The effect of age and obesity on platelet amyloid precursor protein processing and plasma markers of oxidative stress and inflammation

Elsworthy, Richard; Aldred, Sarah

DOI:
10.1016/j.exger.2020.110838

Document Version
Peer reviewed version

Citation for published version (Harvard):
Elsworthy, R & Aldred, S 2020, 'The effect of age and obesity on platelet amyloid precursor protein processing and plasma markers of oxidative stress and inflammation', *Experimental gerontology*, vol. 132, 110838. https://doi.org/10.1016/j.exger.2020.110838

THE EFFECT OF AGE AND OBESITY ON PLATELET AMYLOID PRECURSOR PROTEIN PROCESSING AND PLASMA MARKERS OF OXIDATIVE STRESS AND INFLAMMATION

AUTHORS AND AFFILIATIONS:

RICHARD J ELSWORTHY[a.]
DR SARAH ALDRED[a.]

[a.]School of Sport, Exercise and Rehabilitation Sciences, College of Life and Environmental Sciences, University of Birmingham, UK

RUNNING TITLE:

AGE, OBESITY AND APP PROCESSING

CORRESPONDANCE DETAILS:
Dr Sarah Aldred
School of Sport, Exercise and Rehabilitation Sciences
University of Birmingham
Edgbaston
Birmingham
B15 2TT
UK
TEL: +44 (0)121 414 7284
EMAIL: s.aldred.1@bham.ac.uk

DECLARATIONS OF INTEREST

'Declarations of interest: none'

ABSTRACT

INTRODUCTION: Advancing age is a major risk factor for a range of diseases such as, cardiovascular disease, diabetes, cancer and neurodegenerative diseases. In addition, over a third of the population are overweight and obesity is becoming more prevalent in younger people. Ageing and obesity are both linked to a chronic proinflammatory state and elevated oxidative stress, which have both been implicated in cardiovascular and neurodegenerative diseases. Platelets contain all the necessary machinery to process the Amyloid precursor protein AβPP, a pathway thought to be perturbed in Alzheimer's Disease (AD). The ratio of AβPP isoforms present in platelets, and the amount of alpha secretase ADAM10, that works to process AβPP, appear to be associated with cognitive decline and a diagnosis of Alzheimer's disease. The aim of this study was to assess changes in AβPP ratio, ADAM10 and markers of inflammation and oxidative stress with ageing and obesity.

MATERIALS AND METHODS: Ninety participants were recruited to this study to provide one blood sample. Platelet-rich plasma and platelet lysates were collected and the expression of AβPPr, proADAM10 and mADAM10 was assessed by Western blotting. In addition, markers of inflammation (IL-6) and oxidative stress (8-Isoprostane) were assessed in plasma.

RESULTS: Participants were placed into one of four groups based on their age and body mass index (Young/Lean, Young/obese, Old/Lean and Old/Obese). IL-6 was able to significantly distinguish obese from lean participants (AUC of 0.80, SE = 0.05, $P < 0.001$). Plasma isoprostanes were able to distinguish between both young/ old (AUC of 0.73, SE = 0.05, $P < 0.01$), and obese/ non-obese participants (AUC of 0.66, SE = 0.01, $P < 0.01$). Plasma protein carbonyls could distinguish young and old participants (AUC of 0.69, SE = 0.07 $P < 0.02$). Old Lean participants had significantly lower mADAM10 expression than both Young Lean and Young Obese participants ($p < 0.05$). Old obese participants had significantly lower proADAM10 expression compared to both Young Lean and Young Obese ($p < 0.05$). Both mADAM10 and proADAM10 were significantly decreased with advancing age ($p<0.05$).

CONCLUSIONS: The findings presented in this study provide evidence that blood-based biomarkers related to the pathology of AD are altered with age and obesity in otherwise healthy adults. Ageing was more strongly associated with elevated markers of oxidative stress whereas obesity was associated with elevated inflammatory IL-6.

KEY WORDS: AGEING; OBESITY; AMYLOID PRECURSOR PROTEIN; ADAM10; INFLAMMATION; OXIDATIVE STRESS

1. INTRODUCTION

Better healthcare treatments, advancements in technology and improved living conditions all contribute to the observed increase in human life expectancy. However, this increase has not been matched by health span, or the number of disease-free and functional years of life (Hansen & Kennedy 2016). This disparity is predicted to create an unsustainable economic burden. Advancing age is a major risk factor for a range of diseases including cardiovascular disease, diabetes, cancer and neurodegenerative diseases (Cuthbertson et al 2016, Lennon et al 2015, Smetana et al 2016, Tesauro et al 2017). This association is due to a combination of age-related physiological and behavioural changes. Cellular senescence, chronic inflammation and an imbalance in cellular redox status with a higher probability of engaging in negative lifestyle activities. However, the risk of disease morbidity is modifiable through improving lifestyle factors, such as increasing physical activity, minimising sedentary time and a consuming a healthy, balanced diet (O'Doherty et al 2016). One of the most frequently reported consequences of ageing is a decline in cognitive function. Specifically, executive control, attention, learning and memory are all known to decline with age (Kirova et al 2015). The interaction between the cognitive decline seen in 'normal' brain ageing and modifiable risk factors can significantly influence the likelihood of developing neurodegenerative diseases such as Alzheimer's disease (AD).

1.1 INFLAMMATION AND OXIDATIVE STRESS IN AGEING

Inflammation is a key protective process that is stimulated either by endogenous signalling or invading pathogens to minimise injury, remove necrotic cells and repair damage to tissue (Netea et al 2017). However, with increasing age there is a shift towards a chronic low-grade inflammatory state. The persistent elevation of inflammatory cytokines has been termed 'inflammageing' and is linked with a high susceptibility to chronic morbidity, disability, frailty, and premature death (Ferrucci & Fabbri 2018). In addition, the accumulation of genotoxic and oxidative stress can drive cellular senescence which is associated with organelle dysfunction, protein misfolding, dysregulated autophagy/mitophagy and DNA damage. This cellular dysfunction can then generate excess reactive oxygen

species (ROS) further elevating oxidative damage, contributing to the chronic inflammatory state seen in ageing (Fulop et al 2017). Reactive oxygen species are signalling molecules that are fundamental to normal physiology (Sies et al 2017). To prevent uncontrolled oxidation cascades, antioxidant enzymes and exogenous sources of antioxidant molecules are able to quench ROS to maintain reduction/oxidation (Redox) homeostasis. However, excessive ROS production or reduced antioxidant capacity, as a result of altered metabolic regulation, can have pathophysiological consequences. Chronic inflammation and elevated oxidative stress are intrinsically linked. ROS are a central feature of inflammasome signalling and have a role in the regulation of inflammatory pathways including the nuclear factor κ-light-chain-enhancer of activated B cells (NF-κB). In addition, ROS generation can lead to secondary oxidative modification of carbohydrates, proteins, lipids and DNA which can have deleterious consequences for cellular function (Radi 2018). The accumulation of these oxidative modifications have been implicated in the ageing process in the free radical theory of ageing (Harman 1992) and can be detected as markers of oxidative stress in several diseases such as cardiovascular, metabolic and neurodegenerative disease (Bisht et al 2018, Forrester et al 2018, Liguori et al 2018).

1.2 INFLAMMATION AND OXIDATIVE STRESS IN OBESITY

Obesity is a global health challenge with over a third of the population overweight or obese. In addition, obesity is becoming more prevalent in younger people (Ng et al 2014). People who are obese have an elevated risk of metabolic diseases, cardiovascular disease, cancer and neurodegenerative disease in which inflammation and oxidative stress mechanisms have been implicated (Saltiel & Olefsky 2017). Chronic obesity is associated with a sustained low-grade inflammation which is associated with the development of insulin resistance, hyperglycaemia and dyslipidaemia (Stolarczyk 2017). The alteration in cytokine signalling can also lead to elevated oxidation of biomolecules which have been found to accumulate in tissue specific sites and globally (Hauck et al 2019). An excessive supply of energy substrates to metabolic pathways in adipose and non-adipose tissue can lead to elevated ROS production coupled with mitochondrial dysfunction.

This can lead to disrupted ROS-mediated signalling pathways such as C-jun N-terminal kinases (JNK) and NF-κB which may play a key role in insulin resistance (Bonomini et al 2015). Typically, people who are obese consume a diet much lower in vitamins and antioxidants as well as engage in a more sedentary lifestyle which can further promote a pro- oxidative stress state (Hosseini et al 2017).

Adipose tissue has an important endocrine function controlling the release of several hormones including leptin and adiponectin as well as the secretion of cytokines. Adipose dysfunction caused by excessive adipocyte hypertrophy, impaired lipid metabolism and inadequate vascularisation can further promote a proinflammatory state. In addition, overwhelming adipocytes with lipids can lead to elevated ROS production which prevents 'healthy expansion' and increases ectopic lipid accumulation (Okuno et al 2018). The combination of elevated adipokines, increased ROS production and reduced antioxidant enzyme activity seen in obesity can significantly increase the risk of vascular and cognitive comorbidities (Fernández-Sánchez et al 2011).

1.3 ALZHEIMER'S DISEASE IN AGEING AND OBESITY

Although AD is typically associated with hallmark neuropathology, metabolic dysfunction of peripheral tissue has been identified as contributor to increased risk of the disease. Mid-life obesity, independent of insulin resistance and vascular disease, can significantly increase the risk of AD (Serrano-Pozo & Growdon 2019). However, the presence of these metabolic comorbidities can further increase the risk of developing AD (Caleyachetty et al 2017), possibly mediated by low-grade inflammation and increased oxidative stress (Letra et al 2014, Verdile & Keane 2015). Elevated inflammatory cytokine signalling cascades are a prevalent feature of AD in both peripheral tissue and brain (Ferreira et al 2014). Clustering of activated microglia and astrocytes around areas of the brain with amyloid-β (Aβ) plaques enables increased cytokine signalling and innate immunological action, resulting in neuroinflammation (Heneka et al 2015). Systemic inflammation is thought to interfere and interact with immunological processes in the brain and promote disease progression, therefore, peripheral markers of inflammation have received much attention in the assessment of AD risk.

Increased oxidative stress may also be an early event in the propagation of AD (Caldwell et al 2015, Nunomura et al 2001, Zhu et al 2004). The brain is susceptible to oxidative stress due to its high energy demand, high polyunsaturated fat content and limited antioxidant capacity (Skoumalová & Hort 2012). Several studies have shown that AD is associated with greater protein carbonyl formation, both in the brain (Butterfield et al 2006) and in the periphery in specific proteins linked to cardiovascular dysfunction (Aldred et al 2010). This elevation is even seen in mild cognitive impairment (MCI), which is often seen as a precursor to AD (Bermejo et al 2008, Conrad et al 2000, Greilberger et al 2010, Perrotte et al 2019). The formation of protein carbonyls can be initiated by end products of lipid peroxidation. Due to the brains high poly-unsaturated fat content, small lipophilic molecules with the capability to cross the blood brain barrier can be produced. These lipophilic molecules are then able to oxidise downstream peripheral targets (Skoumalová & Hort 2012). F2 isoprostanes are one of the most reliable markers of lipid peroxidation. These prostaglandin-like molecules arise from the non-enzymatic esterification and hydrolysis of the poly-unsaturated fatty acid, Arachidonic acid. F2-isoprostanes have been found to be significantly increased in AD (Sinem et al 2010).

Although the onset of AD symptoms is typically seen over the age of 65, there is a growing consensus that the underlying biochemical pathology may be present as much as 30 years prior to this. Therefore, it is imperative to develop a greater understanding of how lifestyle factors in younger populations can impact AD. Investigating accessible biomarkers that enable early detection of disease, such as AD is therefore a priority for current and future research.

1.4 PLATELET MARKERS OF AβPP PROCESSINGS IN ALZHEIMER'S DISEASE

A-disintegrin and metalloproteinase 10 (ADAM10) is a secretase protein that may protect against the development of AD. As the major physiologically relevant α-secretase involved in the processing of the amyloid-β precursor protein (AβPP), ADAM10 is known to prevent Aβ formation and liberate the neuroprotective soluble AβPP-α segment (Kuhn et al., 2010; Postina et al., 2004). This is opposed to the 'amyloidogenic pathway' in which AβPP is first cleaved by β-amyloid converting

enzyme-1 (BACE-1) generating sAβPP-β and then further processed to generate the toxic Aβ peptides seen in AD.

ADAM10 expression and activity is reduced in AD, which can impact the development of tau pathology, maintain normal synaptic function and neural network homeostasis (Yuan et al 2017). ADAM10 is a transmembrane anchored protein that is synthesised as a proenzyme (ProADAM10). During trafficking towards the cell membrane, the auto-inhibitory pro domain is removed by proprotein convertases to liberate the catalytically active peptide (mADAM10). The trafficking of ADAM10 is a crucial step in its ability to physically interact with substrates, such as APP, and this is regulated by a network of proteins known as c8-Tetraspanins (TSPAN-C8) (Jouannet et al 2016, Matthews et al 2017). ADAM10 is expressed in many different tissues and cleaves multiple substrates, however its expression in platelets has gained significant interest due to the accessibility of platelets as a sample for biomarker assessment. Platelets co-express AβPP and BACE-1 in addition to neurotrophic growth factors and neurotransmitters and thus, present an excellent opportunity to study these markers of AD when compared to the less accessible or costly alternatives, for example cerebral spinal fluid (CSF), brain imaging and post mortem tissue (Kermani & Hempstead 2019).

Early studies looking at the platelet markers of AβPP processing focused on the expression AβPP isoforms. Longer isoforms of 120-130kDa represent mature AβPP which has been post-translationally modified though N- and O- glycosylation during trafficking to the cell membrane (Schedin-Weiss et al 2014). Shorter isoforms of AβPP at 110kDa are more likely to interact with BACE-1 intracellular vesicles and thus are likely to follow the amyloidogenic pathway. The ratio of AβPP isoforms (AβPPr), 130/120:110kDa has been shown to decrease in people with AD (Bram et al 2018, Colciaghi et al 2004). Similarly, the cleaved sAβPP-α segment is decreased in people with AD indicating a reduction in non-amyloidogenic AβPP cleavage. This may be explained by altered AβPP isoforms or alterations in ADAM10 activity Colciaghi et al., (2002). In a subsequent study ADAM10 expression was decreased in very mild and mild AD compared to healthy controls (Colciaghi et al., 2004). More recently ADAM10 expression has been used to predict scoring on measures of cognitive function (Manzine et al 2014, Manzine et al 2013).

Due to the prodromal nature of AD development, it may be possible to detect change in platelet AβPPr and ADAM10 expression prior to clinical symptoms of dementia. Both age and obesity are known risk factors for the development of AD and may therefore impact platelet markers associated with cognitive decline in addition to inflammatory and oxidative stress markers. Interestingly, cognitively healthy older adults have been shown to have increased ADAM10 expression and activity compared to younger individuals (Schuck et al 2016). The effects of obesity on ADAM10 activity, however, has not been assessed. This may provide a novel link between obesity and AD risk in otherwise healthy participants.

2. MATERIALS AND METHODS

2.1 PARTICIPANTS

Ninety participants were recruited via online recruitment websites, poster advertisement and participant databases. Participants were invited to make one visit to the lab for anthropometric measures and blood collection following a standardised breakfast. Ethical approval was obtained from the Institutional Science, Technology, Engineering and Mathematics ethics panel.

2.2 BLOOD COLLECTION

Blood (6 mL) was collected in EDTA tubes (Vacutainer, BD). The interval between the collection and the processing was a maximum of 30 minutes. The platelet-rich plasma (PRP) was obtained by centrifugation at 100x*g* for 20 min and 1x protease inhibitors were added (Halt protease inhibitor, Thermo Fisher scientific). Platelet lysates were also collected by additional centrifugation of PRP at 7600x*g* for 10 min at room temperature, then washed twice in phosphate buffered saline solution (PBS). Finally, the platelet pellet was suspended in cold lysis buffer (200 mM NaCl, 10 mM EDTA, 10 mM Na_2HPO_4, 0.5% NP40, 0.1% SDS, and 1x protease inhibitors) and incubated for 30 minutes at room temperature. Aliquots of PRP and platelet lysates were then frozen at -80 °C until further use.

2.3 SDS-PAGE AND WESTERN BLOTTING

The protocols used for SDS-PAGE and western blotting were adapted from Manzine et al. (2013) with minor modifications. The necessary volume of platelet lysate containing 10µg of protein, quantified via BCA assay (Smith et al., 1985), was boiled for 5mins at 95°C and loaded into 10% SDS- PAGE gel. To confirm ADAM10 detection, a purified ADAM10 peptide (Abcam 7868) was used to compare samples. Molecular weight markers were also loaded to give context to gel running and transfer (Amersham ECL DualVue, RPN 810). After the gel running, the proteins were transferred to nitrocellulose membranes (Amersham protran 0.2µm) using the Mini Trans-Blot Cell transfer system (BioRad) for 1 hour. Membranes were blocked with 5% milk in TBST 0.5% for 1 hour and washed in 0.05% TBST. Membranes were then incubated in the primary antibody for ADAM 10 (AB1997), AβPP (Merck

MAB348, 22C11) or Actin (Sigma AC40 A3853) followed by incubation with corresponding secondary antibodies (Sigma Anti-mouse IgG FC-specific A0168 or Cell Signalling Anti-rabbit IgG, HRP-linked Antibody 7074). After, the membranes were developed using ECL substrate (Clarity Western, Biorad) according to manufacturer instruction and imaged using C-digit scanner (Licor). Finally. Bands were quantified using Image Studio (Licor).

2.4 QUANTIFICATION OF IL-6 VIA IMMUNOASSAY

IL-6 was measured using a human IL-6 immunoassay (Quantikine HS ELISA, R&D systems) following manufacturer's instructions. IL-6 standards were prepared from a dilution series ranging from 0 to 10pg/ml to create an 8-point standard curve. Standard or sample (100µL) was added to the appropriate wells with assay diluent (100µL) and this was left to bind at room temperature with gentle orbital shaking (500 ± 50rpm) for 2 hours. The plate was then washed 6 times with wash buffer (400µL) before human IL-6 conjugate (200µL) was added and incubated for 2 hours as previously described. Following a second wash step, substrate solution (50µL) was added and allowed to bind for 60 minutes on the benchtop, followed by the addition of the amplifier solution with a 30-minute incubation without shaking. Finally, stop solution (50µL) was added and the absorbance was read at 490nm with 650nm wavelength corrections.

2.4 QUANTIFICATION OF PROTEIN CARBONYL FORMATION

Modification of protein by formation of carbonyl groups was assessed by the method of Carty et al. (2000). Briefly, samples (plasma proteins) and standards (BSA) were diluted in carbonate buffer (sodium carbonate 50mM, pH 9.2) and plated into 96 well plates (50µL at 0.05mg/ml). Protein was allowed to bind for 1h at 37^{O}C before washing with TBS–Tween (300µL, 0.5%). DNPH was added in 2M HCl (1mM, 50µL) and allowed to react for 1h at room temperature before washing as before. Non-specific binding sites were blocked overnight at 4^{O}C with TBS-tween (200µL, 1%). After washing, rabbit anti-DNPH primary antibody (50µL, 1:1000) was applied and incubated for 1h at 37^{O}C and, following washing with TBS/Tween, anti-rabbit IgE conjugated to peroxidase (50µL, 1:5000) was also incubated at 37^{o}C for 1h. The

reaction was visualised by substrate solution (50µL; o-phenylenediamine tablets with hydrogen peroxide (8µl) in citrate-phosphate buffer (10ml)) and stopped by addition of sulphuric acid (50µL, 2N). Absorbance was read at 490nm.

2.5 MEASUREMENT OF LIPID PEROXIDATION

Total 8-isoprostanes were measured using an ELISA kit to assess lipid peroxidation in plasma (8-isoprostane ELISA kit, Cayman Chemical). Prior to this assay the plasma samples were purified following the manufacturer's instructions. Briefly, plasma (100 µL) was added to the 8-isoprostane affinity sorbent (401113, Cayman Chemical) and incubated for 60 minutes with gentle mixing and then centrifuged at 1500xg for 30 seconds to sediment the sorbent. The supernatant was removed and discarded. Eicosanoid Column Affinity buffer (100µL, 400220, Cayman Chemical) was added and placed in the centrifuge at 1500xg for 30 seconds, the supernatant was then removed. This step was repeated by adding ultrapure water (100µL) before centrifugation and discarding the supernatant. Elution solution (100µL, 95% ethanol) was then added to the sediment and evaporated to dryness under nitrogen. Samples were suspended in the ELISA buffer (100µL). Next, standards were prepared from the assay stock solution to create an 8-point standard curve ranging from 0.8 to 500pg/ml, a blank well was used as the 0pg.ml standard. Standard or sample (50µL) was added to the appropriate well with of 8-isoprostane tracer (50µL) and antiserum (50µL). This was incubated for 18hours at 4^{O}C. Ellman's reagent was added to each well (200µL) and left to incubate for 2 hours with gentle agitation. The plate was then read at 420nm.

2.6 ANTIOXIDANT CAPACITY – FERRIC REDUCING ABILITY OF PLASMA

Plasma samples were diluted 1:1 with ultrapure water (Millipore). Standards were freshly prepared using Ascorbic acid to create a 7-point standard curve ranging from 0µM to 1000µM. Sample or standard (10µl) was then added into wells a 96-well microtiter plate in duplicate. Next, the 'FRAP' reagent (300µl) was made by combining acetate buffer (30ml, 300mM), TPTZ solution (3ml, 10.6mM) and ferric chloride solution (3ml, 20mM) and added to each well. The plate was then incubated for 8 minutes at room temperature. Plate reading was completed at 650nm and

values were calculated using linear regression. Values were expressed as μM of antioxidant power relative to ascorbic acid.

2.7 STATISTICAL ANALYSIS

Statistical analysis was undertaken using IBM SPSS statistics version 25. Between-groups comparisons were carried out using a one-way repeated measures ANOVA, with Bonferroni corrections applied. Western blot data for ADAM10 expression was analysed using the Kruskal-Wallis H test with Dunn-Bonferroni corrections. Linear regression analysis was used to assess relationships across the whole sample.

3. RESULTS

3.1 PARTICIPANT CHARACTERISTICS

Participants were placed into one of four groups based on their age and body mass index (BMI). Inclusion criteria for age was under 40 years for the young groups and over 65 years for the older groups. BMI requirements for the Lean group was below 24.9 $kg.m^2$ and over 29.9 $kg.m^2$ for the Obese group. Participants were free from a history of gastric banding, eating disorders, neurological or inflammatory disorders (e.g., rheumatoid arthritis, inflammatory bowel disease, multiple sclerosis, periodontitis) or use of anti-depressant, antihistamine, or anti-inflammatory (e.g., antibiotics) medication during the past 7 days.

Table 1. Characteristics and comparison of the participant sample

ITEM	YOUNG LEAN	YOUNG OBESE	OLD LEAN	OLD OBESE
PARTICIPANTS (N=90)	21	21	24	24
AGE (YEARS)	24.67 ± 3.38	27.90 ± 4.27	71.67 ± 4.30*	69.67 ± 3.53*
BMI ($Kg.M^2$)	21.97 ± 2.69	34.02 ± 3.67#	23.51 ± 2.13	32.96 ± 3.66#
COMORBIDITIES				
CVD	0	0	7	13
DIABETES	0	0	3	5
HYPERTENSION	0	0	6	8
MEDICATION				
STATINS	0	0	6	8
ANTIHYPERGLYCEMIC AGENTS	0	0	2	5

(Data shown as mean ± SD)

**Significantly greater than Young Lean and Young Obese* ($p<.05$)

#Significantly greater than Young Lean and Old Lean ($p<.05$)

3.2 INFLAMMATION

Plasma IL-6 (pg/ml) (*Figure 1**A***) was significantly elevated in young obese (2.87 ± 1.69, p=0.007) and old obese (2.50 ± 1.10, p=.000) compared to young lean participants (1.22 ± 0.58). No significant effect of age was detected (p>.05). Simple regression analysis revealed that BMI was the most influential predictor of plasma IL-6 ((F(1,88)= 20.625, p=.000) R^2 = .190). Plasma IL-6 (pg/ml) was found to increase for every .098 kg.m^2 increase in BMI. In addtion, ROC analysis of plasma IL-6 showed an ability to distinguish between obese and non-obese participants (AUC of 0.80, SE = 0.05, P < 0.001)(*Figure 1**C**.*).

3.3 OXIDATIVE STRESS

Plasma 8- Isoprostanes (pg/ml)(*Figure 1**B**.*) were significantly elevated in old obese participants (52.78 ± 25.09) compared to young lean (25.71 ± 14.51, p = .000)*,* young obese (27.97 ± 15.10, p=.000) and old lean (32.87 ± 14.21, p = .001) participants. Both age ((F(1,88) = 12.371, p = .001) R^2 = .123) and BMI ((F(1,88) = 7.413, p = .008) R^2 = .078) were significant predictors of plasma 8-isoprostane concentration, however, this was greatest for age with an increase in 8-isoprostanes of 1 pg/ml for every .322 increase in years. Plasma 8-isoprostanes were able to distinguish between both young/ old (AUC of 0.73, SE = 0.05, P < 0.01)(*Figure 1**C**.*), and obese/ non-obese participants (AUC of 0.66, SE = 0.01, P < .01)(*Figure 1**D**.*). Sub-group analysis revealed that plasma 8-isoprostanes were significantly elevated in obese participants with a diagnosis of diabetes compared to obese participants without (66.82 ± 29.99, 38.02 ± 17.58, p < .01).

Plasma protein carbonyls (nmol/mg) were significantly elevated in old obese (1.44 ± 0.31) compared to young obese participants (1.05 ± 0.13, p = .000). Further analysis showed that plasma protein carbonyls could distinguish young and old participants (AUC of 0.69, SE = 0.07 P < .02)(*Figure 1**D.***). No significant effects were found for total blood antioxidant status (p > .05).

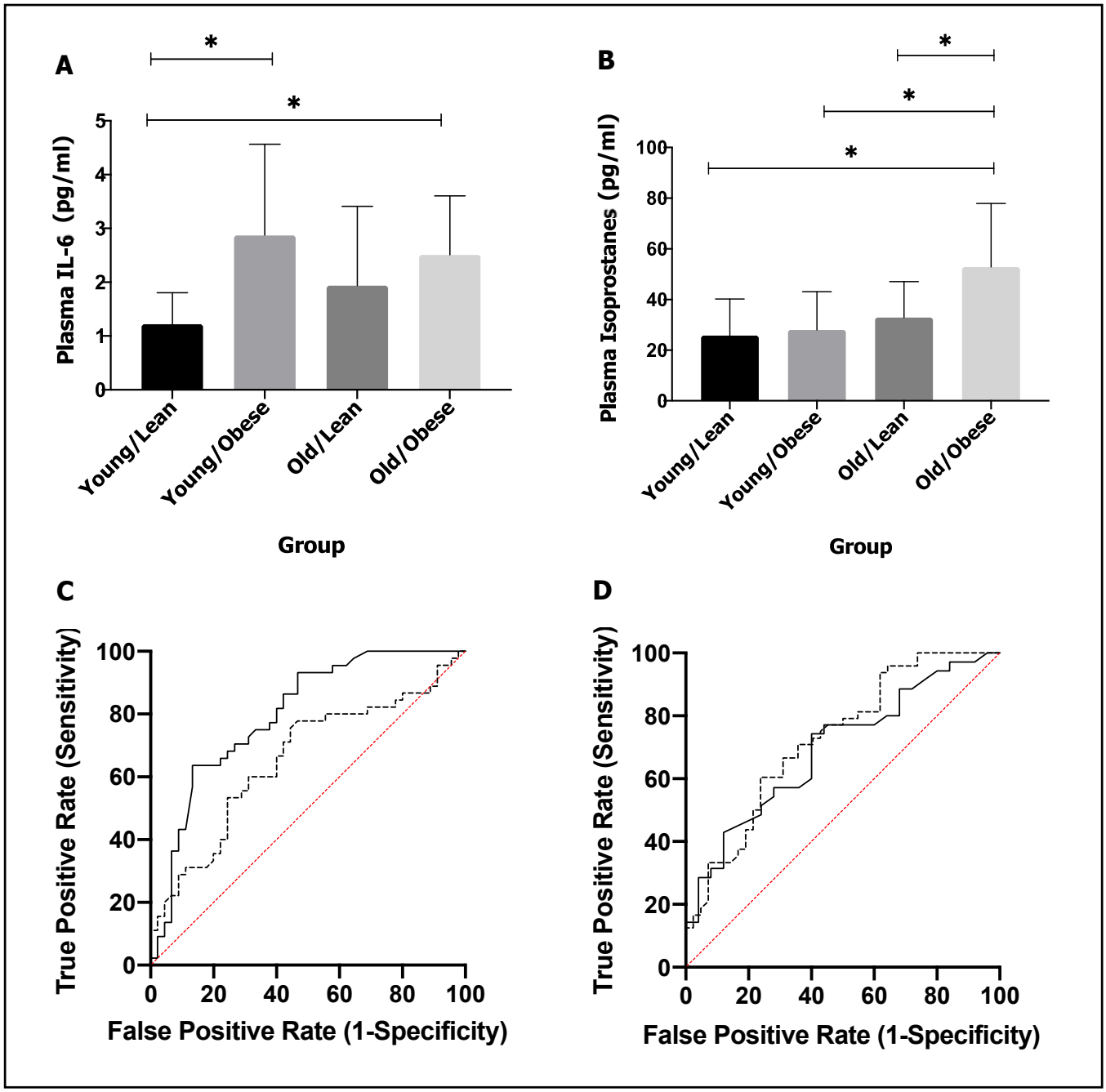

Figure 1. Effect of age (years) and BMI (kg/m^2) on plasma markers of inflammation and oxidative stress. **A**, Plasma IL-6 (pg/ml) measured via ELISA compared between groups. **B**, Concentrated plasma 8-isoprostane (pg/ml) measured via ELISA compared between groups. **C.** ROC analysis for predictive capacity of plasma IL-6 (solid line) and isoprostanes (dashed line) to distinguish between obese and non-obese participants. **D**, ROC analysis for predictive capacity of plasma protein carbonyls (solid line) and isoprostanes (dashed line) to distinguish between young

and old participants. * indicates a significant difference between corresponding groups.

3.4 PLATELET APP PROCESSING MARKERS

A Kruskal-Wallis H test showed that there was a statistically significant difference in Mean Rank platelet mADAM10 protein levels (AU) between groups, $\chi2(3) = 16.734$, $p = 0.001$ (*Figure 2**A***.). Old Lean participants had significantly lower platelet mADAM10 protein levels than both Young Lean ($p = 0.017$) and Young Obese participants ($p = 0.007$). Old obese participants also typically showed lower platelet mADAM10 protein levels compared to Young Lean ($p = 0.083$) and Young Obese ($p = 0.033$). Mean Rank platelet proADAM10 protein levels (AU) was also significantly different between groups, $\chi2(3) = 13.986$, $p = 0.003$ (*Figure 2**B***). Old obese participants had significantly lower platelet proADAM10 protein levels compared to both Young Lean ($p = 0.011$) and Young Obese ($p = 0.030$). Both platelet mADAM10 and proADAM10 were significantly decreased with advancing age ($p<0.05$). Platelet mADAM10 was found to significantly decrease with elevated protein carbonyls. There was no statistically significant difference in platelet AβPPr between groups ($p>0.05$).

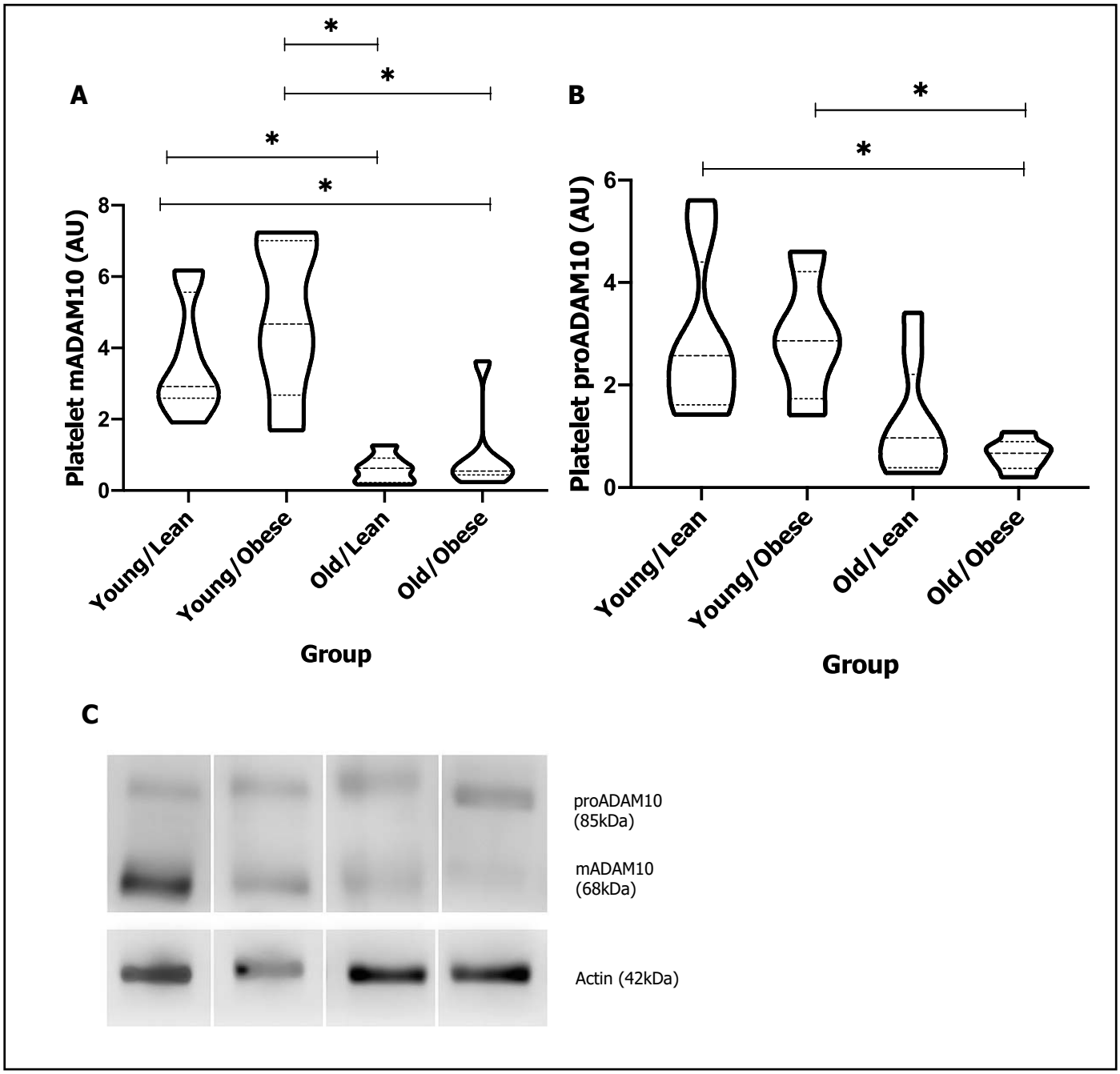

Figure 2 Effect of age (years) and BMI (kg.m^2) on platelet protein levels of AβPP isoforms and ADAM10 measured via western blotting in a sub sample of participants (n=26). **A**, Platelet protein levels of mADAM10 (AU) normalised to Actin compared between groups. **B**, Platelet protein levels of proADAM10 (AU) normalised to Actin compared between groups. **C**, exemplar images of western blotting for ADAM10 (top box), Actin as a control protein (middle box).

*significant difference between corresponding groups.

4. DISCUSSION

This results from this study suggest that age and obesity are differentially associated with inflammation and oxidative stress. Obesity was associated with elevated inflammation irrespective of age. This finding supports other similar studies suggesting that low-grade inflammation is prevalent in people who are obese and may be linked to the presence of insulin resistance, hyperglycaemia and dyslipidaemia (Stolarczyk 2017). The alteration in cytokine signalling seen in obesity can also lead to elevated oxidation of biomolecules, which have been found to accumulate in tissue specific sites and globally (Hauck et al 2019). This is reflected in the current data presented showing that obese participants had the greatest accumulation of 8-isoprostanes.

Older age, not obesity, was more strongly associated with elevated markers of oxidative stress. Although the production of ROS is essential for normal physiological function, the gradual accumulation of damage as a result of redox imbalance is present in ageing. These oxidative markers are typically further elevated in a number of disease pathologies including neurodegenerative diseases (Bisht et al 2018, Forrester et al 2018, Liguori et al 2018). Interestingly, lipid oxidation was associated with obesity however, this was not significantly altered in with younger obese participants. It is possible that younger individuals are able to better tolerate increased ROS production and it is in mid to late-life that obesity significantly impacts oxidative stress (Letra et al 2014).

This present study is the first to measure changes in ADAM10 protein levels in obese individuals. Ageing was linked to lower protein levels of proADAM10, whereas obesity in later life was associated with lower mADAM10. Alzheimer's Disease is argued to exist as a prodromal syndrome long before the onset of clinical symptoms (Dubois et al 2016). Therefore, a major challenge for researchers is to develop biomarkers capable of detecting AD in 'at-risk' populations to enable effective intervention. People who are obese are one example of an at-risk population (Caleyachetty et al 2017, Serrano-Pozo & Growdon 2019). Platelet ADAM10 is an enzyme that acts to preclude Aβ liberation from AβPP and is currently under investigation as a potential biomarker. The protein levels of pro- and mADAM10 was lower in older individuals compared to younger individuals. This contradicts research

by Schuck et al. (2016) who found elevated ADAM10 protein expression and activity in cognitively healthy older adults. A potential explanation for this difference could be that both studies are subject to recruitment bias. The trajectory of ADAM10 expression across the lifespan may be dependent on the individual and therefore, those who age cognitively healthy may have elevated ADAM10 activity from mid-life or younger ages.

In this study there was a significantly lower amount of proADAM10 that only found in old obese participants. This may provide insight into a link between late life-obesity and increased AD risk and warrants further investigation. Although there was a significant reduction in mADAM10, proADAM10 was not significantly lowered in healthy older participants compared to younger participants. Therefore, ageing may alter the trafficking or pro-domain cleavage of the proADAM10 peptide which are both crucial post-translational steps for generation mature ADAM10. The significant reduction of ProADAM10 was only seen in older participants who were also obese. This may suggest a combinatory effect of ageing and obesity on Pro-ADAM10 protein levels and may reflect changes at a protein transcription level.

Future research into the effect of obesity as a co-factor in the development of AD should examine changes in biomarkers over a longitudinal period. No changes in ADAM10 protein levels were found in young obese participants, it is possible this age range precludes the typical onset of AD pathological changes in AβPP processing and therefore, assessing individuals who are obese as they progress into mid-life may hold valuable information. This research also highlights the potential for ADAM10 to be used as a marker in other high-risk AD populations, such as those with metabolic syndrome.

4.1 CONCLUSION

In conclusion, this study provides evidence that blood-based biomarkers related to the pathology of AD are altered with age and obesity. Ageing was more strongly associated with elevated markers of oxidative stress whereas obesity was associated with elevated inflammatory IL-6. Although there was no difference in AβPP, ADAM10 protein levels was significantly reduced in old, obese participants.

This indicates that there may be a reduction in non-amyloidogenic AβPP processing, which has been found to occur in people with AD.

ACKNOWLEDGEMENTS

The authors would like to acknowledge Alzheimer's Research UK for supporting some of the laboratory work undertaken during this research.

FUNDING

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

REFERENCES

Aldred S, Bennett S, Mecocci P. 2010. Increased low-density lipoprotein oxidation, but not total plasma protein oxidation, in Alzheimer's disease. *Clin Biochem* 43: 267-71

Bermejo P, Martín-Aragón S, Benedí J, Susín C, Felici E, et al. 2008. Peripheral levels of glutathione and protein oxidation as markers in the development of Alzheimer's disease from Mild Cognitive Impairment. *Free Radic Res* 42: 162-70

Bisht K, Sharma K, Tremblay M. 2018. Chronic stress as a risk factor for Alzheimer's disease: Roles of microglia-mediated synaptic remodeling, inflammation, and oxidative stress. *Neurobiol Stress* 9: 9-21

Bonomini F, Rodella LF, Rezzani R. 2015. Metabolic syndrome, aging and involvement of oxidative stress. *Aging Dis* 6: 109-20

Bram JMF, Talib LL, Joaquim HPG, Sarno TA, Gattaz WF, Forlenza OV. 2018. Protein levels of ADAM10, BACE1, and PSEN1 in platelets and leukocytes of Alzheimer's disease patients. *Eur Arch Psychiatry Clin Neurosci*

Butterfield DA, Perluigi M, Sultana R. 2006. Oxidative stress in Alzheimer's disease brain: new insights from redox proteomics. *Eur J Pharmacol* 545: 39-50

Caldwell CC, Yao J, Brinton RD. 2015. Targeting the prodromal stage of Alzheimer's disease: bioenergetic and mitochondrial opportunities. *Neurotherapeutics* 12: 66-80

Caleyachetty R, Thomas GN, Toulis KA, Mohammed N, Gokhale KM, et al. 2017. Metabolically Healthy Obese and Incident Cardiovascular Disease Events Among 3.5 Million Men and Women. *J Am Coll Cardiol* 70: 1429-37

Colciaghi F, Marcello E, Borroni B, Zimmermann M, Caltagirone C, et al. 2004. Platelet APP, ADAM 10 and BACE alterations in the early stages of Alzheimer disease. *Neurology* 62: 498-501

Conrad CC, Marshall PL, Talent JM, Malakowsky CA, Choi J, Gracy RW. 2000. Oxidized proteins in Alzheimer's plasma. *Biochem Biophys Res Commun* 275: 678-81

Cuthbertson DJ, Bell JA, Ng SY, Kemp GJ, Kivimaki M, Hamer M. 2016. Dynapenic obesity and the risk of incident Type 2 diabetes: the English Longitudinal Study of Ageing. *Diabet Med* 33: 1052-9

Dubois B, Hampel H, Feldman HH, Scheltens P, Aisen P, et al. 2016. Preclinical Alzheimer's disease: Definition, natural history, and diagnostic criteria. *Alzheimers Dement* 12: 292-323

Fernández-Sánchez A, Madrigal-Santillán E, Bautista M, Esquivel-Soto J, Morales-González A, et al. 2011. Inflammation, oxidative stress, and obesity. *Int J Mol Sci* 12: 3117-32

Ferreira ST, Clarke JR, Bomfim TR, De Felice FG. 2014. Inflammation, defective insulin signaling, and neuronal dysfunction in Alzheimer's disease. *Alzheimers Dement* 10: S76-83

Ferrucci L, Fabbri E. 2018. Inflammageing: chronic inflammation in ageing, cardiovascular disease, and frailty. *Nat Rev Cardiol* 15: 505-22

Forrester SJ, Kikuchi DS, Hernandes MS, Xu Q, Griendling KK. 2018. Reactive Oxygen Species in Metabolic and Inflammatory Signaling. *Circ Res* 122: 877-902

Fulop T, Larbi A, Dupuis G, Le Page A, Frost EH, et al. 2017. Immunosenescence and Inflamm-Aging As Two Sides of the Same Coin: Friends or Foes? *Front Immunol* 8: 1960

Greilberger J, Fuchs D, Leblhuber F, Greilberger M, Wintersteiger R, Tafeit E. 2010. Carbonyl proteins as a clinical marker in Alzheimer's disease and its relation to tryptophan degradation and immune activation. *Clin Lab* 56: 441-8

Hansen M, Kennedy BK. 2016. Does Longer Lifespan Mean Longer Healthspan? *Trends Cell Biol* 26: 565-68

Harman D. 1992. Free radical theory of aging. *Mutat Res* 275: 257-66

Hauck AK, Huang Y, Hertzel AV, Bernlohr DA. 2019. Adipose oxidative stress and protein carbonylation. *J Biol Chem* 294: 1083-88

Heneka MT, Carson MJ, El Khoury J, Landreth GE, Brosseron F, et al. 2015. Neuroinflammation in Alzheimer's disease. *Lancet Neurol* 14: 388-405

Hosseini B, Saedisomeolia A, Allman-Farinelli M. 2017. Association Between Antioxidant Intake/Status and Obesity: a Systematic Review of Observational Studies. *Biol Trace Elem Res* 175: 287-97

Jouannet S, Saint-Pol J, Fernandez L, Nguyen V, Charrin S, et al. 2016. TspanC8 tetraspanins differentially regulate the cleavage of ADAM10 substrates, Notch activation and ADAM10 membrane compartmentalization. *Cell Mol Life Sci* 73: 1895-915

Kermani P, Hempstead B. 2019. BDNF Actions in the Cardiovascular System: Roles in Development, Adulthood and Response to Injury. *Front Physiol* 10: 455

Kirova AM, Bays RB, Lagalwar S. 2015. Working memory and executive function decline across normal aging, mild cognitive impairment, and Alzheimer's disease. *Biomed Res Int* 2015: 748212

Lennon LT, Ramsay SE, Papacosta O, Shaper AG, Wannamethee SG, Whincup PH. 2015. Cohort Profile Update: The British Regional Heart Study 1978-2014: 35 years follow-up of cardiovascular disease and ageing. *Int J Epidemiol* 44: 826-26g

Letra L, Santana I, Seiça R. 2014. Obesity as a risk factor for Alzheimer's disease: the role of adipocytokines. *Metab Brain Dis* 29: 563-8

Liguori I, Russo G, Curcio F, Bulli G, Aran L, et al. 2018. Oxidative stress, aging, and diseases. *Clin Interv Aging* 13: 757-72

Manzine PR, Barham EJ, Vale FA, Selistre-de-Araújo HS, Pavarini SC, Cominetti MR. 2014. Platelet a disintegrin and metallopeptidase 10 expression correlates with clock drawing test scores in Alzheimer's disease. *Int J Geriatr Psychiatry* 29: 414-20

Manzine PR, Barham EJ, Vale FeA, Selistre-de-Araújo HS, Iost Pavarini SC, Cominetti MR. 2013. Correlation between mini-mental state examination and platelet ADAM10 expression in Alzheimer's disease. *J Alzheimers Dis* 36: 253-60

Matthews AL, Szyroka J, Collier R, Noy PJ, Tomlinson MG. 2017. Scissor sisters: regulation of ADAM10 by the TspanC8 tetraspanins. *Biochem Soc Trans* 45: 719-30

Netea MG, Balkwill F, Chonchol M, Cominelli F, Donath MY, et al. 2017. A guiding map for inflammation. *Nat Immunol* 18: 826-31

Ng M, Fleming T, Robinson M, Thomson B, Graetz N, et al. 2014. Global, regional, and national prevalence of overweight and obesity in children and adults during 1980-2013: a systematic analysis for the Global Burden of Disease Study 2013. *Lancet* 384: 766-81

Nunomura A, Perry G, Aliev G, Hirai K, Takeda A, et al. 2001. Oxidative damage is the earliest event in Alzheimer disease. *J Neuropathol Exp Neurol* 60: 759-67

O'Doherty MG, Cairns K, O'Neill V, Lamrock F, Jørgensen T, et al. 2016. Effect of major lifestyle risk factors, independent and jointly, on life expectancy with and without cardiovascular disease: results from the Consortium on Health and Ageing Network of Cohorts in Europe and the United States (CHANCES). *Eur J Epidemiol* 31: 455-68

Okuno Y, Fukuhara A, Hashimoto E, Kobayashi H, Kobayashi S, et al. 2018. Oxidative Stress Inhibits Healthy Adipose Expansion Through Suppression of SREBF1-Mediated Lipogenic Pathway. *Diabetes* 67: 1113-27

Perrotte M, Le Page A, Fournet M, Le Sayec M, Rassart E, et al. 2019. Blood-based redox-signature and their association to the cognitive scores in MCI and Alzheimer's disease patients. *Free Radical Biology and Medicine* 130: 499-511

Radi R. 2018. Oxygen radicals, nitric oxide, and peroxynitrite: Redox pathways in molecular medicine. *Proc Natl Acad Sci U S A* 115: 5839-48

Saltiel AR, Olefsky JM. 2017. Inflammatory mechanisms linking obesity and metabolic disease. *J Clin Invest* 127: 1-4

Schedin-Weiss S, Winblad B, Tjernberg LO. 2014. The role of protein glycosylation in Alzheimer disease. *FEBS J* 281: 46-62

Schuck F, Wolf D, Fellgiebel A, Endres K. 2016. Increase of α-Secretase ADAM10 in Platelets Along Cognitively Healthy Aging. *J Alzheimers Dis* 50: 817-26

Serrano-Pozo A, Growdon JH. 2019. Is Alzheimer's Disease Risk Modifiable? *J Alzheimers Dis* 67: 795-819

Sies H, Berndt C, Jones DP. 2017. Oxidative Stress. *Annu Rev Biochem* 86: 715-48

Sinem F, Dildar K, Gökhan E, Melda B, Orhan Y, Filiz M. 2010. The serum protein and lipid oxidation marker levels in Alzheimer's disease and effects of cholinesterase inhibitors and antipsychotic drugs therapy. *Curr Alzheimer Res* 7: 463-9

Skoumalová A, Hort J. 2012. Blood markers of oxidative stress in Alzheimer's disease. *J Cell Mol Med* 16: 2291-300

Smetana K, Lacina L, Szabo P, Dvořánková B, Brož P, Šedo A. 2016. Ageing as an Important Risk Factor for Cancer. *Anticancer Res* 36: 5009-17

Stolarczyk E. 2017. Adipose tissue inflammation in obesity: a metabolic or immune response? *Curr Opin Pharmacol* 37: 35-40

Tesauro M, Mauriello A, Rovella V, Annicchiarico-Petruzzelli M, Cardillo C, et al. 2017. Arterial ageing: from endothelial dysfunction to vascular calcification. *J Intern Med* 281: 471-82

Verdile G, Keane KC, Vinicius Medic, Sandra Sabale, Miheer Rowles, Joanne Wijesekara, Nadeeja Martins, Ralph Fraser, Paul Newsholme, Philip. 2015. Inflammation and Oxidative Stress: The Molecular Connectivity between Insulin Resistance, Obesity, and Alzheimer's Disease. *Mediators of Inflammation* 26: 1-17

Yuan XZ, Sun S, Tan CC, Yu JT, Tan L. 2017. The Role of ADAM10 in Alzheimer's Disease. *J Alzheimers Dis* 58: 303-22

Zhu X, Raina AK, Perry G, Smith MA. 2004. Alzheimer's disease: the two-hit hypothesis. *Lancet Neurol* 3: 219-26