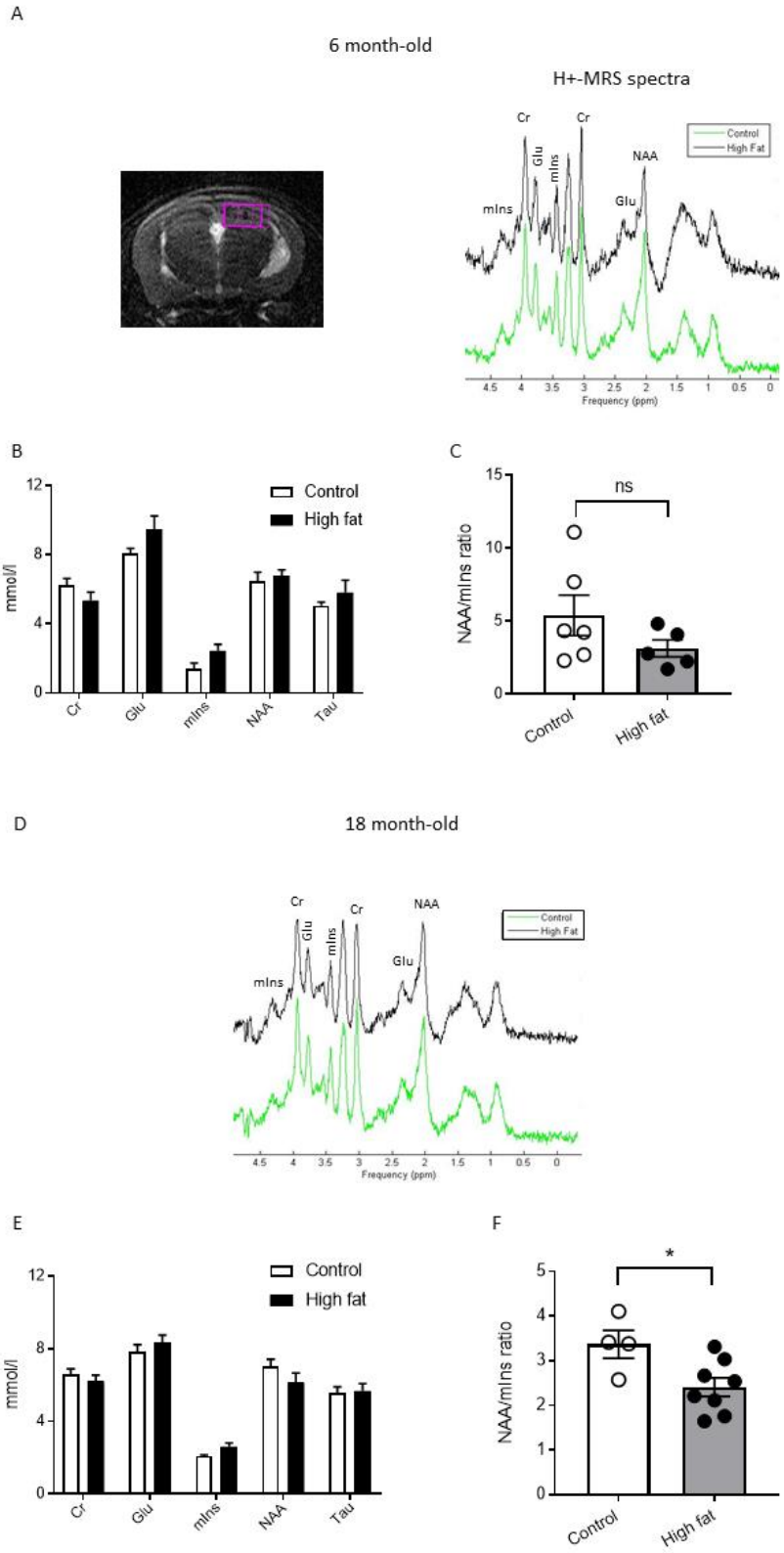

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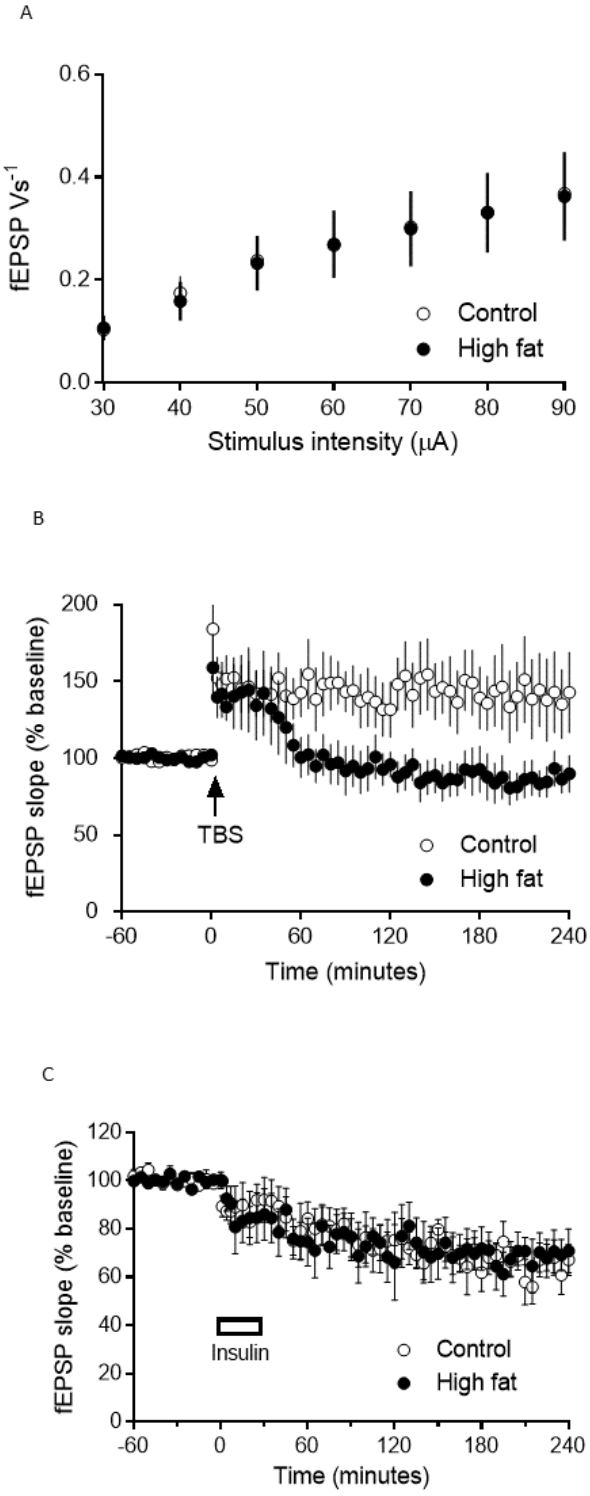
Fig 5: High fat diet treatment triggered an impaired LTP in the CA3-CA1 hippocampal synapses



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