Monocytes exposed to plasma from patients with Alzheimer's disease undergo metabolic reprogramming

- **Running title** Increased glycolysis in THP-1 cells with AD plasma**.**

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ABSTRACT

The search for a blood-based biomarker that reliably identifies Alzheimer's disease (AD) and can replace the current diagnostic tools, continues. Perhaps the most extensively-examined peripheral marker is β-amyloid (Aβ) but the results are not consistent across studies and perhaps do not adequately reflect the changes that take place in the brain. Several studies have assessed possible proteomic signatures also, largely, with inconsistent findings although increases in circulating inflammatory molecules are generally observed. In this study, rather than focus on identifying changes in the circulation, we evaluated the effect of plasma from patients with mild cognitive impairment (MCI) and AD on the monocyte-like cell line, THP-1 cells, and plasma from a mouse model of AD on a mouse monocyte-macrophage cell line, J774.2 cells. The data showed that plasma from AD patients and the AD mouse model increased expression of inflammatory molecules in the cells and that these changes were accompanied by an increase in glycolysis, but no change in oxidative metabolism in the respective cells. Interestingly, plasma from MCI patients exerted no significant effect on THP-1 cells. The possibility therefore exists that evaluating the effect of plasma on IL-8 and TNFα mRNA in THP-1 cells combined with analysis of glycolysis in these cells, may be the basis of an indicator that discriminates between AD and MCI, but is unlikely to be useful in identifying early changes in AD.

Keywords: Alzheimer's disease, MCI, APP/PS1 mice, plasma, J774.2 cells, THP-1 cells, inflammatory markers, glycolysis.

INTRODUCTION

Despite a great deal of research, the search for reliable and robust biomarkers that identify Alzheimer's disease (AD), and also mild cognitive impairment (MCI), continues. The ideal is a blood marker that avoids the need for imaging and CSF sampling, which are costly and require specialist services, that reproducibly identifies early changes and, preferably, that predicts the progression from MCI to AD.

A number of groups have attempted to identify a protein signature that distinguishes between controls, MCI and AD, the first of which suggested a panel of 18 proteins that was 90% accurate (Ray et al., 2007), with a later study indicating that a smaller panel of 5 of these was sufficient to differentiate between AD and controls (Gomez Ravetti and Moscato, 2008). However others questioned both the reliability of these signatures (Bjorkqvist et al., 2012), the reported degree of accuracy (Soares et al., 2009) and the ability to differentiate between MCI and AD (Marksteiner et al., 2011). These, and other studies (Doecke et al., 2012; O'Bryant et al., 2011), however, determined that several inflammatory proteins were increased in the samples from AD patients and, consistent with this, meta-analysis of 40 studies concluded that the concentrations of IL-6, TNFα, IL-1β, IL-12 and IL-18 were increased in the plasma of AD patients compared with controls (Swardfager et al., 2010). Despite this, the inconsistency of the data from individual studies remains and, despite the numerous studies, a defined blood-based signature that robustly and reproducibly identifies AD remains to be identified (Dubois et al., 2016).

In an attempt to circumvent the lack of consistency in identifying changes in the circulation, and yet to acknowledge the fact that most, if not all studies, agree that inflammatory mediators are increased in the plasma of AD patients, our approach was to incubate cells with plasma from control, MCI and AD cohorts.

We first showed that plasma from APP/PS1 mice increased expression of inflammatory molecules and increased glycolysis in J774.2 cells and that these changes may be mediated by circulating Aβ. We then assessed the effect of plasma from MCI and AD cohorts on THP-1 cells and show that plasma from AD, but not MCI, patients similarly

increased mRNA expression of IL-8 and TNFα and also increased glycolysis. The data identifies a means of discriminating between MCI and AD and therefore may provide a possible marker of AD.

MATERIALS AND METHODS

Animals

Plasma was obtained from APP/PS1 mice and their wild type littermates (22-24 months; n=9 and 11 respectively), for use in this study. Mice were maintained under veterinary supervision in a specific pathogen-free environment in the Comparative Medicine Unit, Trinity College Dublin, and were housed in groups of 4 or 5 per cage, at 20-22°C with a 12 h light/dark cycle. All animals had free access to food and water and were fed a standard laboratory diet. All mice were maintained according to European Union regulations, and experiments were performed under license from the Department of Health and Children/HPRA and with approval from the Trinity College Dublin Bioresources Ethics Committee.

Blood sampling and plasma analysis

Blood samples were obtained from mice under euthatal-induced anaesthesia by cardiac puncture using EDTA-coated syringes, collected in 1.5 ml sterile Eppendorf tubes and centrifuged (2000 x *g*; 10 min; 22°C). The resultant plasma fraction was aliquoted and stored at -80°C until further use. Samples from some of the cohort were assessed by ELISA for circulating IL-1β, TNFα and CXCL1 and from the remainder were used to incubate with J774.2 cells as detailed below.

MCI, AD and control participants

Patients with a clinical diagnosis of AD (NINCDS criteria; 2 female, 4 male; mean age 63.50 ± 3.89 (SD)) and MCI (Petersen criteria; 5 female, 12 male; mean age 65.65 ± 7.03) were recruited from the BIOMARKAPD project at St. James' and St. Vincent's Hospitals, Dublin, Ireland and Bon Secours Hospital, Tralee, Kerry, Ireland. Control participants (6 female, 14 male; mean age 65.05 ± 8.53) were recruited from the Santry Sports Centre, Dublin, Ireland. Cognitive function was assessed by the MoCA and this revealed a significant difference between controls (26.70 ±1.62 (SD)) and both MCI patients (22.00 ± 4.36; $p < 0.01$) and AD patients (13.25 ± 9.84; $p < 0.001$; 1-way ANOVA and Student Newman-Keuls multiple comparison test). Blood samples (20 ml) were collected in EDTA tubes and centrifuged (2000 x *g*, 10 min, 22°C). Aliquots of plasma were stored at -80°C. This study was approved by the Adelaide and Meath Hospital, Dublin, incorporating the National Children's Hospital/St. James's Hospital Joint Research Ethics Committee and all participants provided informed consent. Exclusion criteria included smokers, those with any significant active medical conditions, those with a history of major psychiatric or neurological condition, epilepsy, diabetes, heart attacks, traumatic brain injury, immunodeficiency, and those that were taking psychoactive medication, immunosuppressants or corticosteroids.

Cell culture

Mouse J774.2 monocytes (passage 14-18; kindly donated by Professor Luke O'Neill, Trinity College Dublin) were maintained in cDMEM at 37°C in a humidified 5% CO: 95% air environment. Cells were counted in a Trypan Blue cell suspension (10%) and seeded onto a 24-well cell culture plate (density: $1x10^6$ cells/ml). J774.2 cells were incubated in the presence or absence of Aβ (5.8μM $Aβ_{1-42}$ and 4.2μM $Aβ_{1-42}$, 4 h) and, in separate experiments, with plasma (1:10, 4 h) from wild type and APP/PS1 mice. For metabolic analysis, cells were seeded onto 24-well Seahorse XF24 cell culture microplates ($1.2x10^6$ cells/ml) pre-coated with poly-D-lysine (50 ng/ml; Merck Millipore, Germany). Cells were harvested and assessed for mRNA expression or bioenergetic profile.

Human THP-1 monocytes (passage 12-15; kindly donated by Professor Andrew Bowie, Trinity College Dublin) were maintained in cRPMI at 37°C in a humidified 5% CO_2; 95% air environment. Cells were counted in a Trypan Blue cell suspension (10%) and seeded onto a 24-well cell culture plate ($1x10^6$ cells/ml) or onto a 24-well Seahorse XF24 cell culture microplate ($1.2x10^6$ cells/ml) pre-coated with poly-D-lysine (50 ng/ml). Cells were incubated with plasma (1:40; 4 h) from control individuals or MCI or AD patients, harvested and assessed for expression of inflammatory markers. In separate experiments, conducted under the same incubation conditions, THP-1 cells were assessed for their bioenergetic profile.

PCR analysis

RNA was isolated from cells (Nucleospin® RNAII kit; Macherey-Nagel GmbH, Germany) and reverse transcribed into cDNA (High-Capacity cDNA Archive kit; Applied Biosystems, UK) according to the manufacturer's instructions. Assay ID's for the mouse genes examined were: CXCL1 (Mm00433859_m1), TNFα (Mm00443258_m1), NOS2 (Mm0040502_m1) and IL-6 (Mm00446190_m1). The assay IDs for the human genes were IL-8 (Hs01567912_g1), TNFα (Hs99999043_m1), iNOS (Hs 01075529_m1) and IL-6 (Hs00985639_m1). Real-time PCR was performed using an ABI Prism 7300 instrument (Applied Biosystems, UK), 18S was the endogenous control and relative gene expression was calculated with reference to untreated cells using the ΔΔCT method.

Analysis of bioenergetic profile of cells

The Seahorse XF Glycolytic Stress test (Seahorse Bioscience, USA) was to assess glycolysis in cells ($1.2x10^6$ cells/ml) that were seeded in Seahorse XF24 cell culture microplates. Samples were incubated (37°C, 1 h), media (150 µl) was added and incubation continued overnight at 37°C in a humidified 5% CO_2; 95% air environment. J774.2 were incubated with plasma from WT and APP mice (1:10, 4 h) and THP-1 cells were incubated with plasma from MCI and AD patients and controls (1:10, 4 h).

Seahorse XF Calibrant solution (1 ml; Seahorse Bioscience, US) was added to each well of the utility plate to hydrate the sensor cartridge and left overnight in a CO_2-free incubator at 37°C. For the assay, cells were washed (Seahorse XF Base Medium supplemented with L-glutamine (2mM); pH 7.35), media was added to each well to give a final volume of 525 µl/well, the plate was placed in a CO_2-free incubator at 37°C for 1 h and loaded onto the Seahorse XF Analyser for calibration. Extracellular acidification rate (ECAR) and oxygen consumption rate (OCR) were measured every 8 min for 96 min and, after the first 3 baseline measurements, glucose (10mM), oligomycin (20µM) and 2-deoxy-D-glucose (2-DG; 500mM; all Sigma-Aldrich, UK) in glycolytic flux assay media were delivered sequentially to allow subsequent determination of glycolysis, glycolytic capacity and glycolytic reserve, which were automatically calculated using the SeaHorse XF24 software.

Analysis of Aβ

The concentrations of $A\beta_{1-40}$ and $A\beta_{1-42}$ were assessed in pooled plasma from wildtype and APP/PS1 mice using a V-Plex Aβ Peptide Panel 1 (4G8) Assay (Meso Scale Discovery, US) as per the manufacturer's instructions. Briefly, diluent 35 was added to the plate (150 µl/well) and samples were incubated (1 h, room temperature). The plate was washed, samples/standards and detection antibody (25 µl/well of each) were added, samples were incubated (2 h, room temperature), washed and 2X Read Buffer T was added (150 µl/well) before reading using a Mesoscale Sector Imager (Meso Scale Discovery, US). Aβ concentrations were calculated relative to the standard curve.

The concentrations of human $A\beta_{1-40}$ was assessed in plasma samples from the control, MCI and AD patients using Human APP/Amyloid Beta A4 Protein ELISA kit (Sigma-Aldrich, UK). Duplicate samples or standards (100µl/well) were added to 96-well plates coated with anti-human $A\beta_{1-40}$ antibody. Plates were incubated (4°C, overnight) washed, detection antibody (100µl biotinylated human $A\beta_{1-40}$ antibody) was added and incubation continued (1h, room temperature). Samples were washed, incubated with streptavidin-horseradish peroxidase conjugate (100µl, 45 min, room temperature) and washed before addition of substrate solution (100µl; ELISA Colorimetric TMB Reagent). After further incubation (30 min, room temperature) in the dark, the reaction was stopped by adding stop solution (50µl), absorbance was measured immediately at 450nm (Biotek, Mason Technology, UK) and the $A\beta_{1-40}$ concentration was calculated according to the manufacturer's instructions.

Statistical analysis

Data were analysed using the student's t-test for independent means and, as appropriate, a 1-way analysis of variance (ANOVA) followed by a Newman Keul's post-hoc test to determine which conditions were significantly different from each other. Data are expressed as means + SEM.

RESULTS

Plasma from APP/PS1, compared with wildtype, mice significantly increased mRNA expression of CXCL1, TNFα, iNOS and IL-6 (*$p < 0.05$; student's t-test for independent means; Figure 1A-D). Our previous data have indicated that inflammatory cells shift metabolism towards glycolysis and, here, we examined the inflammatory signature of J774.2 cells incubated with plasma from APP/PS1 and wild type mice. As indicated by the metabolic profile (Figure 2A), there was a clear genotype-related difference in ECAR and analysis of the data indicated that glycolytic capacity and glycolytic reserve were increased in J774.2 cells that were incubated with plasma from APP/PS1 mice compared with plasma from wild type mice (**$p < 0.01$; ***$p < 0.001$; student's t-test for independent means; Figure 2C,D) although the increase in glycolysis (Figure 2B) did not reach statistical significance. In contrast, OCR was similar in J774.2 cells incubated with plasma from wild type and APP/PS1 mice and no difference was observed in basal respiration or in ECAR:OCR (Figure 2E-G).

Plasma concentrations of IL-1β and CXCL1 were significantly increased in APP/PS1 mice (5.59 pg/ml ± 2.88 (mean ± SEM; n=4) vs 23.84 pg/ml ± 2.61 (mean ± SEM; n=7) for IL-1β, $p < 0.05$, and 214.2 pg/ml ± 57.65 vs 410.0 pg/ml ± 32.64 for CXCL1, $p < 0.01$). An increase in TNFα was also observed (565.6 pg/ml ± 31.63 vs 692 pg/ml ± 41.5) but this did not reach statistical significance. One possible plasma factor apart from these cytokines that may account for these changes is Aβ and the data show that $A\beta_{1-40}$ and $A\beta_{1-42}$ were increased in plasma from APP/PS1 mice compared with wild type mice (Figure 3A). We incubated J774.2 cells with Aβ and observed an increased in mRNA expression of CXCL1, TNFα, iNOS and IL-6 (*$p < 0.05$; ***$p < 0.001$; student's t-test for independent means; Figure 3B-E), mimicking the effect of plasma from APP/PS1 mice.

We then assessed the effect of plasma from MCI and AD patients on inflammatory markers and metabolic changes in the human monocyte cell line, THP-1 cells. The data show that plasma from MCI patients exerted no significant effect on mRNA expression of IL-8, TNFα, iNOS or IL-6 but plasma from AD patients significantly increased expression of IL-8 and TNFα (**$p < 0.01$; 1 way ANOVA; Figure 4A,B). Expression of TNFα mRNA was also significantly greater in THP-1 cells incubated with plasma from AD

patients compared with cells incubated with plasma from MCI patients ($^{++}p < 0.01$). Analysis of the metabolic signature in THP-1 cells indicated that plasma from AD patients increased ECAR as indicated by the metabolic profile (Figure 5A) whereas plasma from MCI patients exerted no marked effect. Similarly glycolysis, glycolytic capacity and glycolytic reserve were increased in cells which were incubated with plasma from AD, though not MCI, patients ($^{*}p < 0.05$; $^{**}p < 0.01$; 1 way ANOVA; Figure 5B-D) but the change in glycolytic capacity did not reach statistical significance. In contrast, oxidative metabolism was similar in THP-1 cells incubated with plasma from control individuals and individuals with MCI and AD; the data revealed that OCR, basal metabolism and ECAR:OCR were similar in the 3 groups (Figure 5E-G). Analysis of plasma concentration of $A\beta_{1-40}$ revealed a significant increase in samples from the AD cohort compared with the other 2 cohorts ($^{*}p < 0.05$; $^{**}p < 0.01$; 1 way ANOVA; Figure 6) and no difference in the concentration between the control and MCI cohorts.

DISCUSSION

The primary objective of this study was to determine the impact of plasma from APP/PS1 mice compared with wild type mice on J774.2 cells and to assess whether this could be paralleled by an effect of plasma from AD patients on a human monocyte cell line. Specifically our interest focussed on investigating whether an inflammatory phenotype could be induced and establishing whether this was accompanied by a shift in metabolism towards glycolysis. The data show that plasma from APP/PS1 mice and plasma from AD patients induce an inflammatory phenotype in a monocyte/macrophage cell line and that this is associated with a glycolytic signature. The evidence suggests that one possible factor which triggers the effect is Aβ.

We predicted that incubating cells in the presence of plasma from APP/PS1 mice would induce a greater response than plasma from wild type mice; the findings concurred with this prediction showing genotype-related increases in mRNA expression of CXCL1, TNFα, iNOS and IL-6. Recent studies have indicated that the metabolic profile of macrophages changes depending on environmental conditions; specifically the default mitochondrial metabolism, which occurs under normal conditions, switches to glycolytic metabolism when cells are exposed to inflammatory stimuli (Kelly and O'Neill, 2015; O'Neill and Pearce, 2016). For example IFNγ increases production of inflammatory cytokines and drives glycolysis in macrophages (Holland et al., 2018) and exerts similar effects in microglia. The present data show that plasma from APP/PS1 mice increased glycolysis in J774.2 cells and this is consistent with the plasma-induced increases in TNFα mRNA and iNOS mRNA; these are considered to be the archetypal markers of the so-called M1 macrophages (Gordon, 2003) and several studies have proposed that this macrophage phenotype are glycolytic (Galvan-Pena and O'Neill, 2014; Holland et al., 2018; Zhu et al., 2015). In contrast to this effect, plasma from APP/PS1 mice did not affect oxidative metabolism.

Several factors in the plasma of APP/PS1 mice may contribute to the changes observed in J774.2 cells including inflammatory mediators and the genotype-related increases in plasma that we report here are in line with other findings (Cornejo et al., 2018; Sanchez-Ramos et al., 2009). We considered that Aβ may play a role and show that $A\beta_{1-40}$ and $A\beta_{1-42}$ were both increased in plasma from APP/PS1 mice compared with wild type mice. In this regard, it is relevant that Aβ also mimicked the effect of plasma from APP/PS1 mice by increasing mRNA expression of CXCL1, TNFα, iNOS and IL-6 in J774.2 cells and our unpublished work has indicated that Aβ also increases ECAR in macrophages.

The consensus is that circulating inflammatory cytokine concentrations are also increased in AD (Brosseron et al., 2014; Zheng et al., 2016) and circulating Aβ has been considered by some as a correlate of AD and/or cognition (Olsson et al., 2016), although the evidence points to it being of little value as a diagnostic tool. Reports of changes in circulating Aβ in MCI and AD lack consistency (Song et al., 2011) and interpretation of changes is confounded by several factors including the variability in patient cohorts, the handling and method of analysis of samples, and the fact that circulating concentrations of Aβ are derived from both peripheral tissues and the brain (Schneider et al., 2009). In our sample, $A\beta_{1\text{-}40}$ concentration was increased in plasma from AD patients although no changes were observed in samples from MCI patients.

In parallel with the data from the animal study, we show that plasma from AD patients increased mRNA expression of IL-8 and TNFα in THP-1 cells but no change was observed in cells incubated with plasma from MCI patients; the lack of change in circulating Aβ in MCI patients provides indirect evidence of a role for Aβ in driving the inflammatory change. Plasma from AD patients also increased glycolysis in THP-1 cells without affecting OCR and no effect of plasma from MCI patients was observed suggesting that Aβ may also be responsible for the metabolic change and also suggesting that this analysis discriminates between AD and MCI.

Few studies have assessed the effect of plasma from MCI and AD patients on cells although proteomic analysis of lysates from a cultured microglial cell line, CHME-5 cells, incubated with pooled plasma samples from AD patients revealed upregulation of a number of proteins involved in glycolysis (Jayasena et al., 2015). These authors also reported that incubating these CHME-5 cells with pooled plasma from 20 AD patients increased ECAR and, although the data are derived from different cells and pooled sample, they are broadly consistent with the present findings.

It has been suggested that metabolic dysfunction, and specifically a reduction in brain metabolism as revealed by fluoro-2-deoxy-D-glucose positron emission tomography, may predict progression from MCI to AD (Cohen and Klunk, 2014; Kennedy et al., 1995). However abnormalities in metabolic function are also reported in the periphery in AD with evidence of a decrease in expression of genes of the mitochondrial respiratory chain in whole blood samples from MCI and AD patients (Lunnon et al., 2012) together with an increase in reactive oxygen species production (Leutner et al., 2005) and decreased mitochondrial respiration (Leuner et al., 2012; Maynard et al., 2015) in lymphocytes from AD patients. The present study circumvents the need to prepare and analyse cells and has the advantage that plasma can be readily stored. The data present the possibility that a combination of evaluating the effect of plasma on IL-8 and TNFα mRNA in THP-1 cells with analysis of glycolysis in these cells, may be the basis of an indicator that discriminates between AD and MCI, but is unlikely to reflect early changes in disease.

FIGURE LEGENDS

Figure 1. Expression of pro-inflammatory markers is increased in J774.2 cells in response to plasma from APP/PS1 mice.

J774.2 cells were incubated with plasma from wild type or APP/PS1 mice (1:10 dilution, 4 h) and gene expression was examined by RT-qPCR. Plasma from APP/PS1 mice significantly upregulated IL-8, TNFα, IL-6 and iNOS mRNA (A-D) in J774.2 cells compared with plasma from wild type mice (*$p<0.05$; Student's *t*-test for independent means; n = 11 and 9 for wild type and APP/PS1 mice respectively). Data are expressed as the mean ± SEM.

Figure 2. Plasma from APP/PS1 mice increases glycolysis in J774.2 cells.

J774.2 cells were incubated with plasma from wild type or APP/PS1 mice (1:10 dilution, 4 h) and a glycolytic stress test performed to examine metabolic function.
A-D. The metabolic profile and analysis of the mean data show significant increases in glycolytic capacity (C) and glycolytic reserve (D) of cells incubated with plasma from APP/PS1 mice compared with wild type mice (**$p < 0.01$; ***$p < 0.001$; Student's *t*-test for independent means; n = 18 and 8 for wild type and APP/PS1 mice respectively).
E-G. OCR was similar in J774.2 cells incubated with plasma from wild type and APP/PS1 mice (E) and no differences were observed in basal respiration (F) or ECAR/OCR (G). Data are expressed as the mean ± SEM.

Figure 3. Aβ increased expression of inflammatory markers in J774.2 cells
Plasma concentration of $A\beta_{1\text{-}40}$ and $A\beta_{1\text{-}42}$ were markedly increased in samples from APP/PS1, compared with wild type mice (A). Incubation of J774.2 cells in the presence of Aβ significantly increased mRNA expression of CXCL1 (B), TNFα (C), iNOS (D) and IL-6 (E; *$p<0.05$, ***$p<0.001$; Student's *t*-test for independent means; n = 14 and 12 for wild type and APP/PS1 mice respectively). Data are expressed as the mean ± SEM.

Figure 4. IL-8 mRNA and TNFα mRNA are increased in THP-1 cells in response to plasma from AD, but not MCI, patients.
THP-1 cells were incubated with plasma from control subjects, MCI patients or AD patients (1:40 dilution, 4 h) and pro-inflammatory gene expression was examined by RT-qPCR. Plasma from MCI patients did not affect expression of any inflammatory marker while incubation with plasma from AD patients significantly upregulated IL-8 mRNA (A) and TNFα mRNA (B; *$p<0.05$, **$p<0.01$; 1 way ANOVA; n = 20, 17 and 6 for controls, MCI and AD respectively). Expression of TNFα mRNA was also significantly greater in THP-1 cells incubated with plasma from AD patients compared with cells incubated with plasma from MCI patients ($^{++}p < 0.01$). No significant changes in mRNA expression of IL-6 (C) or iNOS (D) were observed.

Figure 5. Plasma from AD patients increases glycolysis in THP-1 cells.
THP-1 cells were treated with plasma from control subjects, MCI patients or AD patients (1:40 dilution, 4 h) and a glycolytic stress test was performed to examine metabolic function. The metabolic profile (A) shows that there was no effect on ECAR in cells incubated with plasma from MCI patients but there was an increase in ECAR in cells incubated with plasma from AD patients. Analysis of the mean data indicated that glycolysis (B) and glycolytic reserve (D), though not glycolytic capacity (C) were significantly increased in cells incubated with plasma from AD patients compared with plasma from control subjects (*$p<0.05$, **$p<0.01$; 1 way ANOVA; n = 20, 17 and 6 for controls, MCI and AD respectively). E-G. OCR was similar in THP-1 cells incubated with plasma from control subjects, MCI patients and AD patients (E) and no differences were observed in basal respiration (F) or ECAR/OCR (G). Data are expressed as the mean ± SEM.

Figure 6. Plasma $A\beta_{1\text{-}40}$ concentration was increased in samples from AD, but not MCI, patients.
$A\beta_{1\text{-}40}$ was assessed by ELISA as described in the methods. Analysis of the mean data indicate that $A\beta_{1\text{-}40}$ concentration was increased in samples from AD, but not MCI, patients (*$p < 0.05$; **$p < 0.01$ AD vs controls and MCI respectively; n = 20, 17 and 6 for controls, MCI and AD respectively). Data are expressed as the mean ± SEM.

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CONFLICT OF INTEREST/DISCLOSURE STATEMENT

The authors have no conflict of interest to report.

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Figure 1

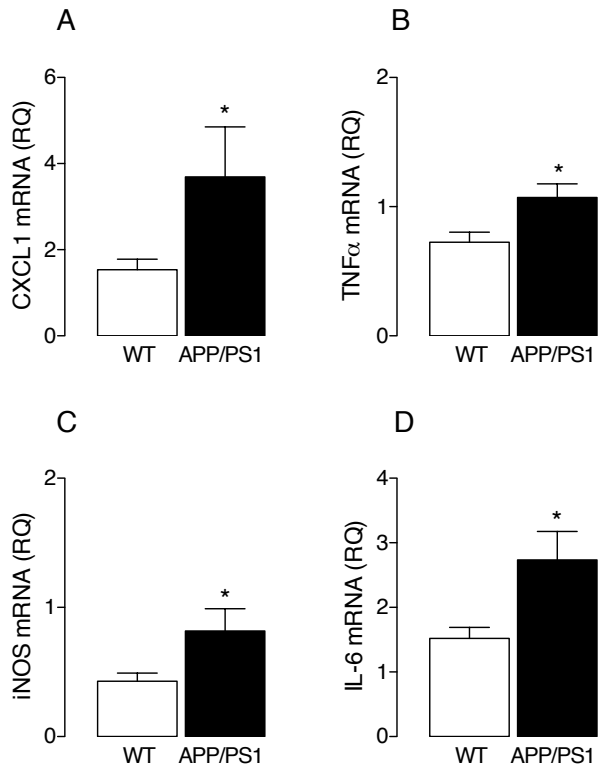
A
CXCL1 mRNA (RQ)
6
4
2
0
*
WT
APP/PS1
B
TNFα mRNA (RQ)
2
1
0
*
WT
APP/PS1
C
iNOS mRNA (RQ)
2
1
0
*
WT
APP/PS1
D
IL-6 mRNA (RQ)
4
3
2
1
0
*
WT
APP/PS1

Figure 2

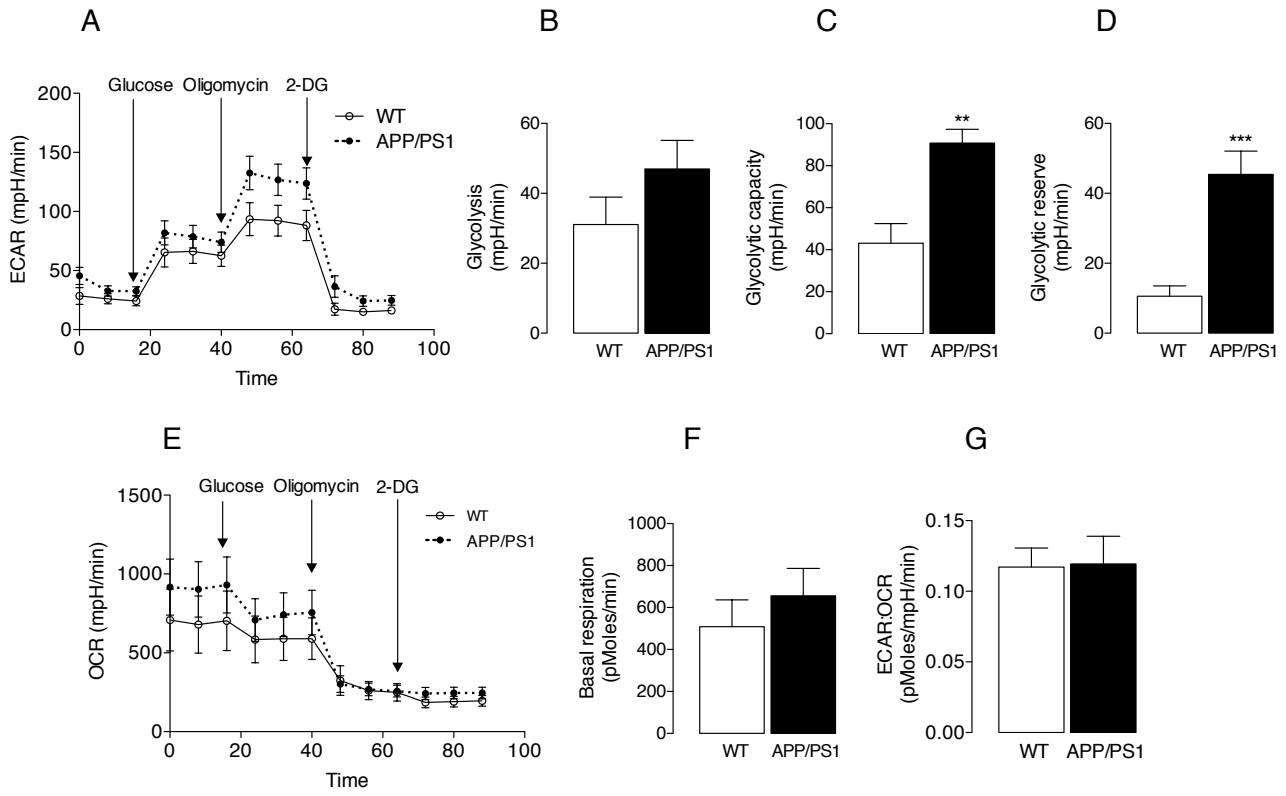
A
B
C
D
Glucose
Oligomycin
2-DG
WT
APP/PS1
ECAR (mpH/min)
Time
Glycolysis (mpH/min)
Glycolytic capacity (mpH/min)
Glycolytic reserve (mpH/min)
**
***
E
F
G
OCR (mpH/min)
Basal respiration (pMoles/min)
ECAR:OCR (pMoles/mpH/min)

Figure 3

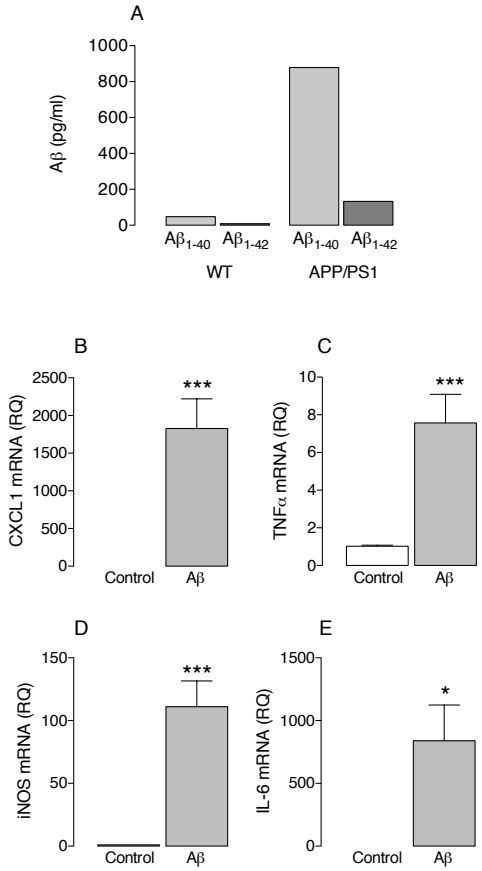
A
1000
800
600
400
200
0
Aβ (pg/ml)
Aβ1-40
Aβ1-42
Aβ1-40
Aβ1-42
WT
APP/PS1
B
2500
2000
1500
1000
500
0
CXCL1 mRNA (RQ)
***
Control
Aβ
C
10
8
6
4
2
0
TNFα mRNA (RQ)
***
Control
Aβ
D
150
100
50
0
iNOS mRNA (RQ)
***
Control
Aβ
E
1500
1000
500
0
IL-6 mRNA (RQ)
*
Control
Aβ

Figure 4

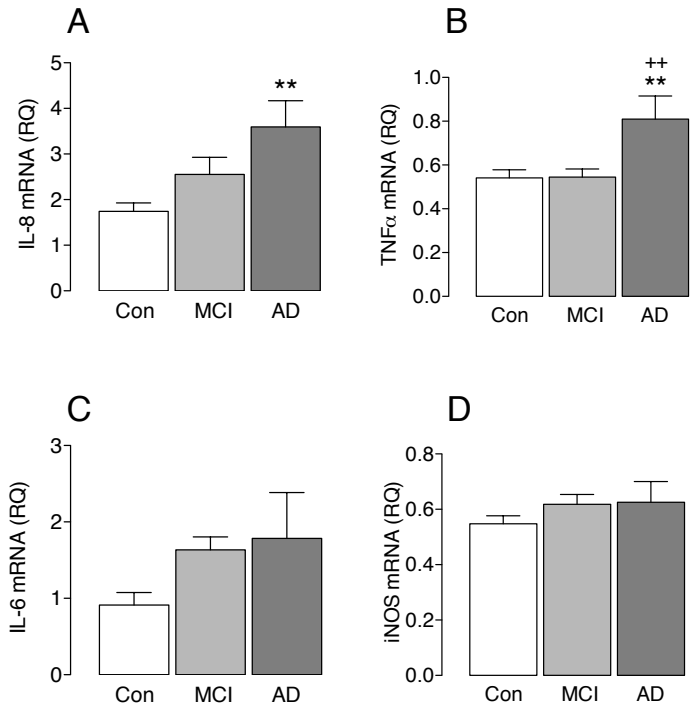
A
IL-8 mRNA (RQ)
0
1
2
3
4
5
**
Con
MCI
AD
B
TNFα mRNA (RQ)
0.0
0.2
0.4
0.6
0.8
1.0
++
**
Con
MCI
AD
C
IL-6 mRNA (RQ)
0
1
2
3
Con
MCI
AD
D
iNOS mRNA (RQ)
0.0
0.2
0.4
0.6
0.8
Con
MCI
AD

Figure 5

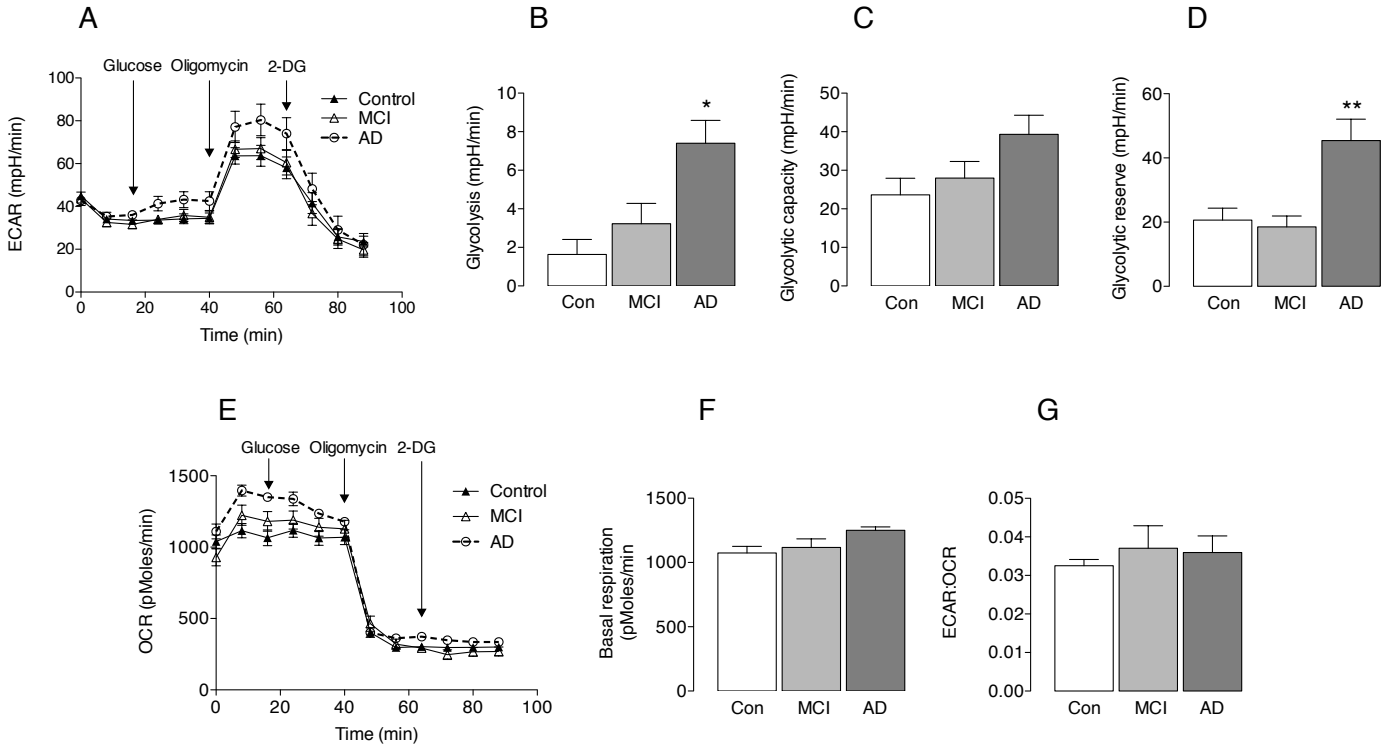
A
Glucose
Oligomycin
2-DG
Control
MCI
AD
ECAR (mpH/min)
Time (min)
B
Glycolysis (mpH/min)
*
Con
MCI
AD
C
Glycolytic capacity (mpH/min)
Con
MCI
AD
D
Glycolytic reserve (mpH/min)
**
Con
MCI
AD
E
Glucose
Oligomycin
2-DG
Control
MCI
AD
OCR (pMoles/min)
Time (min)
F
Basal respiration (pMoles/min
Con
MCI
AD
G
ECAR:OCR
Con
MCI
AD

Figure 6