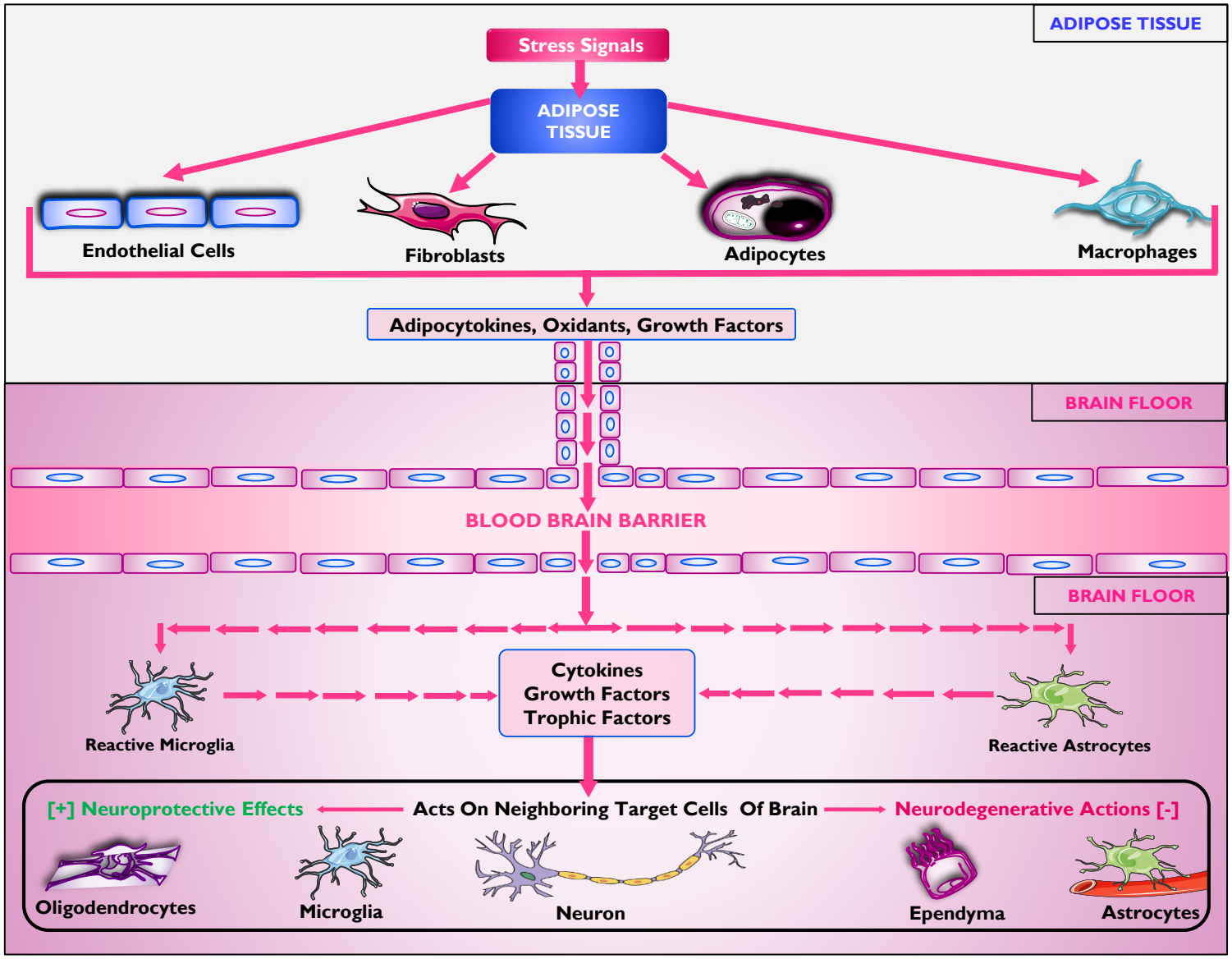
Propriétés anti-inflammatoires de facteurs produits par le tissu adipeux - Applications potentielles dans la neurodégénérescence

Avinash Parimisetty

Thesis For Doctoral Degree (Ph.D)
2015

Anti-Inflammatory Properties Of Factors Produced By The Fat Tissue - Potential Applications In Neurodegeneration



AVINASH PARIMISETTY

UNIVERSITÉ DE LA RÉUNION
S'ouvrir aux mondes

THÈSE

Présenté pour l'obtention du titre de Docteur de l'Université de La Réunion

Spécialité - **Biologie cellulaire, Moléculaire et Biochimie**

Par

Avinash PARIMISETTY

Propriétés anti-inflammatoires de facteurs produits par le tissu adipeux - Applications potentielles dans la neurodégénérescence

Soutenue publiquement le vendredi 19 Juin 2015

JURY:

Mme. Dora Brites, Pr. Université de Lisbonne, Portugal	**Rapporteur**
M. Fabien Gosselet, MC-HDR Université d'Artois	**Rapporteur**
Mme. Marjolaine Roche, MC Université de La Réunion	**Examinateur**
M. Christian Lefebvre d Hellencourt, Pr. Université de La Réunion	**Directeur**

DOCTORAL THESIS

Presented By

Avinash Parimisetty

To the Faculty of Health

Université de La Réunion

For The Award Of

Doctor of Philosophy (Ph.D) in Sciences

On The Research Project Entitled

Anti-Inflammatory Properties of Factors Produced By the Fat Tissue - Potential Applications in Neurodegeneration

Discipline - **Cellular, Molecular Biology and Biochemistry**

Thesis Will Be Publicly Defended at Amphi CHARPAK Grand Auditorium, Faculté des Sciences et Technologies, Saint-Denis on 19th Friday June 2015

JURY:

Mrs. Dora Brites, Prof. University of Lisbon, Portugal	**External Examiner**
Mr. Fabien Gosselet, MC-HDR Université d'Artois	**External Examiner**
Mrs. Marjolaine Roche, MC Université de La Réunion	**Examiner**
Mr. Christian Lefebvre d Hellencourt, Prof. Université de La Réunion	**Thesis Director**

Anti-Inflammatory Properties of Factors Produced By the Fat Tissue

- Potential Applications In Neurodegeneration

By

Avinash Parimisetty

Diabète athérothrombose Thérapies Réunion Océan Indien (DETROI)

Institut National de La Santé et de La Recherche Médicale (INSERM) Unit 1188

Université de La Réunion, CYROI-2, Rue Maxime Rivière, Sainte Clotilde, F-97490, France.

Cover Page Illustration: Relation between Adipocytokines and Glial Cells

(Courtesy of Avinash Parimisetty)

About the cover:

Adipose tissue (part of peripheral immune system) activated in response to exogenous or endogenous stimulus triggers the fabrication of an array of adipocytokines (factors produced by the fat tissue) that will have a high impact in affecting the central nervous system (CNS) mediated via adipocytokine signalling acting in an endocrine fashion. Depending on the factor produced and intensity of the stimulus, the factors may confer either neuroprotective or neurodegenerative actions on the CNS.

Research Thesis Manuscript Written and Composed By: Avinash Parimisetty

Thesis Director and Supervision: Prof. Christian Lefebvre d Hellencourt

Critical Reading of Manuscript: Prof. Christian Lefebvre d Hellencourt,

Prof. Dora Brites,

HDR. Dr. Fabien Gosselet,

Dr. Marjolaine Roche

Cover Page, Text Designer and Photo Researcher: Avinash Parimisetty

Reprints were made with permission from the respective publishers

Printed by Reprographie, University de La Reunion, Saint Denis, France.

With All The Love Devoting This Doctoral Thesis To
My Lovely Friend ***Lakshman Kumar*** *-* లక్ష్మణ్ కుమార్
&
My Adorable Brother ***Sunil Kumar*** *-* సునీల్ కుమార్

The whole secret of existence is to have no fear.
Never fear what will become of you, depend on no one.
Only the moment you reject all help are you freed

…….Swami Vivekananda

Avinash Parimisetty

Avinash an Indian born French researcher was adjudicated by the council of Sri Venkateswara University and conferred University Third Rank Holder title for his Master's study in Biochemistry, 2010. Soon after his Master's, he was funded by fellowships from Conseil Régional de La Réunion, France, 2011 and was recruited as doctoral research student at the University of La Reunion to investigate the impact of factors secreted by the fat tissue on central nervous system vulnerability. Later on he integrated with Prof.Christian Lefebvre d' Hellencourt's Neuroteam under French Institute of Health and Medical Research (INSERM) to advance in his thesis project. The results of his work with in a stipulated period of time enabled Avinash to showcase his recent research findings in two international conferences, one in the RUN-EMERGE - European Union Seventh Research Framework Programme (FP7) Conference, Saint-Denis, 2013 and another at Federation of European Biochemical societies - European Molecular Biology Organization (FEBS-EMBO) hosted by French Society for Biochemistry and Molecular Biology (SFBBM), Paris, 2014. In conjunction with Regional council fellowship, Avinash was also a recipient of Young Scientist Bursary from the French Society for Biochemistry and Molecular Biology (SFBBM), France, 2014 and as well from the Doctoral School in Sciences Technology and Health Fellowship, University of La Reunion, France, 2014. He had been a member of French Society for Biochemistry and Molecular Biology since 2012. He is the author/co-author of 8 peer-reviewed publications in the arena of biochemistry, cellular, molecular biology and neuroimmunology.

Rationale Behind Doctoral Project: Meticulous and painstaking process of understanding the nexus between the factors secreted by the fat tissue (adipocytokines) and its potential impact targeting glial cells under central nervous system (CNS) vulnerability is the primary concern of my research thesis project. Adipose tissue (part of peripheral immune system) activated in response to exogenous or endogenous stimulus triggers the fabrication of an array of adipocytokines (factors produced by the fat tissue) that will have a greater impact towards the negative modulation of glial cells in the CNS. This targeting process can be mediated via adipocytokine signalling acting in an endocrine fashion. Depending on the factor produced and intensity of the stimulus, the factors might confer either neuroprotective or neurodegenerative actions on the CNS. Therefore, our global aim of the research was to investigate the inflammatory properties of the factors produced by the adipose tissue (Autotaxin (ATX) and Adiponectin (ADIPO)) and its potential implication in neuroinflammation and neurodegeneration. The potential of these two factors were evaluated, both *in vitro* (in immortalized microglial and astroglial cell lines) and *in vivo* (in neuroinflammatory, acute hippocampal neurodegeneration mice models). Taken together from in vivo experimental approach, we put forward that fat tissue, inflammatory and oxidative stress factors were expressed in vivo in the brain. In addition to this, our in vitro results authenticate the anti-inflammatory role of ATX in microglial cells and anti-oxidative role of ADIPO in astrocyte cells of the brain.

Research Interests and Future Career Goal: My next specific immediate goal will be to venture deep down into the degenerative disorders that damages the brain and to investigate the involvement of cells, its specific factors and the molecular mechanisms responsible for the onset of this deadly pathology which has become one of the major life threatening menace that affect the human society these days.

Prof. Christian Lefebvre d'HELLENCOURT

Work Address: Diabète athérothrombose Thérapies Réunion Océan Indien (UMR DéTROI INSERM - U1188) - Université de La Réunion - CYROI - 2, rue Maxime Rivière 97490 Sainte Clotilde - La Réunion – France, Tel : (262) 262 93 82 01 Fax : (262) 262 93 82 37, Email: christian.lefebvre-d-hellencourt@univ-reunion.fr

Christian Lefebvre d'Hellencourt has received his PhD degree from the University of Paris 7, France in 1995. He has been assistant professor at the University Of Angers, France from 1996 to 1998. Then from 1999 to 2003, Christian Lefebvre d'Hellencourt performed a postdoctoral training with Jean Harry, in the National Institute of Environmental Health Sciences (NIEHS), National Institutes of Health (NIH), Durham, North Carolina, USA. In 2003, the University of La Réunion, France hired him as assistant professor and habilitation was achieved in 2004. He has been director of the laboratory of Biochemistry and Molecular Genetics (EA2526) in the University of La Réunion from 2005 to 2010. Since 2010, he is the director of EA 4516 (Study Group on Chronic Inflammation and Obesity, GEICO) at University of La Réunion. In 2011, he became full Professor. Christian Lefebvre d'Hellencourt is member of the scientific board and Medicine faculty board of University of La Réunion, research board of the Réunion University hospital (CRBSP) and member of the French National University Board for Biochemistry and Molecular Biology (CNU 64). His research interests reside on inflammation modulation in pathologies, with a main focus on obesity effects on vulnerability of the central nervous system. Effects of peripheral inflammation and factors produced by adipose tissue on neuroinflammation and changed in trauma susceptibility in the central nervous system are some of the recent researches conducted in his laboratory. Glial cells (astrocytes and microglia) are especially studied in different model of neuroinflammation and neurodegeneration, both in vitro (cell cultures) and in vivo in mice. He has successfully supervised 3 PhD students and 9 master thesis.

Thesis By Publications

RESEARCH ARTICLES

- Autotaxin downregulates LPS - induced microglia activation and inflammation. Rana Awada, Jean Sebastien Saulnier-Blache, Sandra Gres, Emmanuel Bourdon, Philippe Rondeau, **Avinash Parimisetty**, Ruben Orihuela, G. Jean Harry, Christian Lefebvre d'Hellencourt, ***Journal of Cellular Biochemistry***, DOI: 10.1002/jcb.24889, Wiley Periodicals, Inc.2014. **http://onlinelibrary.wiley.com/doi/10.1002/jcb.24889/abstract**

- Role of adiponectin on astrocytes in oxidative stress situation. Rachelle Gerges, Rana Awada, **Avinash Parimisetty**, Wildriss Viranaicken, Philippe Rondeau, Christian Lefebvre d'Hellencourt. **[Manuscript to be submitted, 2015]**

- Adiponectin, resistin and autotaxin expression in neuroinflammation and neurodegeneration. **Avinash Parimisetty**, Nicolas Diotel, Cynthia Planesse, Aurelie Catan, Dorothee Girard, Christian Lefebvre d'Hellencourt. **[Manuscript to be submitted, 2015]**

REVIEW ARTICLES

- A molecular web: endoplasmic reticulum stress, inflammation, and oxidative stress. Namrata Chaudhari, Priti Talwar, **Avinash Parimisetty**, Christian Lefebvre d'Hellencourt and Palaniyandi Ravanan, ***Frontiers in Cellular Neuroscience***, Volume 8, Article 213, doi: 10.3389/fncel.2014.00213,2014. **http://journal.frontiersin.org/Journal/10.3389/fncel.2014.00213/abstract**

- Secret talk between adipose tissue and central nervous system - An emerging frontier in the neurodegenerative research. **Avinash Parimisetty**, Nicolas Diotel, Christian Lefebvre d'Hellencourt. [**Manuscript to be submitted, 2015**]

BOOK CHAPTERS

- Influence of obesity on neurodegenerative diseases. Rana Awada, **Avinash Parimisetty,** Christian Lefebvre d'Hellencourt, ***Neurodegenerative diseases,*** Chapter 16, p.p 381-401 ISBN: 978-953-51-1088-0,*DOI:10.5772/53671,2013.* **http://www.intechopen.com/books/neurodegenerative-diseases/influence-of-obesity-on-neurodegenerative-diseases**

Communications

ORAL PRESENTATIONS

- *In vivo* hippocampal and *in vitro* astroglial LPS induced neuroinflammation, **Avinash Parimisetty,** Dorothee Girard, Aurelie Catan, Cynthia Planesse, Rana Awada, Wildriss Viranaicken, Philippe Rondeau, Christian Lefebvre d'Hellencourt. Scientific Days on Emerging Infectious Diseases in the South-Western Indian Ocean, ***RUN - EMERGE - European Union Seventh Research Framework Programme*** **[FP7] Conference,** Saint-Denis, France - 2013.

POSTER PRESENTATIONS

- Autotaxin and adiponectin expression in neuroinflammation and its effects on microglial cells, **Avinash Parimisetty,** Rana Awada, Dorothee Girard, Aurelie Catan, Cynthia Planesse, Philippe Rondeau, Nicolas Diotel, Christian Lefebvre d'Hellencourt, ***Federation of European Biochemical societies - European Molecular Biology organisation [FEBS - EMBO] hosted by French Society for Biochemistry and Molecular Biology*** **[SFBBM],** Paris, France - 2014.

Structure of the Thesis

Abstract

Globally obesity is one of the greatest public health challenges of 21st century, and is considered a major health risk factor. Obesity is responsible for the onset of various kinds of disorders including diabetes, cardiovascular diseases and cancer. Adipose tissue (AT) is a highly active endocrine organ which has intense secretory activity producing an assortment of over 600 factors that have versatile biological activities. Some of these factors are named adipocytokines and have gain an intensive focus on current metabolic and disease recent research. Accumulating data on adipocytokine research strongly suggest that adipose tissue is the key player in promoting chronic inflammation. Many chronic neurodegenerative diseases such as Amyotrophic lateral sclerosis, Alzheimer's and Parkinson's diseases have been associated with inflammation in the Central Nervous System (CNS) in which microglia and astrocytes (glial cells) play a decisive role. Autotaxin (ATX) and Adiponectin (ADIPO) are mediators secreted by the AT. The role of these mediators in metabolic activities have been well studied but the potential role of these adipocyte secreted factors and its precise mechanisms in CNS vulnerability remains to be determined.

Here we used, *in vivo,* two distinct inflammatory stimuli, lipopolysaccharide (LPS) and trimethyltin (TMT), to characterize the expression of inflammatory mediators in mouse CNS. Acute intraperitoneal (ip) injection of LPS (100µg/Kg bwt) mimics gram negative bacterial infection, while acute ip injection of organometal TMT (2mg/kg bwt), induces hippocampal neurodegeneration. Microglia and astrocytes are the major source of inflammatory factors in the brain. To investigate, *in vitro,* the role of ATX and ADIPO in inflammatory and oxidative stress condition, we generated stable over-expressing transfectant in murine microglia BV2 cells for ATX and murine astrocyte CLTT cells for ADIPO. BV2 and CLTT stably transfected overexpressing clones were treated with LPS (1 µg/mL) and H_2O_2 (100µM).

Our *in vivo* results demonstrated that ATX and ADIPO were expressed in the brain and LPS induced a transient neuroinflammatory response in three distinct regions of the brain hippocampus (HIP), cortex (COR) and cerebellum (CER). Besides this it was also found that with this mild dosage of 100 µg LPS/Kg bwt of mice, microglia and astrocytes were not activated in the brain **(Project-1)**. Our *in vitro* results authenticate the anti-inflammatory effects of ATX in microglial cells demonstrated by the downregulation of microglial activation markers (CD11b, CD14, CD80 and CD86) and pro-inflammatory cytokine expression and secretion (TNF-α and IL-6) **(Project-2).** Likewise, ADIPO put forth its anti-oxidant role in astrocyte cells mediated via significant mitigation of ROS, and as well by the significant down and upregulation of pro-oxidative inducible nitric oxide synthase (iNOS) and cyclooxygenase-2(COX-2) and anti-oxidative enzymes mRNA expression levels superoxide dismutase (SOD) and catalase (CAT) respectively **(Project-3)**.

Overall these results suggest that peripheral inflammation induced by infection will not induce neurodegeneration (unless a massive infection) but could prime the glial cells and make them more responsive to the next stimulation. ATX and ADIPO may play a role in the regulation of neuroinflammation by regulating glial activation in stressed situations. Further investigations will be needed to better understand the molecular mechanisms regulating brain inflammation and lead to new therapeutic strategies to combat neurodegenerative diseases.

Key words: Adipose Tissue, Autotaxin, Adiponectin, Neuroinflammation, Neurodegeneration.

Résumé

L'obésité est l'un des plus grands défis de santé publique du 21ème siècle et est considérée comme un facteur de risque majeur pour la santé. L'obésité est responsable de l'apparition de divers troubles, notamment du diabète, des maladies cardiovasculaires et de certains cancers. Le tissu adipeux (TA) est un organe endocrine très actif qui a une activité sécrétoire intense produisant un assortiment de plus de 600 facteurs qui ont des activités biologiques variées. Certains de ces facteurs sont appelés adipocytokines et font l'objet d'un intérêt particulier dans les recherches récentes sur le métabolisme et les pathologies associées. De nombreuses données sur les adipocytokines suggèrent fortement que le tissu adipeux est un élément clé dans le développement d'une inflammation chronique. De nombreuses maladies neurodégénératives chroniques telles que la sclérose latérale amyotrophique, la maladie d'Alzheimer et la maladie de Parkinson ont été associées à une inflammation du système nerveux central (SNC), dans lequel la microglie et les astrocytes (cellules gliales) jouent un rôle déterminant. L'autotaxin (ATX) et l'adiponectine (ADIPO) sont des médiateurs sécrétées par le TA. Le rôle de ces médiateurs dans les activités métaboliques a été bien étudié, mais leur rôle potentiel ainsi que les mécanismes précis dans la vulnérabilité du CNS restent à déterminer.

Ici, nous proposons d'utiliser, *in vivo*, deux stimuli inflammatoires distincts le lipopolysaccharide (LPS) et le triméthylétain (TMT) pour caractériser l'expression de médiateurs de l'inflammation du SNC chez la souris. Une injection intrapéritonéale (ip) aiguë de LPS (100 µg/kg de poids corporel) mime une infection bactérienne Gram négative, tandis que l'injection ip aiguë de TMT (2 mg/kg de poids corporel), induit une neurodégénérescence hippocampique. Les microglies et les astrocytes sont les principales sources de facteurs inflammatoires dans le cerveau. Afin de rechercher, *in vitro*, le rôle de l'ATX et de l'ADIPO sur ces cellules dans un état inflammatoire et de stress oxydatif, nous avons généré des tansfectants stables sur-exprimant l'ATX dans des cellules microgliales murines (BV2) et l'ADIPO dans des cellules astrocytaires murines (CLTT). Les clones BV2 et CLTT surexprimant ces facteurs ont été traitées avec du LPS (1 µg/ml) et du H_2O_2 (100µM).

Nos résultats *in vivo* ont démontré que l'ATX et l'ADIPO sont exprimés dans le cerveau et que le LPS pourrait induire une réponse neuroinflammatoire transitoire dans trois régions distinctes du cerveau l'hippocampe (HIP), le cortex (COR) et le cervelet (CER). En outre, il a été également constaté qu'à cette dose modérée de 100µg de LPS / kg de poids corporel de la souris, la microglie et les astrocytes ne sont pas activés dans le cerveau **(Projet-1)**. Nos résultats *in vitro* démontrent les effets anti-inflammatoires de l'ATX dans les cellules microgliales observables par la baisse d'expression des marqueurs d'activation microgliale (CD11b, CD14, CD80 et CD86) et d'expression et de production de cytokines pro-inflammatoires (TNF-α et IL-6) **(Project- 2)**. De même, nous avons montré que l'ADIPO a un rôle anti-oxydant dans les astrocytes via l'atténuation significative de ROS, une inhibition d'enzymes pro-oxydantes (iNOS et la COX-2) et une régulation positive d'enzymes anti-oxydantes (SOD et CAT) **(Projet-3)**.

Dans l'ensemble, ces résultats suggèrent qu'une inflammation périphérique induite par une infection ne provoque pas de neurodégénérescence (à moins d'une infection importante), mais pourrait sensibiliser les cellules gliales et augmenter leur réponse à la stimulation suivante. L'ATX et l'ADIPO pourraient jouer un rôle dans la régulation de la neuroinflammation en régulant l'activation gliale dans un contexte de stress. Des travaux supplémentaires seront nécessaires afin de mieux comprendre les mécanismes moléculaires régulant l'inflammation du SNC et aboutir à de nouvelles stratégies thérapeutiques pour combattre les maladies neurodégénératives.

Mots clés: tissu adipeux, autotaxin, adiponectine, neuroinflammation, neurodégénérescence.

List of Abbreviations

6-OHDA	6-hydroxydopamine
AA	Arachidonic Acid
ACRP30	Adipocyte Complement-Related Protein of 30 kDa
AD	Alzheimer's Disease
ADIPO/APN	Adiponectin
AdipoQ	Adipocyte, C1q and collagen domain-containing protein
AdipoR	Adiponectin Receptor
ADP	Adenosine Di Phosphate
ALS	Amyotrophic Lateral Sclerosis
AMPK	5-AMP-activated protein kinase
AOD	Anti-Obesity Day
APC	Antigen Presenting Cells
AT	Adipose Tissue
ATMs	Adipose Tissue Macrophages
ATP	Adenosine Tri phosphate
ATX	AutoTaXin
BAT	Brown Adipose Tissue
BBB	Blood–Brain Barrier
BDNF	Brain Derived Neurotrophic Factor
bFGF	Basic Fibroblast Growth Factor
BLS	Bare Lymphocyte Syndrome
BMI	Body Mass Index
BMP	Bone morphogenic protein
BV2	Microglial Cell line
C.L.T.T	Astrocyte Cell line
CA	Cornu Ammonis
cAMP	cyclic Adenosine MonoPhosphate
CAT	Catalase
CCL2	Chemokine C-C Motif Ligand 2
CD11b	Cluster of Differentiation 11b
CD200	Cluster of Differentiation 200
CD45	Cluster of Differentiation 45
CD68	Cluster of Differentiation 68
CD80	Cluster of Differentiation 80
CD86	Cluster of Differentiation 86
CDH13	T-cadherin
CER	CERebellum
CNS	Central Nervous System

CNTF	Ciliary Neurotrophic Factor
CoQ10	Coenzymes Q10
COR	CORtex
COX	CycloOXygenase
CSF	Cerebro Spinal Fluid
CT	Computed Tomography
CXCL2	Chemokine C-X-C motif ligand 2
DA	Dopamine
DAG	Diacylglycerol
DAP12	DNAX activation protein of 12 kDa
db/db	Diabetic Mice
DM	DextroMethorphan
DPI	DiPhenylIodonium
DIABESITY	ObeSITY induced type 2 DIAbetes mellitus
EAAT	Excitatory Amino Acid Transporter
ECM	Extracellular Matrix
ECS	Extracellular Space
EDG	Endothelial Differentiation Gene
EGF	Epidermal Growth Factor
ENPP2	Ectonucleotide pyrophosphatase/phosphodiesterase family member 2
ER	Endoplasmic Reticulum
ESC	Embryonic Stem Cells
ETC	Electron Transport Chain
FDG	FluoroDeoxyGlucose
FFA	Free Fatty Acids
FGF	Fibroblast Growth Factor
FTO	FaT mass and Obesity-associated gene
GABA	Gamma-Amino Butyric Acid
GBP28	Gelatin binding protein of 28 kDa
GCaMP	Green fluorescent protein (GFP), Calmodulin, and M13, a Peptide sequence from myosin light chain kinase
GDM	Gestational Diabetes Mellitus
GDNF	Glial Cell Line-Derived Neurotrophic Factor
GDP	Guanosine Di Phosphate
GFAP	Glial Fibrillary Acidic Protein
GLAST	GLutamate ASpartate Transporter
GLT-1	Glutamate Transporter
GPCR	Guanine-nucleotide-binding Protein Coupled Receptors
GPI	Glycophosphatidylinositol

GPX	Glutathione Peroxidase
GSH	Glutathione
GSSG	Oxidized form of glutathione
H_2O_2	Hydrogen Peroxide
HGF	Hepatocyte Growth Factor
HIP	HIPpocampus
HMW	High Molecular Weight forms
HSP	Heparan Sulphate Proteoglycans
IB4	Isolectin IB4 conjugate
ICAM	InterCellular Adhesion Molecule
IFN-γ	Interferon Gamma
IGF-1	Insulin-like Growth Factor 1
IL-1 β	InterLeukin-1 β
IL-6	InterLeukin-6
IL-10	InterLeukin-10
iNOS	Inducible Nitric Oxide Synthase
INSEE	National Institute of Statistics and Economic Studies
IRS-1	Insulin Receptor Substrate 1
IS	Ischemic Stroke
JAK-STAT	Janus-Kinase/Signal Transducer and Activator of Transcription-3
LCAT	Lecithin Cholesterol Acyltransferase
L-DOPA	L-3, 4-DihydrOxyPhenylAlanine
LMW	Low Molecular Weight Forms
LPA	Lysophosphatidic Acid
LPA1	Lysophosphatidic Acid Receptor 1
LPAR	Lysophosphatidic Acid Receptors
LPC	LysoPhosphatidylCholine
LPL	LysoPhosphoLipids
LPS	LipoPolySaccharide
LXA4	LipoxinA4
LysoPLD	LysoPhosphoLipase D
MAO	Monoamine Oxidase
MAP-kinase	Mitogen-Activated Protein kinase
MCP-1	Monocyte Chemoattractant Protein
MDA	MalonDiAldehyde
MHC	Major Histocompatibility Complex
MIP-1	Macrophage Migration Inhibitory Factor
MMW	Medium Molecular Weight Forms

Mn-SOD	Mitochondrial SuperOxide Dismutase
MOMO	Macrosomia Obesity Macrocephaly Ocular abnormalities
MPTP	1-methyl-4-phenyl- 1, 2, 3, 6-tetrahydropyridine
MRI	Magnetic Resonance Imaging
mRNA	messenger RNA
MS	Multiple Sclerosis
Nampt	Nicotinamide phosphoribosyltransferase
NFkβ	Nuclear Factor kappa Beta
NGF	Nerve Growth Factor
NO	Nitric Oxide
NOX	NADPH oxidase
NSAID	Non-Steroidal Anti-Inflammatory Drugs
NUC	Carboxy-terminal nuclease-like Domain
O_2	Oxygen
ob/ob	Obese Mouse
Ob-Rb	Leptin Receptor
OECD	Organization for Economic Co-Operation and Development
PA	Phosphatidic Acid
PAI-1	Plasminogen Activator Inhibitor-1
PAMP	Pathogen-Associated Molecular Patterns
PBEF	Pre-B-cell colony-Enhancing Factor
PC	PhosphatidylCholine
PCB	Polychlorinated Biphenyl
PD	Parkinson's Disease
PDE	PhosphoDiEsterase
PDNP2	PhosphoDiesterase I/Nucleotide Pyrophosphatase gene
PE	PhosphatidylEthanolamine
PET	Positron Emission Tomography
PG	ProstaGlandins
PHOX	PHagocytic OXidase
PI3K	Phosphoinositide 3 kinase
PLA1	Phospholipase A1
PLC	PhosphoLipase C
POMC	ProOpioMelanoCortin
PPAR γ	Peroxisome Proliferator-Activated Receptor Gamma
PRR	Pattern Recognition Receptors
PS	PhosphatidylSerine
PS-PLA1	PhosphatidylSerine-specific PhosphoLipase A1
PTM	Post Translational Modification
PTP	Permeability Transition Pore

RANTES	Regulated on Activation, Normal T cell Expressed and Secreted
RBP4	Retinol Binding Protein-4
RES	Resistin
RGD	Arginylglycylaspartic acid
RIP	Receptor-Interacting Protein kinases
RNS	Reactive Nitrogen Species
ROS	Reactive Oxygen Species
SAID	Steroidal Anti-Inflammatory Drugs
SCD-1	Steroyl CoA Desaturase-1
SFRP5	Secreted Frizzled-Related Protein 5
SHH	Sonic Hedgehog
SMB	SomatoMedin B
SOD	Superoxide Dismutase
sPLA2	Secretory PhosphoLipase A2
SVF	Stromal Vascular Fraction of cells
SVZ	Sub Ventricular Zone
TACE	TNF-Alpha Converting Enzyme
TAG	TriAcylGlycerols
TBI	Traumatic Brain Injury
TCR	T-cell Receptor
TGFβ	Transforming growth factor beta
TLR	Toll Like Receptor
Tnc	Tenascin C
TNFR	TNF Receptors/death Receptor
TNFα	Tumor Necrosis Factor-α
TRADD	Tumor Necrosis Factor Receptor Type 1-Associated Death Domain Protein
TRAF	TNF Receptor Associated Factors
TREM-2	Triggering receptor expressed on myeloid cells 2
TZD	ThiaZolidineDione
UCP1	UnCoupling Proteins
VEGF	Vascular endothelial growth factor
VZ	Ventricular zone
WAT	White Adipose Tissue
WDTC1	Adipose gene
W.H.O	World Health Organization
WHR	Waist to Hip Ratio
XO	Xanthine Oxidase

List of Figures

List of Tables

Review Of Scientific Literature

I. GLOBESITY - GLOBAL EPIDEMIC OF OVERWEIGHT AND OBESITY

II. ADIPOSE TISSUE - KEY PLAYER IN CHRONIC INFLAMMATION

III. CANDIDATE GENES: AUTOTAXIN AND ADIPONECTIN

Results

Annex

REVIEW OF SCIENTIFIC LITERATURE

I. GLOBESITY
- Global Epidemic Of Overweight And Obesity

Obesity is a non-communicable chronic disease. It is not just the problem of an individual but the problem of the complete population in this lonely planet earth. Sedentary lifestyles such as passing most of the times in front of television (Prentice, 2006), increased consumption of energy dense food rich in saturated fats and sugars, reduced physical activity, immovable lifestyles, increased urbanization, industrialization and often the disappearance of traditional lifestyles (WHO, 2000) adds up to aggravate this obesity disorder. Major factors for this obesity pandemic are transmitted through multinational companies by providing cheap materials such as poorly refined fats, oils, and carbohydrates, labor-saving mechanized devices and affordable motorized transport. As a companion to these factors, now it is well appreciated that the progression to obesity represents the complex interaction between dietary, physical, genetical and metabolic activity levels.

I. A. Body Mass Index (BMI) And Waist To Hip Ratio (WHR) As A Predictor Of Obesity:

Generally speaking overweight and obesity are defined as abnormal or excessive fat accumulation that may impair health. Clinically speaking, obesity can be defined by the measurements of body mass index (Mei et al., 2002) or waist circumference and waist to hip ratio (WHR) (Eckel et al., 2005).

I. B. BMI Cut-Off Levels:

Body mass index (BMI) is a simple index to classify overweight and obesity (Mei et al., 2002) and can be defined as the person's weight in kilograms divided by the square of person's height in meters which can be deduced by the formula;

$$BMI = kg/m^2$$

A person with a BMI of 30 or more is generally considered obese and a BMI equal to or more than 25 is considered overweight (Table I-1).

TABLE I-1 Classification of Body Mass Index [BMI]	
Category	**BMI Range – kg/m^2**
Very severely underweight	less than 15
Severely underweight	15.0 to 16.0
Underweight	16.0 to 18.5
Normal (healthy weight)	18.5 to 25
Overweight	25 to 30
Obese Class I (Moderately obese)	30 to 35
Obese Class II (Severely obese)	35 to 40
Obese Class III (Very severely obese)	over 40

Table Reference: (WHO, 2011)

I.C. WHR Cut-Off Levels:

The cut-off levels of WHR greater than 0.85 for women and 0.90 for men are generally indexed as obese (Prakash Shetty, 2008).

Up to certain extent, obesity can be effectively preventable by the change of habitual lifestyles that requires long term strategic plans. The overall picture of the obesity in industrialized and developing countries reflects their social, cultural and economic problems faced by that particular country. Investigating the primary factors responsible for the onset of this obesity pandemic at an earlier stage is crucial for the predictions about the future impact in developing countries for a preventive action (WHO, 2000).

I.D. Over Weight and Obesity - A Global Health Observatory and Demographical Study:

Worldwide obesity has nearly doubled since 1980. As per the WHO statistics, highest prevalence of overweight and obesity were recorded in the United States of America in which 62% of the people were found to be overweight in both sexes and 26% for obesity and the lowest prevalence of overweight and obesity were reported in south east Asia in which 14% were overweight in both sexes and 3% for obesity (WHO, 2015b).

The world health organization (WHO) has recently released its Global Status Reports on non-communicable diseases by 2014 (Figure I-1, I-2) to demonstrate the prevalence of obesity in males and females in different countries around the world. The worldwide obesity maps (Men) depicts the red countries like the US, the UK, Canada, Australia, and others have more than 25% prevalence of obesity in men aged 18 years and over.

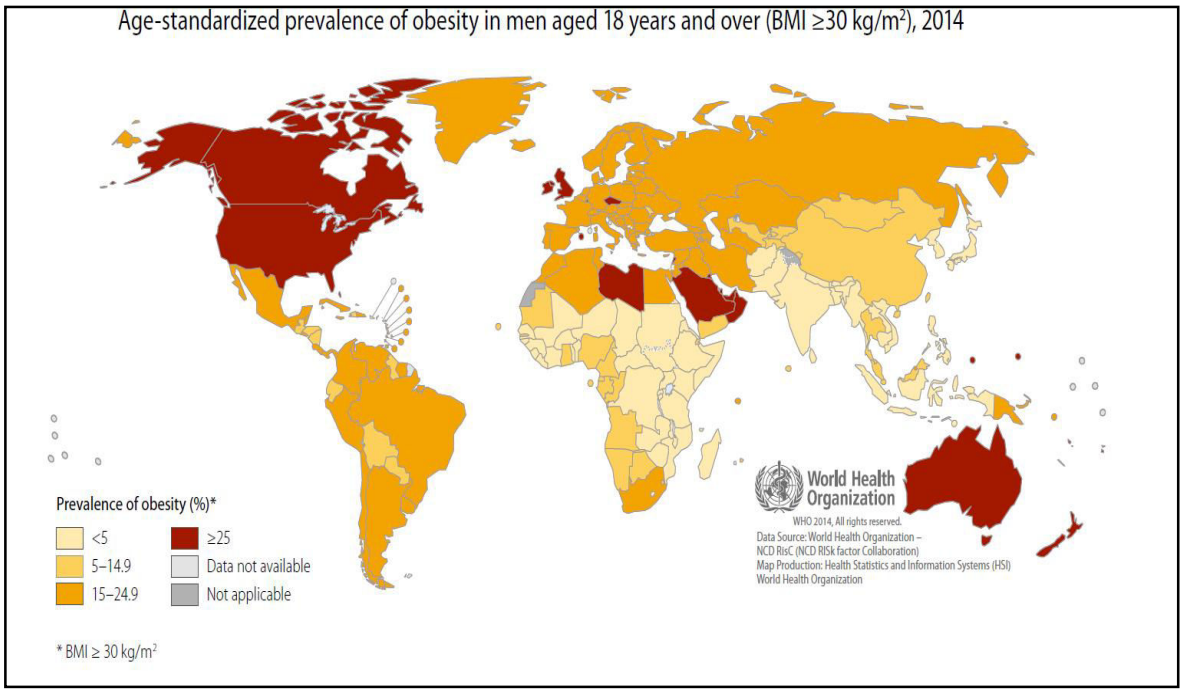


FIGURE I-1 Global Prevalence Of Obesity In Men
Image Reference: (World Health Organization, 2014)

Most interestingly, in all WHO regions, women are more likely to be obese than men. As per the WHO reports, women have roughly double the obesity prevalence of men in the African, South-East Asia, and Eastern Mediterranean regions (Figure I-2).

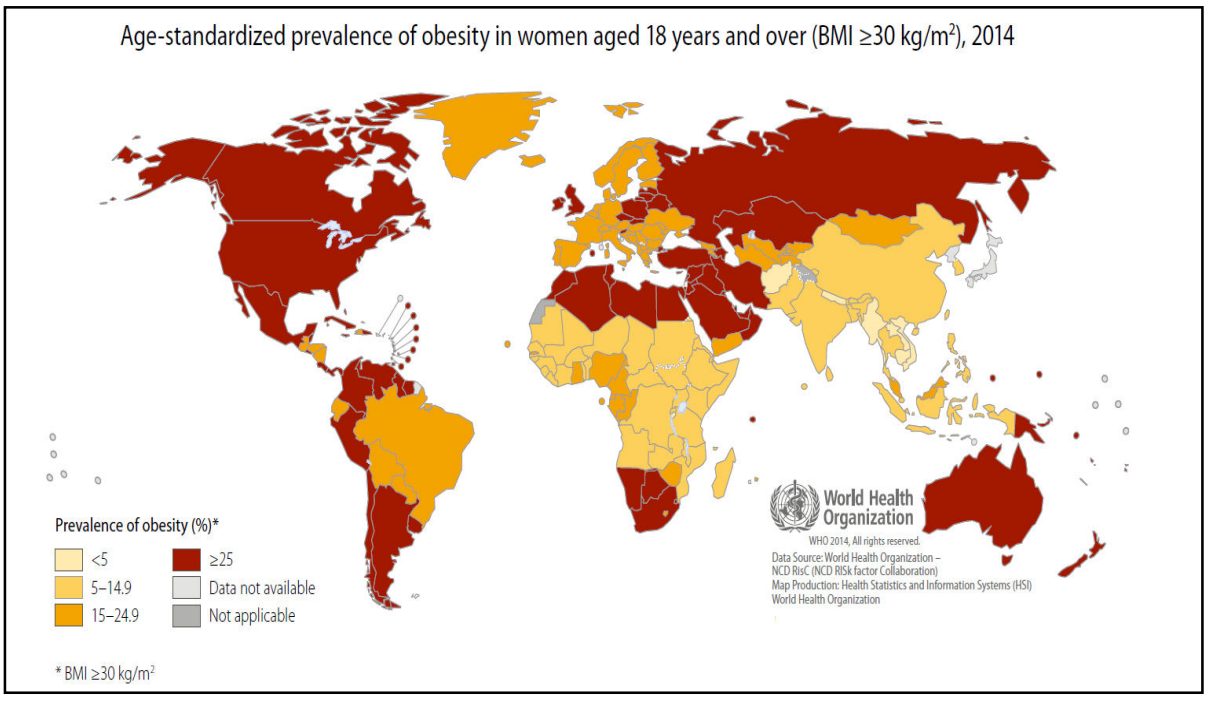


FIGURE I-2 Global Prevalence Of Obesity In Women
Image Reference:(World Health Organization, 2014)

At least 3.4 million people die each year worldwide as a result of being overweight or obese with direct and indirect causes. The increase in rate of deaths are directly proportional to the increasing degrees of overweight. The World Health Organization (WHO) in 2005 estimated the prevalence of obesity to be more than 1 billion overweight adults, with at least 500 million reaching the level of obese. In 2014, more than 1.9 billion adults, 18 years and older, were overweight. Of these over 600 million were obese. As this continues to increase, by 2015, WHO estimates the number of overweighed adults will balloon to 2.3 billion with more than 700 million obese. Worldwide, obesity is currently responsible for 2-8% of health care costs and approximately 10-13% of deaths (WHO, 2015a). Apart from WHO, Organization for economic co-operation and development (OECD) affirms that education also plays a greater role in controlling obesity rate. Women who are poorly educated are likely to be twice obese than the educated women and a similar association had also been reported that men with less education are 1.8 times more likely to be obese than more educated men (OECD, 2014).

I. D. a) Prevalence Of Obesity In USA:

Until 2013, the United States of America had the highest obesity rate. Mexico had surpassed and crossed that figure by 2013 (Althaus, 2013). Obesity affected more than 34.9% of adults and approximately 17 percent of youth in the United States. In numbers it translates by 78 million adults and 12 million children were considered obese (Trogdon et al., 2012).

I. D. b) Prevalence Of Obesity In France:

The first study carried out in France concerning obesity within the French population was by National Institute of Statistics and Economic Studies (INSEE) between 1980 and 1991 (Maillard et al., 1999). The survey result showed that, the obesity prevalence was 6.1% in 1980 and 6.5% in 1991. The subjects for the survey were adults (50% men and 50% women). The conclusions of this study were thus not alarming. Indeed, France's obesity is the lowest in Europe, at about 10%, compared to the UK's 22% and the US's 33% (Rozin et al., 2003). Recent survey from the reports of organization for economic co-operation and development (OECD) reported that one in eight adults is obese in France, and 40% are overweight and obese.

I. D. c) Prevalence Of Obesity In India:

November 26th is celebrated as Anti-Obesity Day (AOD) in various parts of the world, with several healthcare organizations and leading media primarily in India marking the day with activities to highlight how obesity is a public health hazard. On this day, doctors organizes special health camps in order to advice people to practice a healthy lifestyle. Obesity is one of the greatest public health challenges of 21st century in developing countries like India. The prevalence of obesity is increasing at an alarming rate in all the populations of India including women, men, and children. A study conducted by Sidhu et al on 1,000 urban and rural adult males aged 20-50 years of Amritsar district of Punjab revealed that 55.8% were overweight and 36.4% were obese (Sidhu, 2006). Another study lead by Nirmala on Southern Andhra Pradesh, India on 1119 individuals (456 males and 663 females), aged 18 to 75 years demonstrated that the BMI of the participants ranged from 12.6 to 35.1 in males and from 12.3 to 34.2 in females. Followed by this anthropometric study, there is an observed increasing trend in mean BMI until

about 50 years, followed by a decline. It was also recorded that women living in urban areas tends to accumulate increasing amounts of fat and became more obese than the women living in rural areas. Socioeconomic status, increased urbanization, behavioral variables, gender, physical and habitual activities were found to be the cardinal factors responsible for these obesity fluctuations (Reddy, 1998).

I. E. Obesity And Its Consequences:

Obesity is a medical state that may have a greater negative impact in affecting a person's health status leading to reduced life expectancy and is responsible for causing various pathologies (Haslam and James; Awada et al., 2013a) which includes cardiovascular diseases (angina, myocardial infarction) (Yusuf et al., 2004; Poirier et al., 2006), high blood pressure, obesity related type 2 diabetes (DIABESITY), osteoarthritis (Haslam and James), pregnancy complications (Heslehurst et al., 2008), neurological ischemic stroke (Haslam and James), migraine (Bigal and Lipton, 2008), dementia (Beydoun et al., 2008), multiple sclerosis (Munger et al., 2009), various kinds of cancers (Reeves et al., 2007) and preventable causes of death (Allison et al., 1999; MokDad et al., 2004; Barness et al., 2007)

I. F. Obesity And Its Multifactorial Origin:

Obesity is the result of interplay between biological, behavioral, genetical, cultural, molecular and environmental factors (Barness et al., 2007). Energy imbalance over a long period of time results in obesity.

I. F. a) Diet And Energy Imbalance:

Disproportionate match of calories in your body, which is taking in more calories than you burn due to reduced physical inactivity can lead to obesity (Lau et al., 2007a) (Table I-2).

TABLE I-2 **Energy Balance Sheet**

Fluctuations in Weight	Calories Balance Sheet
Weight Loss	Calories consumed < Calories used
Weight Gain	Calories consumed > Calories used
No Weight Change	Calories consumed = Calories used

Table Reference: (Drexel, 2015)

I. F. b) Family History And Complex Interaction Of Genetic Metabolism:

Genetics and heredity plays a pivotal role in regulating obesity. Overweight and obesity tend to run in families because the persons in the same family also share the same food, daily habitual and physical activities and a link also exists between the genes and the family environment (NHLBI, 2012). Fat mass and obesity-associated protein also known as alpha-ketoglutarate-dependent dioxygenase (FTO) is an enzyme that is encoded by the FTO gene located on chromosome 16 is the principal gene responsible for this obese genetic alterations in humans (Loos and Yeo, 2014).

Genes can directly cause obesity in disorders such as Bardet-Biedl syndrome and Prader-Willi syndrome, cohen syndrome, MOMO syndrome Macrosomia (excessive birth weight), Obesity, Macrocephaly (excessive head size) and Ocular abnormalities (Walley et al., 2009). But it is also worth remembering that genes alone itself do not always predict the future health, but both the genes and the behavioral activities (diet, lifestyles) of an individual determines a person health towards the overweight or obesity progression.

I. F. c) Gut Flora And Infectious Agents:

Gut flora differs from lean to obese subjects in humans which affects the physiology and metabolical rate (DiBaise et al., 2008). In support of this statement, a similar hypothesis linking inflammation and obesity were supported by Burcelin's group which involves the intestinal flora equilibrium. In this model, a high fat diet is proposed to increase the gram-negative bacteria proportion in the intestine; this increases intestine permeability and the absorption of lipopolysacharide (LPS; the wall component of the gram negative bacteria). Upon this increased absorption, Toll Like Receptor (TLR) activation leads to an upregulation of the inflammatory response (Cani et al., 2008; Nicholson et al., 2012).

I. F. d) Auxiliary Obesity Devoting Factors:

Apart from these contributing factors, hormonal changes, lack of sleep, smoking, pregnancy and medicines are other factors that adds on to obesity (NHLBI, 2012)

I. F. d. i) Hormonal Effects - Leptin Resistance: obese people tends to accumulate increasing amounts of fat with proportional levels of leptin production, but leptin resistance is one of the critical factors for obese pathogenicity.

I. F. d. ii) Sleep: Lack of sleep increases the risk of obesity. Sleep maintains the balance between hormones that makes you feel hungry (ghrelin) or full satiety feel (leptin). Altered sleeping patterns mismatches the hormonal equilibrium (ghrelin goes up and leptin goes down). This makes the people hungry even at a well-rested state.

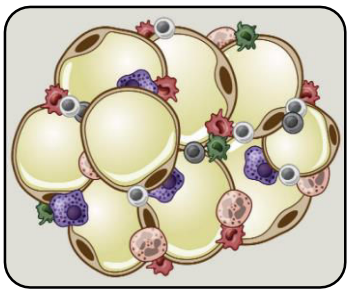
I. F. d. iii) Smoking: Nicotine increases the rate of calorie burning, when smoking is cut down the person will burn only few calories. Another reason for weight gain is that food often tastes and smells better after quitting smoking.

I. F. d. iv) Pregnancy: Women gain weight to support their baby's growth and development. Post pregnancy women find it hard to lose the weight that leads to obesity.

I. F. d. v) Medicines: Corticosteroids, antidepressants, and seizure medicines slowdowns calorie burning, increase the appetite and causes your body to hold on to extra water that leads to the promotion weight gain.

Since obesity leads to the accumulation of fat in the white adipose tissue (WAT) and white adipose tissue was found to be the key player in chronic inflammation, this thesis focusses mainly on the factors (Adipocytokines) produced by the WAT and their potential implication in neuroinflammation and neurodegeneration.

II. ADIPOSE TISSUE
- Key Player In Chronic Inflammation



II. A. What Is Adipose Tissue And Where Is It Derived From?

Adipose tissue (AT) is an anatomical term for loose connective tissue which is derived from preadipocytes. Preadipocytes arises from mesenchymal stem cell lineage. AT is majorly composed of adipocytes. Apart from adipocytes, they are also composed of preadipocytes, fibroblasts, vascular endothelial cells and adipose tissue immune macrophages (ATMs) (Depicted in figure II-1) altogether collectively known as stromal vascular fraction of cells (SVF).

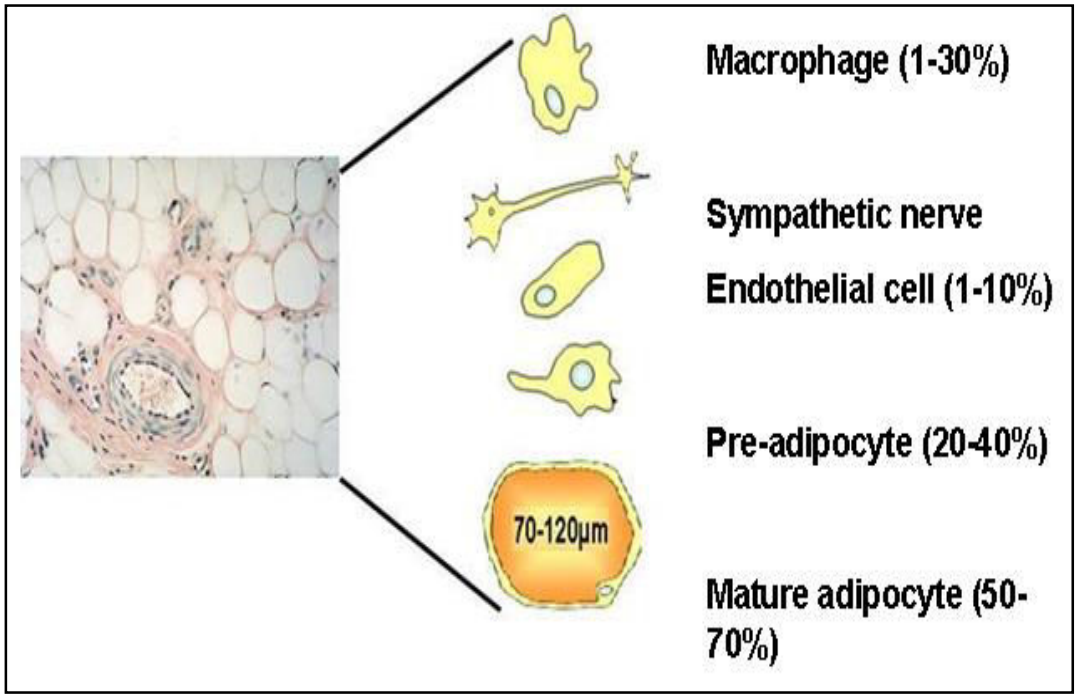


FIGURE II-1 Cell Types Present In The Fat Tissue
Image Reference: (Awada et al., 2013a)

II. B. What Are Adipocytes Doing Inside Our Body?

Adipocytes are the only cells that are specially designed and perfectly adapted to store excess calories/energy such as lipids in the form of triacylglycerols (TAG) by the process of lipogenesis during the periods of abundant energy supply. The more the storage, the larger the cell becomes. Besides its protective padding and storing capacity, it also helps in the mobilization of the stored lipids to destined organs via lipolysis when there is a calorie deficit without compromising its functional integrity (Aarsland et al., 1997; Fonseca-Alaniz et al., 2007).

II. C. How The Fat Tissue Are Distributed? (Adipose Tissue Storehouse)

Predominantly adipose tissue will be deposited at four distinct anatomical locations: Abdominal fat (fat packed around abdomen - Panniculus or Pannus), subcutaneous fat (fat packed beneath skin), epicardial fat (packed fat around heart) and ectopic fat (storage of fat in tissues other than adipose tissue such as - eye balls, kidney, pancreas, muscles, and bone marrow) (Can be seen in figure II-2). The adipose tissue wrapped around these organs serves as a layer of protection and absorbs external shock.

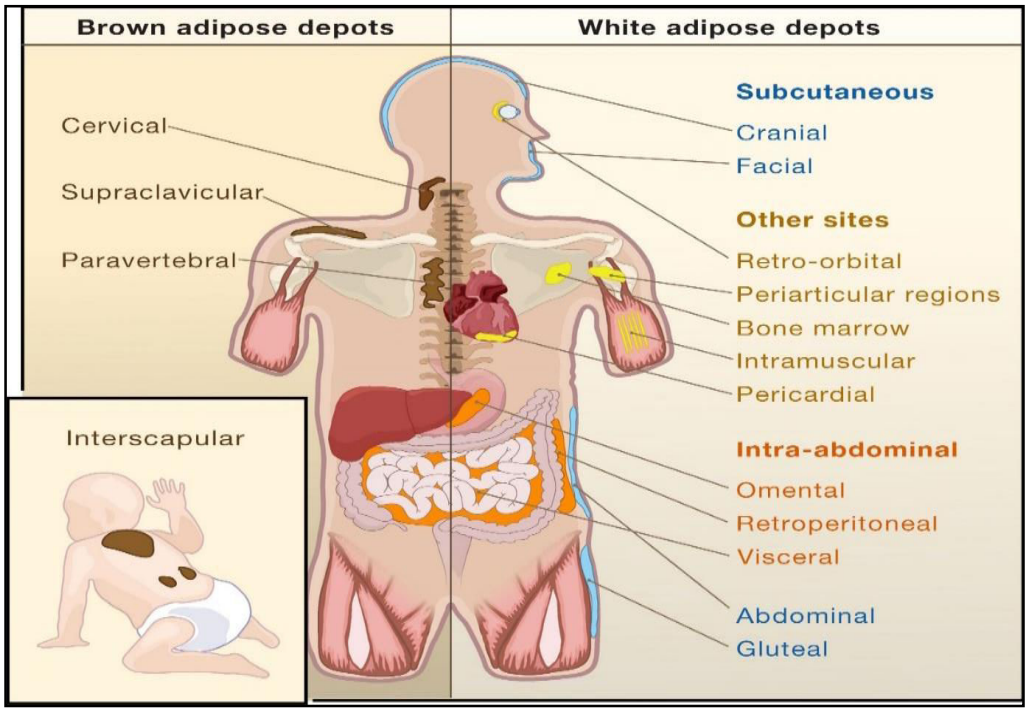


FIGURE II-2 Distribution of Fat: In humans, depots of white adipose tissue are found in areas all over the body, with subcutaneous and intra-abdominal depots representing the main compartments for fat storage. Brown adipose tissue is abundant at birth and still present in adulthood but to a lesser extent. **Image Reference:** (Gesta et al., 2007)

Depending on its anatomical location, adipose tissue exhibits different functional properties. For example, a correlation exists between visceral obesity and increased risk of insulin resistance and cardiovascular diseases, while an increase of subcutaneous fat is associated with favorable plasma lipid profiles (Wronska and Kmiec, 2012). Women tend to accumulate more subcutaneous fat than men due to the differences in the sex hormones produced by them.

II. D. Subcutaneous And Visceral Fat:

Visceral fat is different from subcutaneous fat. Subcutaneous fat is present beneath the skin (thighs, hips and abdomen), whereas visceral fat or intra-abdominal fat is located inside the abdominal cavity (liver, intestine, kidneys, skeletal muscles, and gonads). The fat thus deposited around these organs were found to be metabolically active and secretes various kinds of bioactive molecules that interferes and plays a critical role on that particular and surrounding organ's functionality (Mazurek et al., 2003). Genetic and behavioral factors greatly affects the deposition of these fats at different anatomical locations. Proper maintenance of physique and

maintaining optimum body weight levels are associated with enhanced organ functionality (Snel et al., 2012).

Mammalian adipose tissue can be functionally and histologically divided into two categories; white adipose tissue (WAT) and brown adipose tissue (BAT). WAT is specialized in the storage of excess energy in the form of triacylglycerols (TAG's - Lipogenesis) and acts as a mechanical cushion. Besides this, presence of stem cells in the WAT opened new perspectives and have led to extensive study of adipose tissue's potential in therapeutic reparation and even for the treatment of obesity and metabolic disorders (Roche et al., 2007; Tran and Kahn, 2010; Cawthorn et al., 2012).

BAT is highly vascularized and has a plethora of mitochondria and cytochromes which confers the characteristic brown colored appearance of BAT. It has a strong innervation with sympathetic nervous terminals and acts on β-adrenoreceptors (Fruhbeck et al., 2009). In humans, until recently, it was thought that brown fat was only present in the new-borns and infant (Cannon and Nedergaard, 2004).The extensive use of positron emission tomography (PET) in cancer medical imaging has changed this dogma. An evaluation of fluorodeoxyglucose PET (FDG-PET) data from adult cancer patients indicated a high level of glucose consumption in specific body regions corresponding to brown fat (Cannon and Nedergaard, 2004), presumably in order to maintain normal body temperature.

Despite of its difference in origin, structure and function in these two kinds of AT, the most common cells present in both types of fat are adipocytes and the formation of WAT are controlled by the adipose gene (WDTC1) which is associated with obesity (Suh et al., 2007; Lai et al., 2009).

II. E. Brown Adipose Tissue & Thermogenesis:

Brown adipose tissue with the help of thermogenin or uncoupling proteins (UCP1) across the inner mitochondrial membrane via the process of oxidative phosphorylation generates heat instead of generating ATP molecules when protons are pushed down to form an electrochemical gradient. This complete process is known as thermogenic process (Delineated in figure II-3) which is crucial for hibernating animals and as well for the neonates (Cinti, 2006) to maintain their body temperature and to protect themselves from shivering (Himms-Hagen, 1990). Thermogenesis in BAT is stimulated in response to cold exposure, sympathetic stimulation and energy intake.

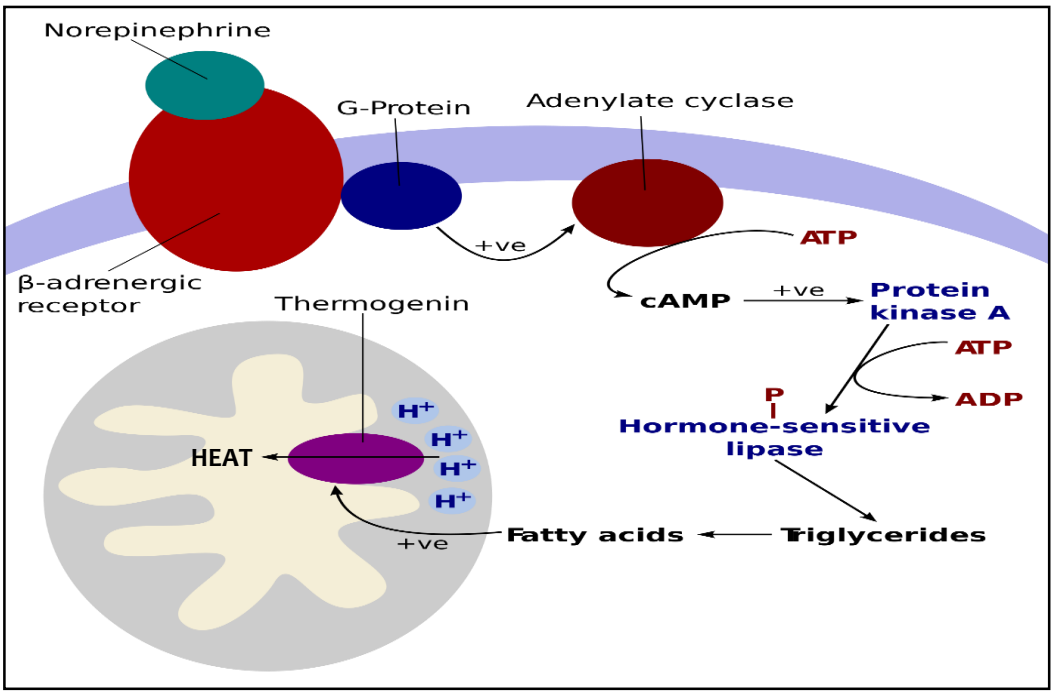


FIGURE II-3 Thermogenin Cascade Activation In Brown Adipose Tissue: During the process of thermogenesis, substances such as free fatty acids (FFA) which are derived from triacylglycerols remove purine (ADP, GDP and others) inhibition of thermogenin (uncoupling protein-1), which causes an influx of H^+ into the matrix of the mitochondrion and bypasses the ATP synthase channel. This uncouples oxidative phosphorylation, and the energy from the proton motive force is dissipated as heat rather than producing ATP from ADP, which would store chemical energy for the body's use. In a last step thermogenin inhibition is released (thermogenin activation) through the presence of FFA. The cascade is initiated by binding of norepinephrine to the cells β3-adrenoceptors. **Image Reference:** (Wikipedia, 2009)

II. F. Beige/Brite Adipocytes:

Brown adipocytes appearing in white adipose tissue are known as inducible, beige or brite adipocytes. These beige adipocytes are derived from mesenchymal stem cells and have a derivation lineage much closer to the white adipocyte lineage (Giralt and Villarroya, 2013) produced in response to various signaling factors and plays a vital role in reducing metabolic diseases such as obesity in mice and correlate with leanness in humans (Harms and Seale, 2013).

TABLE II-1 Characteristic Features of Adipose Tissue - A Comparative Study

Characteristics	White Adipose Tissue (WAT)	Brown Adipose Tissue (BAT)
Location	Subcutaneous abdominal, perirenal, inguinal, gonadal and retroperitoneal	Interscapular, paravertebral, axillary and perineal
Cell Shape	Polyhedral to spherical	Polygonal
Cell Size	25 µm Up To 200 µm (May increases their volume by 1000-fold)	15–60 µm
Cell Color	White (light ivory to strong yellow)	Brown (light pink - dark red)
Multicellularity	High presence of other cell types such as preadipocytes, mature adipocytes, macrophages, endothelial cells, fibroblasts.	Low presence of other cell types
Nuclear Shape	Flattened or cup-shaped	Round or oval shaped
Nuclear Position	Peripheral occupying 2–3% of the cell volume	Centrally located
Lipid Droplets	Unilocular single large lipid droplet that occupies 90% of the cell volume	Multilocular with abundant small lipid droplets
Mitochondria	Few, small, elongated	Abundant, large, round (responsible for the brown color)
Endoplasmic Reticulum (ER)	Sparse cisternae of rough ER, few tubules of smooth ER	Poorly developed ER
Tissue Organization	Densely packed cells	Glandular structure
Vascularization	Modest blood supply	Rich blood supply
Adipokine Secretory Activity	Higher secretory activity of adipokines and growth factors	Lower secretory activity of adipokines and growth factors
Leptin	High levels	Present at birth, not in adults
Uncoupling Proteins	UCP2	UCP1, UCP2, UCP3

Table Reference: (Fruhbeck et al., 2009)

II. G. Adipose Tissue – Dynamic Endocrine Organ Secreting Adipocytokines:

Adipose tissue is highly active metabolic and endocrine organ. The dogma that adipose tissue just acts as a storage organ has been completely revisited by the discovery of leptin. Adipose tissue receives signals and acts as endocrine organ producing an assortment of factors including hormones, inflammatory mediators such as cytokines (TNFα, IL-6, Leptin, Adiponectin), chemokines (MCP-1, MIP-1, RANTES) or adipo lipokines (LPA via Autotaxin) (Blüher and Mantzoros, 2015). The cellular signaling factors (cytokines) produced by the adipose tissue are popularly known as Adipocytokines (Portrayed in figure II-4). To date nearly 600 adipocytokines (Lehr et al., 2012) have been discovered. These factors are secreted by the different cell types of adipose tissue such as adipocytes, immune cells, fibroblasts or endothelial cells (Kelesidis and Mantzoros, 2006; Kelesidis et al., 2010; Dalamaga et al., 2012; Bluher, 2014). These adipocytokines have versatile biological activities in specified host system (Gale et al., 2004; Sahin-Efe et al., 2012; Bluher and Mantzoros, 2015).

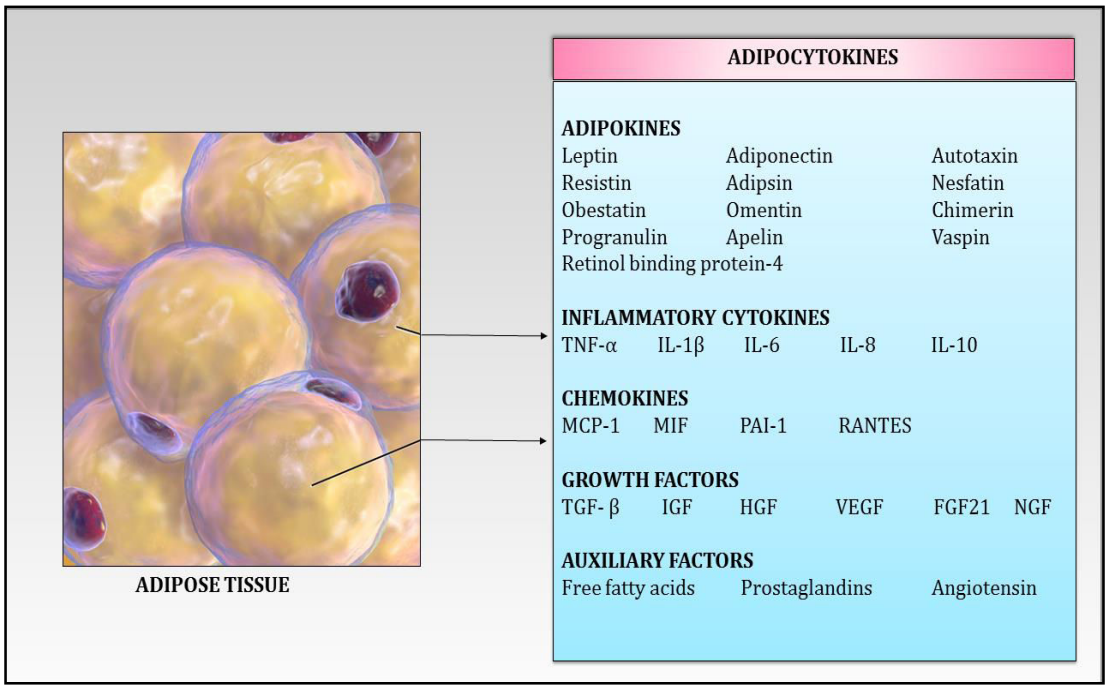


FIGURE II-4 Adipose Tissue Secreting Adipocytokines

Adipocytokines greatly influences the regulation of whole-body energy metabolism such as appetite, satiety, energy expenditure, insulin sensitivity and secretion, glucose and lipid metabolism, fat distribution, endothelial function, hemostasis, blood pressure, neuroendocrine regulation, inflammation and functions of the immune system (Van Gaal et al., 2006; Catalan et al., 2009; Bluher, 2012; Sahin-Efe et al., 2012; Bluher, 2014) in target organs including the brain, immune system, liver, skeletal muscle, vasculature, heart, and pancreatic β-cells (Bluher and Mantzoros, 2015). With the versatile functionality of these adipocytokines affecting various target organs acting at physiological level, led adipocytokines gained an intensive focus on current metabolic and disease research nowadays.

Adipocytokines were found to be key players involved in displaying a wide variety of functions in inflammation (TNF-α, adiponectin, monocyte chemoattractant protein-1, IL-1β, IL-6,

IL-10, CRP, osteopontin, progranulin, chemerin), immune response (interleukins, adipsin/ complement factor D, acylation-stimulating protein, serum amyloid A3) and glucose metabolism (leptin, adiponectin, dipeptidyl peptidase-4, fibroblast growth factor 21, resistin, vaspin), insulin sensitivity (leptin, adiponectin, chemerin), hypertension (angiotensinogen), cell adhesion (PAI-1), vascular growth and function (VEGF), atherosclerosis development (cathepsins, apelin), adipogenesis and bone morphogenesis (BMP-7), growth (IGF-1, TGF-β, fibronectin), lipid metabolism (CD36), regulation of appetite and satiety (leptin, vaspin), eating disorders such as anorexia nervosa (leptin, adiponectin, resistin) and allied biological processes (Catalan et al., 2009; Sahin-Efe et al., 2012; Bluher, 2014). Dysfunctioning of adipose tissue leads to the deregulation of the adipocytokines network that could contribute to different kinds of disease progression in the body (Ouchi et al., 2012).

Besides adipose tissue and various immune cells, adipocytokines are also produced in the human's breast milk. Breast milk is rich in a variety of nutrients, cytokines, peptides, enzymes, cells, immunoglobulins, proteins and steroids which are especially suited to meet the needs of newborn infants (Hawkes et al., 2004; Savino et al., 2010). In addition to these vital supplements, several peptides and hormones have recently been identified in human breast milk, including leptin, adiponectin, resistin, obestatin, nesfatin, irisin, adropin, copeptin, ghrelin, pituitary adenylate cyclase-activating polypeptide, apelins, motilin and cholecystokinin (Savino and Liguori, 2008; Aydin et al., 2013; Catli et al., 2014).

II. G. a) Adipocytokines As Molecular Cues:

Adipocytokines produced by adipocytes can be used as molecular cues to study, diagnose and monitor the etiology of discrete pathologies. This include testing of both pro and anti-inflammatory cytokines. Pro-inflammatory cytokines worsens the immune system by producing inflammation, fever, tissue destruction, shock and even death, whereas anti-inflammatory cytokines improvises and tranquilizes the immune system by neutralizing inflammation and by the promotion of cellular repair (Dinarello, 2000). Besides this, it is also worth remembering that controlled inflammation may be beneficial to fight against pathogens and overexpression of anti-inflammatory factors may also be detrimental.

Cytokines include chemokines, interferons, interleukins, and tumor necrosis factor. They act via intracellular and extracellular receptors, and are especially important in regulating the growth, maturation and responsiveness of particular cell populations. Some cytokines enhance or inhibit the action of other cytokines in complex ways, which plays an indispensable role in health and disease, specifically in response to infection, immune response, inflammation, trauma, sepsis, cancer, and reproduction (Dinarello, 2007). In fact, the amount of cytokine, the nature of the target cell, nature of the activating signal, nature of produced cytokines, their timing, sequence of cytokine action and even the experimental model are the parameters which greatly influence the action of cytokine properties (Cavaillon, 2001). These cytokines are released by immune cells such as microglia, macrophages, mast cells, astrocytes, B-lymphocytes, T-lymphocytes and various kinds of other cell types including endothelial cells and fibroblasts (Lacy and Stow, 2011) that dictates the behavior of other cells in autocrine, (acting on its own Cell itself) paracrine (nearly

located cell), juxtacrine (contact dependent signaling) and endocrine fashion (far away located cells) (Kim and Moustaid-Moussa, 2000).

TABLE II-2 Adipocyte Secretory Profile	
Adipocytokine	**Biological Activity**
LEPTIN	Promotes energy expenditure, represses food intake, and controls appetite via CNS anorexigenic effect.
TNF-α (Tumor Necrosis Factor-α or Lymphotoxin-α)	Pro-inflammatory adipocytokine, negatively regulates hepatic and skeletal muscle insulin sensitivity via IRS-1 phosphorylation and by GLUT4 expression.
ADIPONECTIN	Insulin sensitivity promoting factor, increases glucose uptake, fatty acid oxidation, decreases gluconeogenesis, modulates food intake and energy expenditure, anti-atherogenic, anti-inflammatory, anti-diabetic adipocytokine
AUTOTAXIN	Cell motility, migratory and proliferating factor, role in parturition.
RESISTIN	Insulin resistance aggravating factor, endothelial dysfunction, pro-atherogenic, pro-inflammatory, pro-diabetic adipocytokine
MCP-1 (Monocyte Chemotactic Protein-1)	Promotes inflammation, insulin sensitivity antagonist
MIF (Macrophage Migration Inhibitory Factor)	Immunoregulator with paracrine action in WAT
PAI-1 (Plasminogen Activator Inhibitor-1)	Inhibits plasminogen activation, fibrinolysis
PG (Prostaglandins)	Regulates various cellular processes, blood coagulation, active during inflammation, ovulation and secretion of gastric acid
IL-1 β (Interleukin-1 β)	Pro-inflammatory adipokine
IL-6 (Interleukin-6)	Acute phase response, B-cell proliferation, thrombopoiesis, synergistic with IL-1 and TNF on T cells
IL-10 (Interleukin-10)	Inflammatory antagonist
RANTES (Regulated on activation, normal T cell expressed and secreted)	Pro-inflammatory, pro-obese, augments Insulin resistance
	Monocyte chemotactic activity

VISFATIN or PBEF or Nampt (Nicotinamide Phosphoribosyltransferase)	Nampt-mediated systemic NAD biosynthesis is critical for β cell function Stimulates insulin secretion (Insulinomimetic)
ADIPSIN	Activates alternative complement pathway
NESFATIN	Anorexigenic effect
OBESTATIN	Anorexigenic effect
OMENTIN	Promotes insulin-stimulated glucose transport and Akt phosphorylation in human adipocytes
CHEMERIN	Regulator of adipogenesis, potent anti-inflammatory agent on macrophages
PROGRANULIN	Promotes adipose tissue inflammation
APELIN	Glucose lowering effects by BAT uncoupling proteins - UCP1, thermogenin
VASPIN	Insulin sensitivity agonist
RBP4 (Retinol Binding Protein 4)	Pro-inflammatory and promoter of Insulin resistance and visceral fat distribution.
FGF21 (Fibroblast Growth Factor 21)	Promotes thermogenesis, energy expenditure, fat utilization and glucose uptake into adipocytes
TGF-β (Transforming Growth Factor β)	Regulates the proliferation, differentiation, development and apoptosis of adipocytes
IGF-1 (Insulin-Like Growth Factor 1)	Stimulates proliferation and differentiation of adipocytes
HGF (Hepatocyte Growth Factor)	Stimulates differentiation and development of adipocytes
VEGF (Vascular Endothelial Growth Factor)	Stimulates vascular proliferation angiogenesis
SFRP5 (Secreted Frizzled-Related Protein 5)	Inflammatory antagonist and modulates Wnt signalling

II. G. b) Leptin:

The adipocyte produced Leptin has been discovered 20 years ago (Friedman, 2014; Blüher and Mantzoros, 2015; Friedman and Mantzoros, 2015). In Greek language Leptos means Thin. Experiments conducted by the researchers during early 1950's observed that non sense mutations caused by the disruption of functional polypeptide leptin in ob/ob mice negatively aggravates the feeding behavior (Table II-2), body weight and developed diabetic phenomenon (Campfield et al., 1995; Halaas et al., 1995; Pelleymounter et al., 1995). Whereas to confirm and to counteract this observed pathology, ob/ob mice were administered with leptin that significantly reduced the food intake behavior, body weight and reversed the diabetic

phenotype which confirmed the significance of leptin in *ob*/*ob* mice phenotype (Tartaglia et al., 1995a; Chen et al., 1996; Lee et al., 1996a).

Leptin is an adipose tissue derived signal which acts as a negative feedback loop for the maintenance of energy homeostasis in the brain and other tissues. It controls the appetite and feeding behavior of a living system by acting on the leptin receptors (OB-Rb) of hypothalamic arcuate nucleus, ventromedial hypothalamic nucleus, and dorsal medial hypothalamic nucleus of the CNS which are responsible in regulating the feeding behavior and acts via Janus-Kinase/Signal Transducer and Activator of Transcription-3 (JAK-STAT) pathway (LA et al., 1995) (Figure II-5).

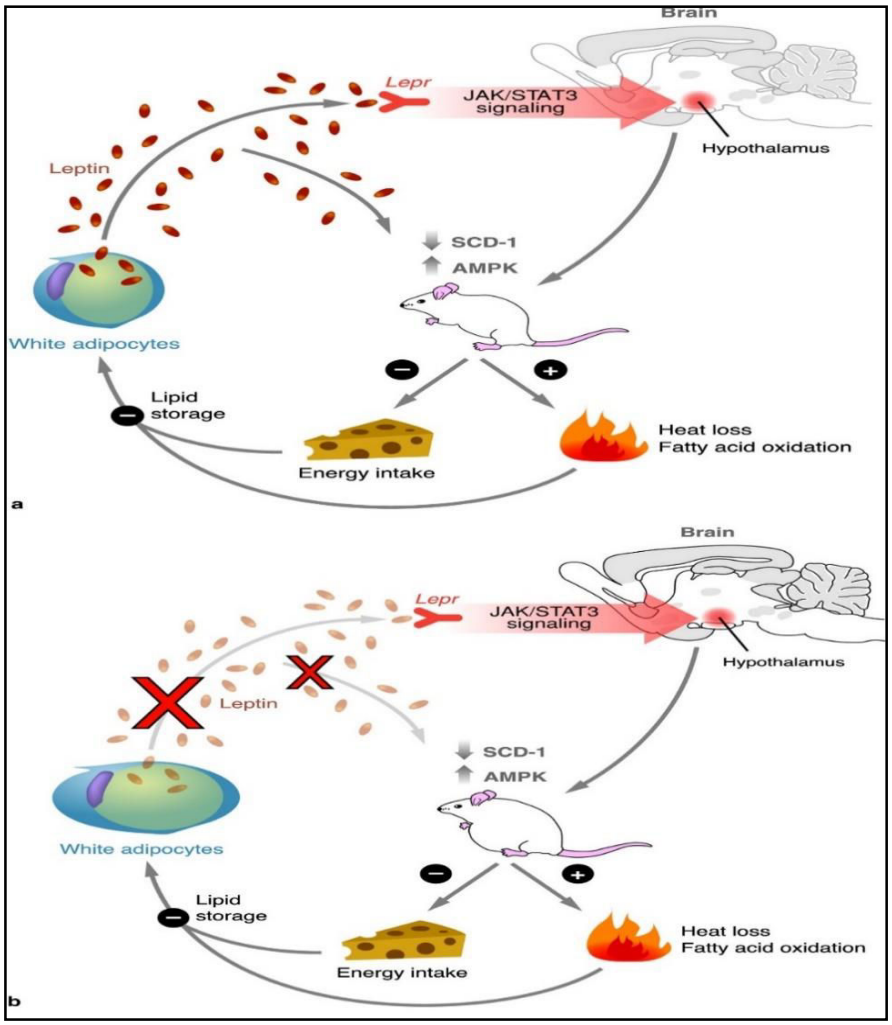


FIGURE II-5 Effect of Leptin on Feeding Behavior, Energy Expenditure, and Adiposity. (a) The net effects of leptin action are decreased food intake and increased energy expenditure, resulting in less lipid storage in adipocytes. **(b)** ob/ob mice are deficient in leptin: Loss of negative feedback from leptin results in increased adiposity. **Image Reference:** (Waki and Tontonoz, 2007b)

Besides its effects on regulating food intake, leptin is also known to have beneficial effects in the promotion of energy expenditure (Figure II-5) (Cohen and Friedman, 2004) by the suppression of Steroyl CoA desaturase-1 (SCD-1) expression in liver (Coleman DL et al.,

1969; Cohen P et al., 2002), prevents the ectopic accumulation of lipid molecules in pancreatic β cells (Colombo et al., 2002) and also stimulates the oxidation of fatty acids in muscle and liver by activating 5-AMP-activated protein kinase (AMPK) in the central nervous system (Combs et al., 2003).

The expression and secretion levels of leptin in the majority of obese patients and obese mouse models are characterized by elevated leptin levels in the circulation, in which cells fails to utilize the produced leptin (leptin resistance) (De Benedetti F et al., 1997; Eitzman DT et al., 2000; Eren et al., 2002). Leptin therapy in this context is ineffective due to its unclear precise mechanism, but transport across the blood-brain barrier and intracellular signaling are likely to be altered in leptin resistance (Farooqi et al., 2002; Fain et al., 2003; Farooqi IS et al., 2005).

After 20 years of intensive research efforts, recombinant leptin and the analog of human leptin Metreleptin (trade name Myalept) are available in the market as a therapeutic agent where administration and replacement of leptin in leptin deficient subjects improvises congenital leptin deficiency, hyperglycemia and hyperlipidemia in patients with lipodystrophy (Davis R. J et al., 2000; Oral et al., 2002; Friedman and Mantzoros, 2015).

II. G. c) Tumor Necrosis Factor-α (TNF-α):

TNF-α was first identified as an endotoxin-induced serum factor that caused necrosis of tumors (Trayhurn P, 2004) and was later found to be identical to cachectin (Trujillo ME, 2005) which shows its effects in inducing septic shock, cachexia and inhibits lipogenesis in adipocytes and various tissues. It is a biologically active trimer formed after the cleavage of transmembrane precursor protein produced by the action of TNF-α converting enzyme (TACE, also called ADAM17). It is a 26 kDa transmembrane secreted protein (Hotamisligil et al., 1993).

TNF-α is a factor that is highly expressed in and secreted from adipose tissue (Table II-2) (Hotamisligil et al., 1993) and overexpressed in case of obese mice and human subjects (Wallach et al., 1999). Exposure of cells and animals to TNF-α for a prolonged period of time causes insulin resistance. In contrast, neutralization of TNF-α leads to an increase in uptake of peripheral glucose in response to insulin as observed in a mice and rat model of obesity (Hotamisligil et al., 1993). TNF-α shows its mode of action by reducing the tyrosine-phosphorylation of IRS-1 activity by insulin (Uysal et al., 1997), which is stimulated by serine-phosphorylation of IRS-1. Besides this in another way TNF-α activates serine/threonine kinase IKKβ which in turn leads to serine-phosphorylation of IRS-1 (Insulin receptor substrate 1) (F et al., 2002), thereby augments the levels of free fatty acid (FFA) in circulation and suppresses the expression of adiponectin (Vaughan, 2005). TNF-α executes its functions by binding to TNF receptors/death receptor (TNFR) regulating a wide range of cellular processes. (For example cell-survival in the CNS via TNF-R1, TNF-R2) (Pickering et al., 2005; Montgomery and Bowers, 2012; Arnoldussen et al., 2014). These receptors in turn cooperates with adaptor proteins such as Tumor Necrosis Factor Receptor Type 1-Associated Death Domain Protein (TRADD), TNF Receptor Associated Factors (TRAF),

Receptor-Interacting Protein Kinases (RIP) that determines the inflammatory and apoptotic response.

Tumor necrosis factor alpha (TNF-α) is a pro-inflammatory circulating cytokine well-known for its role in chronic peripheral and central inflammation (Thaler et al., 2012; Arnoldussen et al., 2014). It is mainly produced by macrophages and its expression is increased at the mRNA and protein levels in obese and in type 2 diabetes models (Hotamisligil et al., 1993). Besides its detrimental role in several tissues, TNF-α also demonstrates protective effects in the brain. It notably acts on neurogenesis, synaptic transmission and plasticity (Arnoldussen et al., 2014). Thus, TNF-α was notably described for its neuroprotective roles on hippocampal neurons by suppressing accumulation of ROS and by maintaining intracellular levels of calcium (Barger et al., 1995). In addition, TNF-α modulates glutamatergic transmission (Beattie et al., 2002). Furthermore, TNF-α favors neural progenitor cells survival by notably mediating anti-apoptotic signals via TNFR2 (Marchetti et al., 2004). In rat, TNF-α appear to promote the survival of stroke-generated hippocampal and striatal neurons (Heldmann et al., 2005). In addition, TNF-α knock-out mice show cognitive impairment (i.e: significant poorer learning, retention and spatial learning), suggesting a strong role for TNF-α on these mechanisms (Baune et al., 2008).

In the previous section, we have seen the versatile biological activities of the WAT in health and disease, and the coming section focusses majorly on the impact of factor produced by the WAT (ATX and ADIPO) which are potentially involved in obesity induced neurodegeneration.

It is also worth remembering that these adipocytokines (ATX and ADIPO) and their receptors are not only present in the adipose tissue, but they are also well expressed in the CNS.

III. Candidate Genes: AUTOTAXIN and ADIPONECTIN

III. A. AUTOTAXIN

TABLE III-I	III. A a) AUTOTAXIN Portfolio
Gene Name	ENPP2
Protein names	Ectonucleotide pyrophosphatase/phosphodiesterase family member 2
Defining Autotaxin	A protein that is a translation product of ENPP2 gene
Alternative names	Autotaxin, Extracellular lysophospholipase D, LysoPLD
Synonyms	Npps2, Pdnp2
Lineage	Eukaryota › Metazoa › Chordata › Craniata › Vertebrata › Euteleostomi › Mammalia › Eutheria › Euarchontoglires › Glires › Rodentia › Sciurognathi › Muroidea › Muridae › Murinae › Mus › Mus
Organism	Mus musculus (Mouse)
Chromosomal Locus	Chromosome-8 (Humans) Chromosome-15 (Mouse)
Gene Details	**DNA:** ~81118 b.p **mRNA*:** 2772 - 3124b.p **Protein:** 863 a.a
Molecular Mass	125 kDa
Diversified forms	Isoform -1 or β, Isoform - 2 or α, Isoform -3 or γ.
Subunit interactions	Interacts with zinc and copper co-factors
Secretion	Cancerous cells and Adipocytes.
Principal Involvement	Acts as Cell Motility, Migratory, proliferating factor, parturition
Therapeutic Activity	Anti-cancer therapy
Signaling pathway	Follows LPA signaling pathway

*mRNA transcript variants length depends on alternative splicing

TABLE III-2 **III. A b) Functionality of AUTOTAXIN**

Catalytic Activity:
Autotaxin has Lysophospholipase D (LysoPLD) activity that converts lysophosphatidylcholine (LPC) into lysophosphatidic acid (LPA), which is responsible for its cell proliferating effects (Giganti et al., 2008; Prestwich et al., 2008). The interdomain disulfide bond between Cys-413 and Cys-805 is essential for catalytic activity (Nishimasu et al., 2011a; Kawaguchi et al., 2013).

Lysophospholipase D (LysoPLD) activity:
N-glycosylation, but not furin-cleavage, plays a critical role on secretion and LysoPLD activity (Pradère et al., 2007).

Secretory Activity:
Secretion requires simultaneous glycosylation on Asn-53 and Asn-410, while probable glycosylation of Asn-410 has a preferential role on LysoPLD activity (Not O-glycosylated) (Pradère et al., 2007).

Anti-Inflammatory and Anti-oxidative Activity:
Antagonizes and counteracts the effects of TNF-α by negatively regulating its expression (Awada et al., 2014) and inhibits intracellular reactive oxygen species (ROS) (Awada et al., 2012), and inflammation mediated nuclear factor kappa beta (NFkβ) signalling.

Miscellaneous Activities:

Chemotaxis	Lipid degradation	Lipid metabolism

Molecular Functionality:
Lysophospholipase activity (Nishimasu et al., 2011a)
phosphodiesterase-I activity (Bachner et al., 1999)
nucleotide diphosphatase activity (Bachner et al., 1999)
Zinc and Calcium binding activity (Nishimasu et al., 2011a)
Polysaccharide binding activity (Houben et al., 2013)
Scavenger receptor activity (Jansen et al., 2009)

Biological Processivity:
Positively Regulates:

Cell Motility	Cell proliferation	Cell development

Negatively Regulates:

Inflammation	Oxidative stress
Tumor necrosis factor production	Nuclear Factor kappa B-Cell (NF-kB) signalling

Subcellular Location:

Extracellular space	Integral component of plasma membrane
Cell surface	Cell periphery

Post translational modification (PTM): Formation of Disulfide bonds, Glycoprotein groups, undergoes Glycosylation (Pradère et al., 2007; Nishimasu et al., 2011a).

III. A. c) Autotaxin Alias Lysophospholipase D:

Autotaxin alias Lysophospholipase D (LysoPLD) is a type II ectonucleotide pyrophosphate phosphodiesterase secreted enzyme that catalyzes the transformation of albumin bound or membrane-derived lysophosphatidylcholine (LPC) to produce equimolar amounts of bioactive lysophosphatidic acid (LPA) and choline (Tokumura et al., 2002; Umezu-Goto et al., 2002) with the help of Lysophospholipase D activity.

Autotaxin is a protein of 125 kDa, which is anchored in the membrane by its amino-terminus, and released into the extracellular medium by proteolytic cleavage (Figure III-1). It is a member of the nucleotide pyrophosphatase/ phosphodiesterase family of ectoenzymes (E-NPP). ATX has a catalytic site in its extracellular portion that hydrolyzes phosphodiester bonds of various nucleotides such as ATP or ADP, and that turns lysophosphatidylcholine (LPC) into lysophosphatidic acid (LPA) (Stracke et al., 1992; Clair et al., 1997; Bollen et al., 2000; Goding et al., 2003; Saulnier-Blache, 2004).

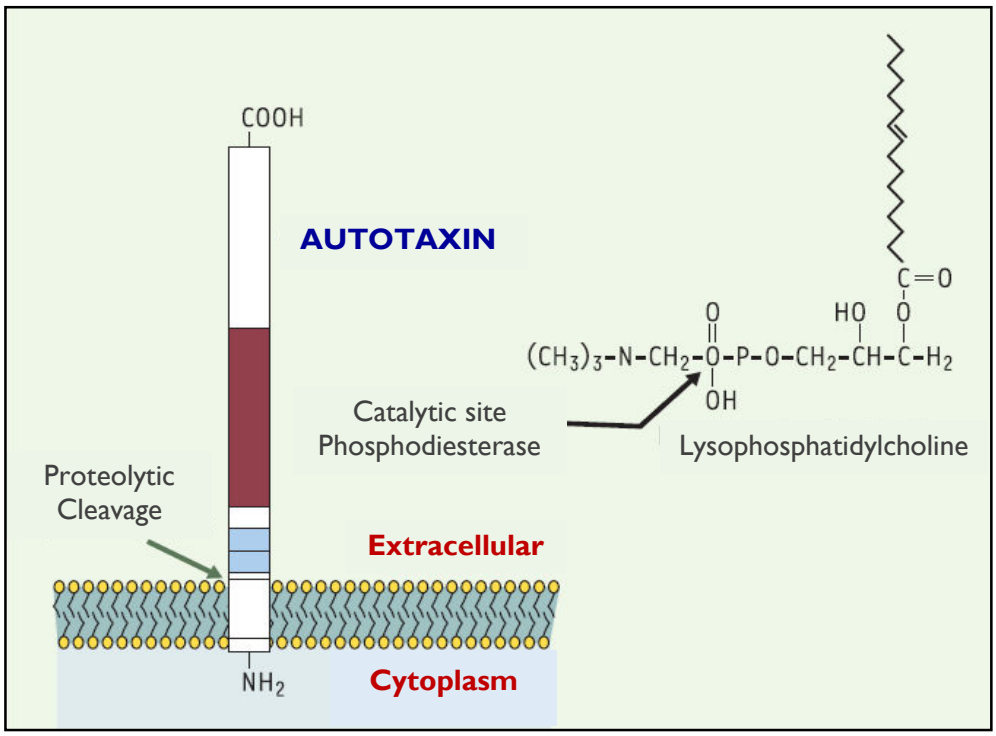


FIGURE III-1 Structure of membrane anchored ATX protein
Image Reference: (Saulnier-Blache, 2004)

ATX is secreted in most of the body fluids including: blister fluid (Mazereeuw-Hautier et al., 2005) and cerebrospinal fluid (Hammack et al., 2004; Sato et al., 2005), whereas the catalytic activity of Autotaxin is present at high concentrations in biological fluids such as plasma, serum and seminal plasma (Aoki, 2004) in various species including rabbit (Tokumura et al., 2002), bovine (Umezu-Goto et al., 2002), and humans (Masuda et al., 2008; Nakamura et al., 2008). ATX or LPA is also produced by adipocytes and cancerous cells during differentiation but not pre-adipocytes (Gesta et al., 2002).

III. A. d) Bioactive Phospholipid - "The Lysophosphatidic Acid (LPA)":

The lysophosphatidic acid (LPA) is a phospholipid consisting of glycerol-phosphate backbone which is able to generate growth factor-like activities in a wide Variety of normal and malignant cell type. ATX removes the choline head group from LPC and thereby produces LPA. So the bioactive LPA thus produced via ATX catalysis acts through six distinct guanine-nucleotide-binding protein (G protein) coupled receptors (GPCRs) termed as LPA. It shows both overlapping and distinct tissue distribution and signalling properties. Major G protein-linked effector pathways induced by LPA includes: the mitogenic RAS–extracellular signal-regulated kinase pathway; the phosphoinositide 3 kinase (PI3K)-AKT survival pathway; RHO and RAC-mediated cytoskeletal remodeling and cell migration; and phospholipase C (PLC) activation, leading to Ca2+ mobilization (Figure III-2).

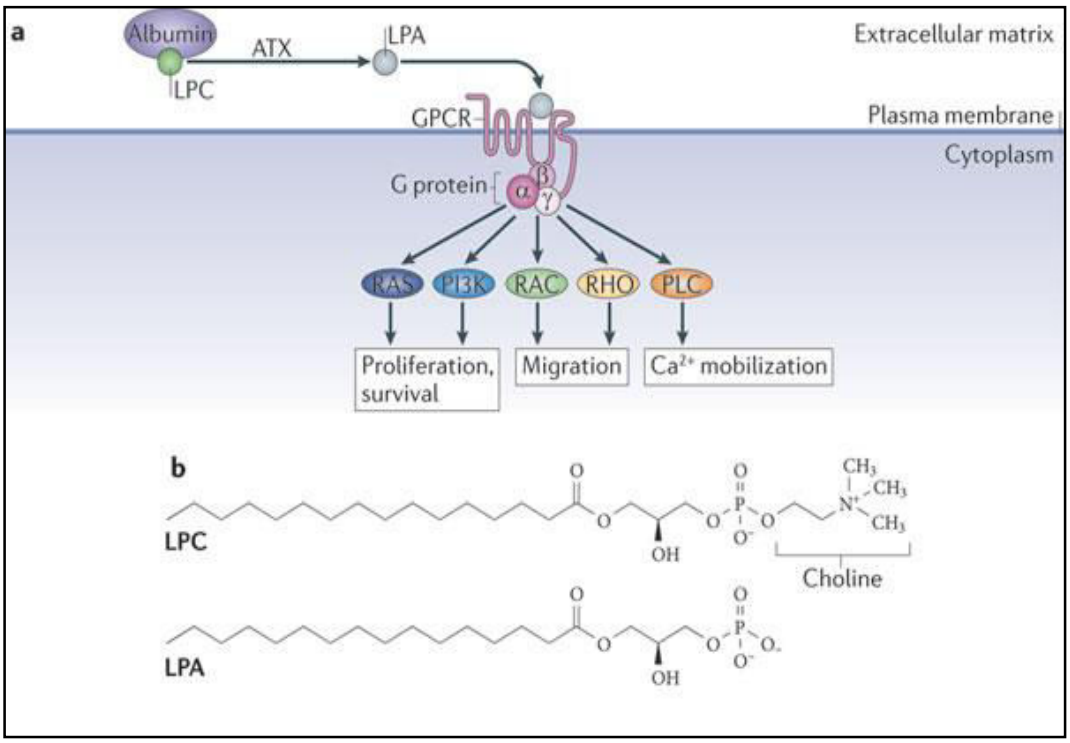


FIGURE III-2 LPA Signal Transduction: a) ATX-LPA receptor signalling; **b)** Structures of LPC and LPA. **Image Reference:** (Moolenaar and Perrakis, 2011)

III. A. e) Two Major Synthetic Pathways Engendering Bioactive LPA:

LPA can be generated via two major synthetic pathways. In the first pathway, the precursor phospholipids (phosphatidylcholine, phosphatidylserine, or phosphatidylethanolamine) will be converted to their corresponding lysophospholipids such as lysophosphatidylcholine (LPC), LPS, or LPE. In platelets, this occurs via phosphatidylserine-specific phospholipase A1 (PS-PLA1) or secretory phospholipase A2 (sPLA2) activity. In plasma, LPC is produced by LCAT and PLA1 activity. In either location, lysophospholipids can then be converted to LPA via autotaxin (ATX) activity. In the second major pathway, phosphatidic acid (PA) is first produced from phospholipids through phospholipase D or from diacylglycerol (DAG) through diacylglycerol kinase. Then, PA is converted directly to LPA by the actions of either PLA1 or PLA2 (See Figure III-3).

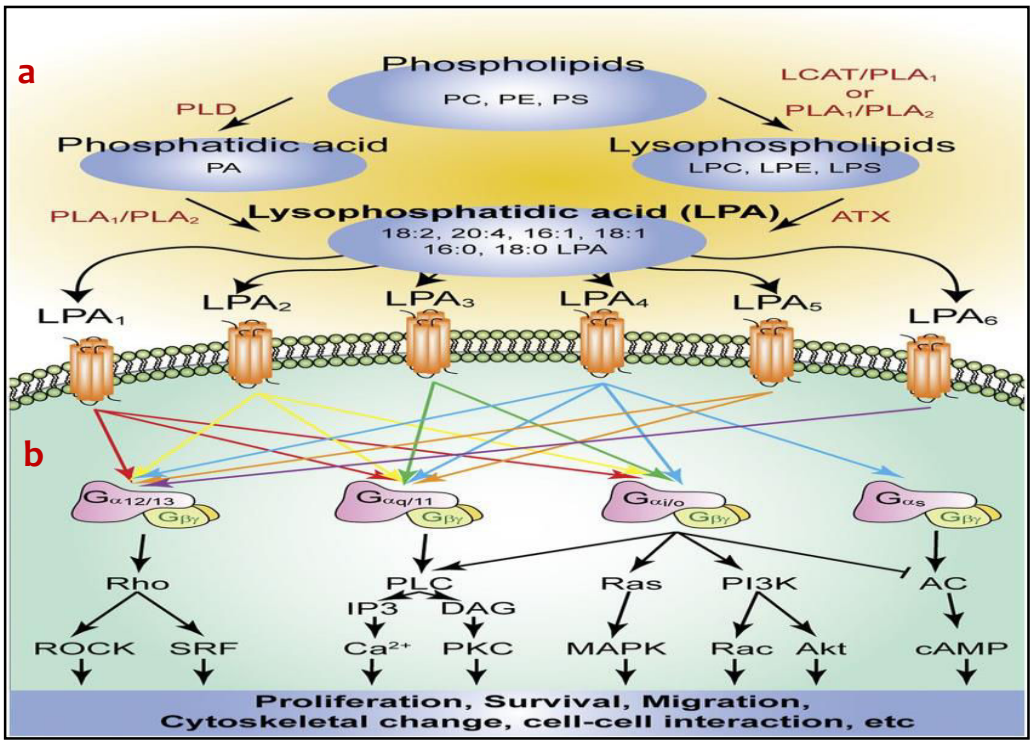


FIGURE III-3 LPA Synthetic Pathways: a) Two major synthetic pathways engendering bioactive LPA; **b)** Summary of the major routes of LPA synthesis and the activated signaling pathways via the six cognate LPA receptors. Phosphatidylcholine (PC); phosphatidylethanolamine (PE); phosphatidylserine (PS); phospholipase D (PLD), lecithin cholesterol acyltransferase (LCAT); phospholipase A1 (PLA1); phospholipase A2 (PLA2). **Image Reference:** (Yung et al., 2014)

III. A. f) Expression Of Autotaxin's Isoforms:

The ATX gene is located on chromosome 8 in humans and chromosome 15 in the mouse. It has an intricate gene structure and contains 27 exons and have greater number of alternative splicings. The mRNA transcript variants length of Autotaxin usually depends on alternative Splicing. (Murata et al., 1994). Autotaxin (ATX) has three isoforms, Isoform-1 or β, Isoform-2 or α, Isoform-3 or γ. Isoform alpha expresses the intron 12 and has a cleavage site which is primarily responsible for the rapid catabolism of this particular isoform (Giganti et al., 2008). The ATX isoforms are differentially expressed. High levels of ATX β mRNA expression are detected in peripheral tissues, whereas lower expression levels are observed in the central nervous system. In contrast, the highest levels of mRNA expression for the ATX γ variant are detected in total brain, whereas significantly lower expression levels are observed in peripheral tissues. Among the three isoforms, ATX-α exhibits the lowest expression levels in both the central nervous system and peripheral tissues. The α isoform is very unstable because it contains an extra exon (exon 12) compared to isoforms β, and γ (Giganti et al., 2008).

III. A. g) Autotaxin's Architectural Domain:

Autotaxin has various domains (As seen in figure III-4), N-terminal intracellular domain (targeting to the plasma membrane), signal peptide, transmembrane domain, extracellular domain comprises two cysteine-rich somatomedin B (SMB) like domains located adjacent to a hydrophobic domain containing an RGD tripeptide (cell-extracellular matrix interactions), catalytic domain (lysophospholipase D activity), central PDE domain and nuclease-like domain located at its C-terminus NUC domain. *N*-Glycosylation of Asn53 and Asn410 and signal peptide cleavage are the key elements in the regulation of the enzyme secretory and catalytic activity (Murata et al., 1994). ATX has phosphodiesterase, pyrophosphatase and ATPase activities with a low catalytic capacity (Lee et al., 1996b) .

FIGURE III-4 Domain Organization of Autotaxin
Image Reference: (Nishimasu et al., 2012)

III. A. h) Autotaxin's LPA Receptors (LPAR):

So far six bona fide LPA receptors (LPAR1 to LPAR6) and several other intracellular PPARγ (An et al., 1998; Bandoh et al., 1999) putative or accepted LPA receptors (GPR87 and p2Y) (Noguchi et al., 2003; Yanagida et al., 2009) and five sub-types of receptors other phospholipid bioactive, sphingosine-1-phosphate (Saulnier-Blache, 2004) have been identified. These receptors with seven transmembrane domains G protein-coupled belongs to the EDG family (Endothelial differentiation gene). Few biological roles of LPA can be mediated by ATX. ATX can interact with target cells via specific cell-surface molecules such as integrins and heparan sulphate proteoglycans (HSPs), as well as by direct membrane association, to facilitate LPA release near to its cognate receptors. In this way, ATX not only drives the formation of LPA but also ensures specificity in LPA signalling (Moolenaar and Perrakis, 2011).

LPA receptors are expressed in immune cells, including lymphocytes (Goetzl et al., 2000; Zheng et al., 2000) and dendritic cells (Panther et al., 2002; Chen et al., 2006b), and in lymphoid organs such as the spleen and thymus (Ishii et al., 2004; Kotarsky et al., 2006; Oh et al., 2008).

III. A. i) LPA Receptor Expression in CNS:

Apart from immune cells, adipocytes and cancer cells, central nervous system is another major hotspot for the LPA receptor expression (Weiner et al., 1998; Contos et al., 2000; Fukushima et al., 2001; Ishii et al., 2001) which regulates the activity of various neural cell types, such as neural cell lines, neural progenitors, primary neurons, oligodendrocytes, Schwann cells, astrocytes, and microglia (Choi et al., 2010). LPA levels are increased during pathological conditions of the brain, such as in response to injury, cerebral ischemia, and following disruption of the blood-brain barrier (Fukushima et al., 2002; Fukushima, 2004).

III. A. j) Autotaxin's Protein Stability:

The optimum enzymatic activity of ATX can be noticed at pH 8, whereas the optimum temperature activity is 40 degree centigrade.

III. A. k) Autotaxin in Pathophysiology:

III. A. k. i) ATX in Obesity:

Autotaxin is predominantly secreted by adipocytes whose expression is substantially upregulated in obese, diabetic db/db mice (Boucher et al., 2005).

III. A. k. ii) ATX in Diabetes:

Autotaxin expression was upregulated by treatment with TNF-α (insulin resistance-promoting cytokine), and downregulated by rosiglitazone treatment (insulin-sensitizing compound) in 3T3F442A adipocytes. Adipose tissue autotaxin expression was significantly upregulated in patients exhibiting both insulin resistance and impaired glucose tolerance. Finally, it is showed that type 2 diabetes in humans is also associated with upregulation of adipocyte autotaxin expression. These observations suggested a possible involvement of autotaxin in the normal or pathological development of adipose tissue and/or pathologies associated with obesity (Ferry et al., 2003).

III. A. k. iii) ATX in Fetal Development:

ATX expression at the developing embryo and ATX-mediated LPA production and subsequent G-protein coupled receptor (GPCR) signaling are essential for vascular branching morphogenesis and chorio-allantoic fusion, which seem to be the primary causes of embryonic death in the absence of ATX. It also plays a key role in fetal development and mice deleted of the ATX gene are not viable (Tanaka et al., 2006; van Meeteren et al., 2006).

III. A. k. iv) ATX in Neuropathology:

It is worth noticing that ATX and its LPA receptors has also been expressed in CNS. That is why ATX role and activity has been mostly linked to various central nervous system diseases, oligodendrocyte function, myelination, wound healing and so on. One such includes neuropathic pain (Inoue et al., 2004) through its transformation of LPC into LPA in the spinal cord. Another such includes Autotaxin downregulates LPS-Induced microglia activation and pro-inflammatory cytokines production (Awada et al., 2014). Besides this, Autotaxin expression is enhanced in frontal cortex of Alzheimer-type dementia patients as reported by (Umemura et al., 2006) and cell type ATX specific expression in the brain has been upregulated during development and after neurotrauma (Savaskan et al., 2007)

III. A. k. v) ATX As A Biomarker And A Therapeutic Target:

Ample of evidences confirmed that ATX expression has been significantly upregulated in various tumors such as breast cancer, renal cell cancer, hepatocellular carcinoma and thyroid cancer (Yang et al., 1999; Zhang et al., 1999; Zhao et al., 1999; Euer et al., 2002; Yang et al., 2002; Kehlen et al., 2004) which suggests the potential role of Autotaxin as a chemotherapeutic target. In order to inhibit the dynamic enzymatic activity of ATX in various

forms of cancer, histidine (Clair et al., 2005), LPA analogues (Baker et al., 2006; Federico et al., 2008), albumin (Morishige et al., 2007) and cyclic phosphatidic acid can be used as a natural molecules that regulates this ATX activity. FTY720 (van Meeteren et al., 2008), an immuno modulator at a concentration of 200 nM has been reported as the first compound that has anti-cancer activity and shows its effect by inhibiting the ATX's uncontrolled enzymatic activity.

III. B. ADIPONECTIN

TABLE III-3	III. B. a) ADIPONECTIN Portfolio
Gene Name	AdipoQ
Protein names	Adiponectin
Defining Adiponectin	A protein that is a translation product of AdipoQ gene
Alternative names	Adipocyte complement-related protein of 30 kDa (ACRP30) Gelatin binding protein of 28 kDa (GBP28) Adipocyte, C1q and collagen domain-containing protein Adipose most abundant gene transcript 1 protein Adipocyte-specific protein AdipoQ
Synonyms	Acrp30, GBP28, adipo, APN Acdc, Apm1
Lineage	Eukaryota › Metazoa › Chordata › Craniata › Vertebrata › Euteleostomi › Mammalia › Eutheria › Euarchontoglires › Glires › Rodentia › Sciurognathi › Muroidea › Muridae › Murinae › Mus › Mus
Organism	Mus musculus (Mouse)
Chromosomal Locus	Chromosome-3 (Humans) Chromosome-16 (Mouse)
Gene Details	**DNA:** ~11493 b.p **mRNA*:** 947-1292 b.p **Protein:** 247 a.a
Molecular Mass	90 kDa (Basic Unit) 180 kDa (LMW) 360 kDa (HMW)
Diversified forms (Adiponectin Bouquet)	Monomer, dimer, trimer, hexamer, 12 to 18-mers which associates to form LMW, MMW or HMW complexes.
Subunit interactions	Aggregates via non-covalent interactions of the collagen-like domains in a triple helix and hydrophobic interactions within the globular C1q domain.
Secretion	Synthesized by adipocytes and secreted into plasma.
Principal Involvement	Regulates fat metabolism and insulin sensitivity
Therapeutic Activity	Anti-diabetic, anti-atherogenic and anti-inflammatory.
Signaling pathway	Follows AMPK pathway.

*mRNA transcript variants length depends on alternative splicing (Murata et al., 1994)

TABLE III-4 **III. B. b) Functionality of ADIPONECTIN**

Insulin-Sensitizing Activity:
Hydroxylation and glycosylation of the lysine residues within the collagen like domain of adiponectin are critically involved in regulating the formation and secretion of HMW complexes of adiponectin that contributes to the insulin-sensitizing actions of adiponectin in hepatocytes. HMW complexes are more extensively glycosylated than smaller oligomers. (Wang et al., 2002b)

Anti-Inflammatory Activity:

a. Antagonizes and counteracts the effects of TNF-α by negatively regulating its expression in various tissues such as liver and macrophages. (Ouchi and Walsh, 2008; Moschen et al., 2012)

b. Inhibits endothelial nuclear factor kappa B (NFkB) signalling through cAMP dependent pathway.(Zhang et al., 2013)

Miscellaneous Activities:
Cell growth Angiogenesis
Tissue remodeling in association with various growth factors with distinct binding affinities.

Molecular Functionality:
Hormonal activity (Berg et al., 2001)
Receptor binding activity (Tsao et al., 2002)
Identical protein binding (Suzuki et al., 2007)
Sialic acid binding activity (Richards et al., 2010)

Biological Processivity:
Positively Regulates:
Brown fat cell differentiation Insulin sensitivity
Glucose homeostasis Fatty acid beta oxidation
cAMP dependent protein kinase activity.

Negatively Regulates:
Inflammation Oxidative stress
Nuclear Factor kappa Beta (NFkβ) signalling Tumor necrosis factor production
Gluconeogenesis Fat cell differentiation

Subcellular Location:
Cell surface Cell periphery
Endoplasmic reticulum Extracellular space
Collagen trimer Perinuclear region of cytoplasm

Post translational modification (PTM): Formation of disulfide bonds, glycoprotein groups, undergoes hydroxylation (Wang et al., 2006).

III. B. c) ADIPONECTIN - Anti-Obese & Anti-Diabetic Adipocytokine:

Adiponectin (Adipo) is a 30kDa adipocyte secretory protein (Pajvani et al., 2003) (adipocytokine) with direct anti-diabetic and anti-inflammatory properties (Yamauchi et al., 2001). Besides its exclusive secretion from adipose tissue, adiponectin is also secreted by the placenta during pregnancy (Chen et al., 2006a) into the blood stream. It structurally belongs to the complement 1q family, hence the name AdipoQ. Adiponectin circulates in human plasma as middle molecular weight (MMW) hexamer and a high molecular weight (HMW) multimer. The major actions of adiponectin includes increased glucose uptake (Diez and Iglesias, 2003), improvises insulin sensitivity, β-oxidation, triglyceride clearance, weight loss (Nedvidkova et al., 2005), reduced levels of TNF-α, and increased levels of uncoupling proteins (Bauche et al., 2007) (Detailed under Table III-4). Adiponectin mRNA transcripts were highly expressed in both preadipocytes and adipocytes but relatively a greater degree of expression levels were reported in preadipocytes than adipocytes (Matsuzawa et al., 2004).

III. B. d) Adiponectin's Architectural Domains:

Adiponectin have four distinct regions: a) Signal sequence (17a.a) that directs and targets the hormone for secretion outside the cell, b) variable domain (28a.a), a short region that lies next to signal sequence whose formation is highly variable from species to species, c) collagen like domain (65a.a) has a similarity to collagenous proteins d) and the last one is a globular domain (137a.a) (Goldstein et al., 2009b). Globular adiponectin (gAd) is produced after the proteolytic cleavage of full length adiponectin monomers by neutrophil elastase and circulates in human plasma (Thundyil et al., 2012a) (Figure III-5).

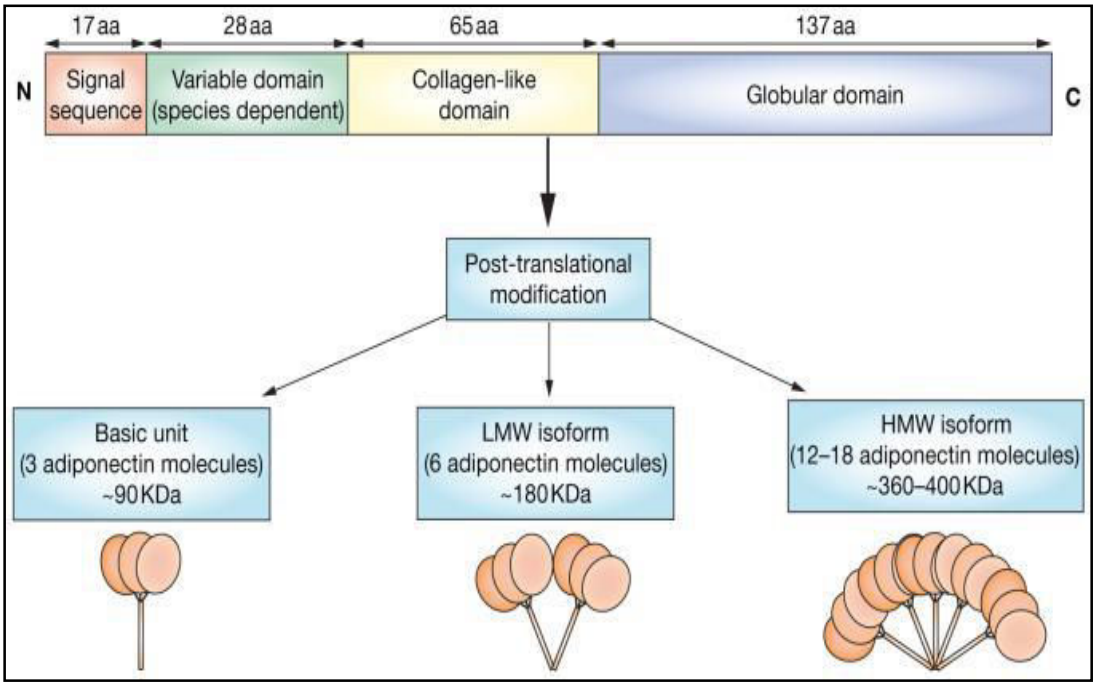


FIGURE III-5 Structure of ADIPO.
Image Reference: (Goldstein et al., 2009a)

III. B. e) Adiponectin's Isoforms:

Majorly Adiponectin molecules in humans are secreted from adipocytes as trimers (90 kDa; the basic unit), low molecular weight hexamers (180 kDa) and high molecular weight isoforms (12-18-mers; >400 kDa) (See Figure III-6). Adiponectin has the potentiality to automatically self-associate itself into larger molecular forms, where the three molecules of adiponectin binds together to form a homotrimer. This trimers continue to self-aggregate and forms hexamers or dodecamers. When compared to the different isoforms of adiponectin, HMW adiponectin has greater biological activity on glucose homeostasis than LMW, MMW molecular forms (Oh et al., 2007).

III. B. f) Oligomerization/Multimerization of Adiponectin:

Manufacturing the oligomeric forms of adiponectin are crucially dependent on the formation of disulfide bonds mediated by Cys-39. Mutations of Cys-39 results in trimers that are subject to proteolytic cleavage in the collagenous domain. So Cys-Ser mutation at amino-terminus, which are incapable of forming multimers more than a trimer were reported to diminish the effects of adiponectin AMPK pathway in hepatocytes. Hence this data suggests that impaired multimerization and impaired secretion of adiponectin were found to the pivotal reasons for the onset of diabetes (Pajvani et al., 2003). In connection to this, not only the total concentrations of adiponectin should be considered, but also the multimer distribution of adiponectin in circulation should also and always be considered as an important parameter in determining plasma adiponectin levels in both health and disease (Waki et al., 2003).

III. B. g) Post Translational Modifications (PTMs) of Adiponectin:

Post-translational modifications such as hydroxylation and glycosylation plays an essential role towards the regulation of adiponectin multimerization, secretion, biological activity and as well for its full length production. This process includes the hydroxylation of multiple conserved proline (Pro71, Pro76, and Pro95), lysine residues (at position - 68, 71, 80, and 104) and glycosylation of hydroxylysines (Hyl65, Hyl68, and Hyl77). Hydroxylysines arises from the post-translational hydroxy modification of lysine. Mutation of modified lysine residues in the collagenous domain prevented the formation of HMW multimers which are related with anomalous functioning of adiponectin multimerization associated pathologies including T2DM (Richards et al., 2006; Wang et al., 2006) (Figure III-6).

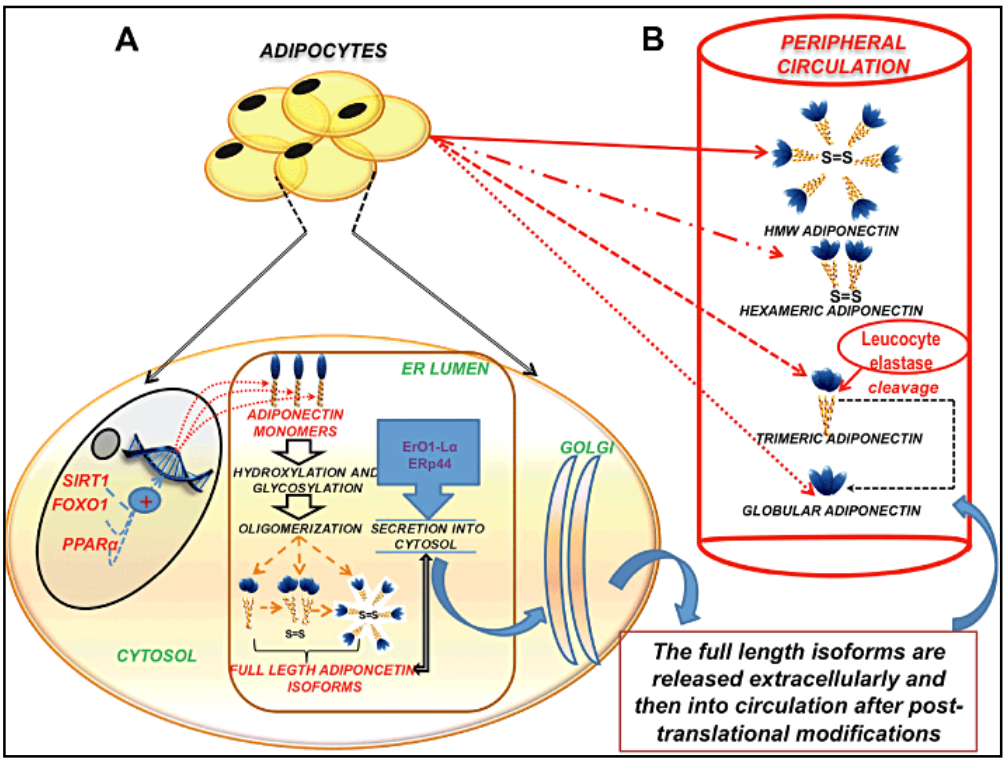


FIGURE III-6 Regulation of synthesis, secretion and circulation of adiponectin: (A) Adipocytes synthesize adiponectin mRNA in its monomeric form within the nucleus. This transcription is regulated and promoted by SIRT1/FOXO-1 and PPARα. Once transcribed, the adiponectin protein monomer is released into the ER, where it undergoes various post-translational modifications, regulated by ER chaperones like ERp44 and ErO1- Lα to form trimers, hexamers and HMW (full-length adiponectin) isoforms. **(B)** Following their packaging in the Golgi, the adiponectin isomers are released into the peripheral circulation. The HMW isomer is the most abundant and biologically active form of adioponectin. Another form of circulating adiponectin is the gAD leukocyte elastase-mediated cleavage of the globular domain of the trimeric adiponectin. **Image Reference:** (Thundyil et al., 2012a)

III. B. h) Adiponectin's Stability:

HMW adiponectin are very stable under basic conditions (pH 7-14), but they are labile under acidic conditions below pH 7 in both mouse and humans (Schraw et al., 2008).

III. B. i) Adiponectin's Adipo Receptors (AdipoR):

So far two bona fide receptors have been identified for adiponectin (Figure III-7) in which one receptor have a homology similar to GPCRs (AdipoR1, AdipoR2) and another receptor similar to cadherin family (T-Cadherin-CDH13). Adiponectin receptor serves as an integral membrane proteins with an internalized amino-terminus and membrane externalized carboxy terminus, which is opposite to the topology of G-protein coupled receptors thereby binds to adiponectin molecules and further induces signal transduction. Whereas T-cadherin is a Glycophosphatidylinositol (GPI) anchored membrane receptor protein which binds to hexameric and HMW adiponectin in endothelial and smooth muscle cells (Buechler et al., 2010a). AdipoR1 and AdipoR2 belong to the progestin and AdipoQ receptor (PAQR) family named after the two initially described ligands, progestin and adipoQ (Tang et al., 2005).

AdipoR1 mainly binds to gAd, while AdipoR2 binds to full-length adiponectin. Both AdipoR1 and AdipoR2 exhibits identical effects but also have their own individual signalling preferences, in which AdipoR1 is more prominent in AMP-activated protein kinase (AMPK) phosphorylation, and AdipoR2 is involved in PPARα activation (Yamauchi et al., 2003a; Yamauchi et al., 2007; Lee et al., 2008).

III. B. j) AdipoR Expression In CNS

Adiponectin has the potentiality to cross the blood-CSF-barrier (BCB) and the blood-brain barrier (BBB) where the concentration levels of this adipocytokine in human cerebrospinal fluid (CSF) were found to 1000-fold less concentrated than in serum (Kos et al., 2007; Kusminski et al., 2007b; Neumeier et al., 2007). There are substantial evidences which suggests that adiponectin receptors are expressed widely in the brain and their expression has been detected in regions of the mouse hypothalamus, brainstem, cortical neurons and endothelial cells, as well as in whole brain and pituitary extracts. Cerebral blood vessels in the brain have tightly regulated membrane permeability which allows the selective passage of only the trimers, hexamers and possibly globular forms of adiponectin into the CNS. Whereas in contrast to this context, the peripheral counterparts even provides the access for HMW adiponectin molecules including trimers, hexamers and globular forms of adiponectin as well (Thundyil et al., 2012b). In addition to this, both AdipoR1 and AdipoR2 receptor proteins are expressed in the hypothalamus and the paraventricular nucleus of the brain with AdipoR1 being more pronounced thereby further suggesting that adiponectin exerts a specific role in the brain (Buechler et al., 2010b). Different adiponectin isomers bind to the AdipoRs with different binding affinities. For instance, gAd has greater binding affinity to AdipoRs when compared to the trimeric forms of adiponectin (As demonstrated by the thickness of the arrows in Figure III-7).

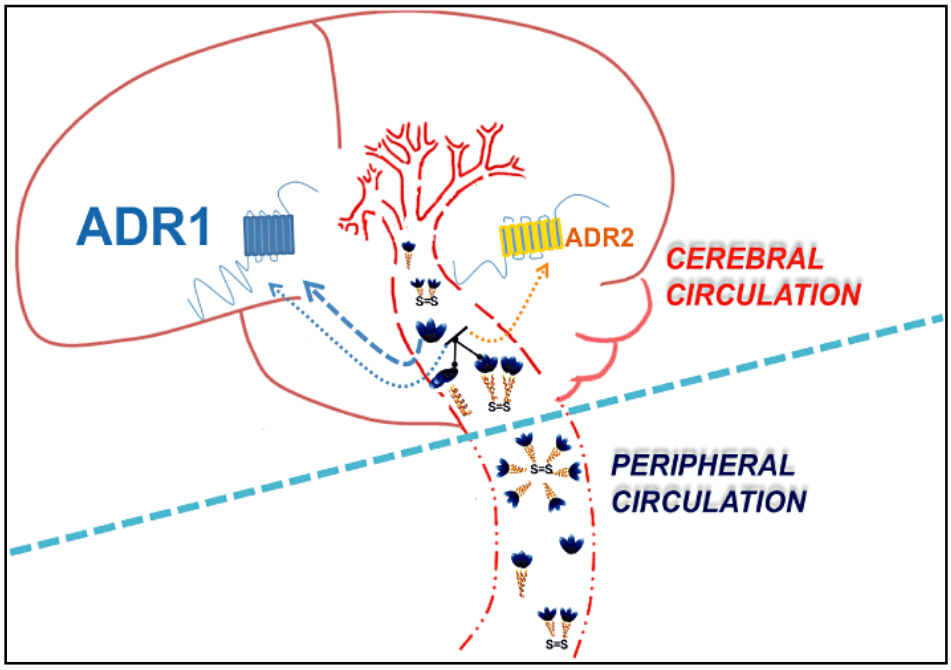


FIGURE III-7 Peripheral and CNS cerebral circulation of Adiponectin signalling
Image Reference: (Thundyil et al., 2012a)

III. B. k) Adiponectin In Pathophysiology:

III. B. k. i) ADIPO In Obesity:

Adiponectin circulation levels are inversely proportional to the weight reduction. It was also recorded that significant weight loss which corresponds to a mean of 14% decrease in BMI through intensive lifestyle changes can also result in increases in adiponectin levels. Adiponectin exerts some of its weight reducing effects via the brain. This is similar to the action of leptin [14] where it is transported into the brain, binds to its cognate receptors in the hypothalamus and activates the JAK-STAT pathway leading to the suppression of orexigenic peptides (neuropeptide Y and agouti-related protein - which normally increase food intake), and elevation of anorexigenic peptides (proopiomelanocortin (POMC) and corticotrophin-releasing hormone, which normally decrease food intake) (Somogyi et al., 2011). Both adiponectin and leptin can have synergistic effects in the brain (Nedvidkova et al., 2005). In addition to the multiple peripheral effects of adiponectin in ameliorating insulin resistance, intracerebroventricular administration of adiponectin decreases body weight by stimulating energy expenditure thereby preventing obesity in rodents (Buechler et al., 2010a).

III. B. k. ii) ADIPO In Diabetes:

Adiponectin gene is located in chromosome 3q27, a susceptibility locus for T2DM and metabolic disorders (Hara et al., 2002). Adiponectin levels are reduced in diabetics when compared to non-diabetics. High-molecular-weight adiponectin was found to be associated with a lower risk of diabetes. Administration of adiponectin in combination with leptin has been shown to completely reverse insulin resistance as demonstrated in mice. In addition to this, it was also reported that supplementation by differing forms of adiponectin were found to improve blood glucose and triglyceride levels in mouse models (Chandran et al., 2003). Lindsay et al. in 2002 found that individuals with high concentrations of adiponectin protein were less likely to develop type II diabetes than with low concentrations of adiponectin in Indian patient subjects (Lindsay et al., 2002). Low levels of maternal plasma adiponectin is a predictive of gestational diabetes mellitus (GDM), a condition that is biochemically similar to type II diabetes. A study led by Williams et al in 2004 demonstrated that Adiponectin concentrations were statistically significantly lower in women with GDM than controls and conclusively suggested an association between hypoadiponectinemia and risk of type II diabetes (Williams et al., 2004).

III. B. k. iii) ADIPO In Neuropathology:

Adiponectin apart from its defined role in metabolic syndromes such as obesity and T2DM, it also plays a contributing role in neurodegenerative disorders including Alzheimer's disease (Song and Lee, 2013). Besides the expression of adiponectin receptors in skeletal muscle and liver, adipo receptors are also expressed in the hypothalamus and vascular endothelial cells of brain (Kubota et al., 2007; Psilopanagioti et al., 2009) and adiponectin was shown to be present in the cerebrospinal fluid (CSF) of rodents (Reaven, 1998; Qi et al., 2004) and human (Kusminski et al., 2007a; Une et al., 2011). Adiponectin also plays a promising role in immune system in the CNS where adiponectin decreases the expression of pro-inflammatory cytokines such as tumor necrosis factor-α (TNF-α) (Yokota et al., 2000) and increases the expression

of anti-inflammatory molecules such as interleukin (IL)-10, IL-1 receptor antagonist (Wolf et al., 2004) and decreases the activation of the pro-inflammatory signal pathway such as nuclear factor-κB pathway (NF-κB) (Detailed under Table III-2).

Various studies in the central nervous system suggest the neuroprotective actions of adiponectin (Jung et al., 2006; Jeon et al., 2009). One such includes Adiponectin protects hippocampal neurons against kainic acid-induced excitotoxicity and another such includes the protective role of adiponectin on astrocytes subjected to oxidative stress situation. Besides this Adiponectin also regulates severe brain inflammation in mild cognitive impairment and Alzheimer's disease (Hivert et al., 2008; Forlenza et al., 2009; Diniz et al., 2010). It is also interesting to note that exercise induced release of adiponectin were associated with augmented hippocampal growth and reported to shown anti-depressive symptoms in mice (Yau et al., 2014).

Glucose is the major energy source of brain in which Adiponectin plays a vital role in modulating brain metabolism and insulin sensitivity to maintain optimum glucose levels for a coordinated whole body energy metabolism including brain. So the dysregulation of insulin have been associated with reduced glucose utilization in brain which ultimately leads to the formation of neurofibrillary tangles and increased amyloid β aggregates by insulin degrading enzyme inhibition (Craft et al., 1999a; Craft et al., 1999b; Park et al., 2000; Kern et al., 2001; Plum et al., 2005; van der Heide et al., 2006). Accumulation of β Amyloid aggregates induces oxidative stress and mitochondrial dysfunction, and these dysfunctions induces Alzheimer's disease pathogenesis (Moreira et al., 2009; Bonda et al., 2010) in which Adiponectin has been reported to be protective against amyloid β neurotoxicity in Alzheimer's disease (Chan et al., 2012).

Conclusively to sum up, chronic neurodegenerative disorders such as Alzheimer's and Parkinson's disease have been associated with inflammation and oxidative stress and accumulating data on adiponectin revealed it as an anti-inflammatory and anti-oxidative adipocytokine, which correlatively suggests that adiponectin could be a promising target for treating neurodegenerative disorders like AD and PD which are associated with neuroinflammatory process.

III. B. k. iv) ADIPO As A Biomarker And A Therapeutic Target:

Administration of adiponectin has been shown to improve insulin sensitivity in mouse models of diabetes Besides this, intraperitoneal (i.p) injection of full-length adiponectin resulted in a significant reduction of glucose levels in both wild-type and type-1, type-2 diabetic mice models (Chandran et al., 2003).

Despite of the evidences supporting the beneficial effects of adiponectin administration in mice, but these methods were found to be difficult to achieve adiponectin as a therapeutic drug in clinical practices due to its larger protein structure and the need for its post translational processing. Henceforth, the indirect approach of upregulating adiponectin levels which includes the upregulation of adiponectin receptors and the use of adiponectin receptor agonists have become the focal point of therapeutic research in treating various adiponectin

associated pathologies such as T2DM and neurodegenerative diseases (Kadowaki et al., 2006). For instance, treatment of human subjects with thiazolidinedione (TZD) results in elevated circulating levels of HMW adiponectin in which TZD may have a direct impact on the synthesis and secretion of adiponectin by adipocytes (Phillips and Kung, 2010) (Depicted in Figure III-8). Apart from thiazolidinediones, statins and angiotensin converting enzyme (ACE) inhibitors are also the potential drugs that augments adiponectin production levels. Niacin acting via GPR109A receptor (Plaisance et al., 2009) and Zetia (Hiramitsu et al., 2010) (apart from TZD) have also been reported to have positive effects on circulating adiponectin levels humans.

In addition to this, evidences supporting the upregulation of adiponectin was corroborated by the studies in 3T3L1 cells treated with thiazolidinediones which have shown an increase in the promoter activity of adiponectin along with an increase in the gene expression and secretion of adiponectin (Iwaki et al., 2003). In spite of these trials, the mechanism by which thiazolidinedione therapy and other drugs which results in an increase in circulating adiponectin levels are still unclear.

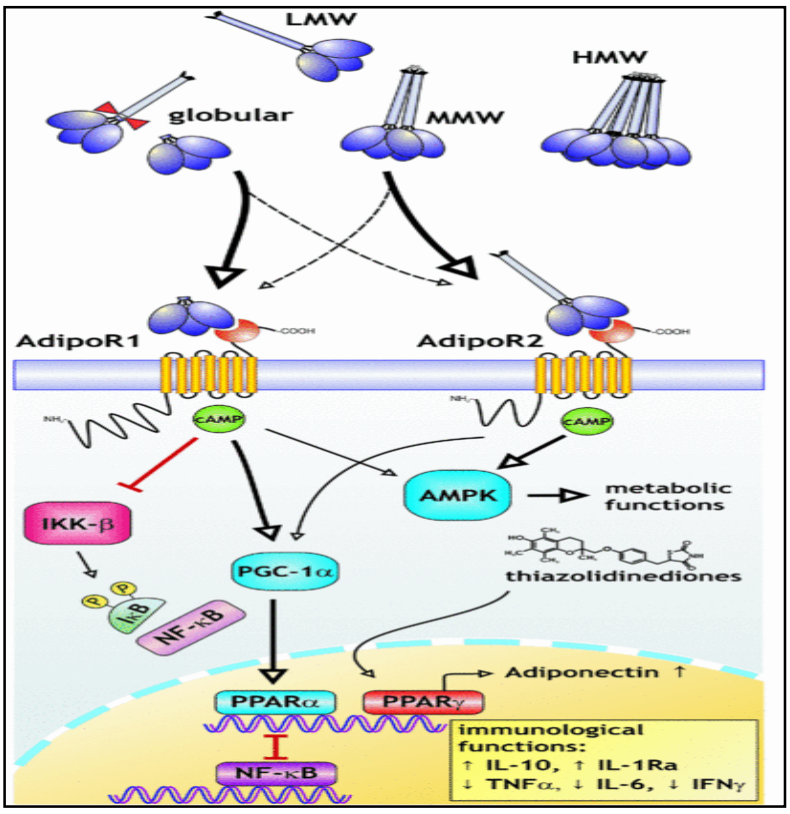


FIGURE III-8 Adiponectin's Interaction with Thiazolidinediones (TZD): Adiponectin interacts with at least two known cellular receptors (AdipoR1 and AdipoR2). Adiponectin expression is strongly up-regulated by thiazolidinediones by the activation of PPAR-γ. Activation of AdipoR1 and/or AdipoR2 by adiponectin stimulates PPAR-α, AMPK and p38 MAPK activation. Adiponectin regulates several pro- and anti-inflammatory cytokines. Its main anti-inflammatory function might be related to its capacity to suppress TNF and IFN-γ synthesis, and to induce anti-inflammatory cytokines, such as IL-10 and IL-1RA. **Image Reference:** (Tilg and Moschen, 2008)

Regardless of the enormous accumulation of scientific documentation on adiponectin suggests that administration of adiponectin have been proven to improvise insulin resistance and hypoadiponectinemia disorders in animal models, but still the experimental clinical trials in humans were still in its infancy. So the extensive efforts are being made to understand how adiponectin levels can be elevated, in which the processing of post translational modifications and further secretion of adiponectin could be the promising areas for adiponectin as a potential therapeutic target to treat multitude of obesity-associated disorders.

Exaggerated inflammatory and oxidative stress responses are the two different, reticulate but intertwined physiological cascades that wires up and drives the brain to a more pathological state manifested by the presence of inflammatory (pro-inflammatory cytokines) and oxidative stress (pro-oxidative cytokines, ROS) bio markers in the brain.

In this regard, the peripheral effects of ATX and ADIPO were well studied, but the central actions of these factors remains poorly delineated. A major part of this thesis was concentrated on investigating the role of ATX on inflammatory conditions and ADIPO under oxidative stress conditions respectively.

IV. Candidate Physiological Process Monitored: Inflammation And Oxidative Stress

IV. A. INFLAMMATION

Before going on to have a knowledge on the implication of glial cells in inflammatory process which is responsible for the onset of various neurodegenerative disorders, first of all it is necessary to have a thorough insight on: what is inflammation, what the inflammation does in the mammalian CNS context refers to, what are the cells involved in it and the probable signaling mechanisms responsible for the onset of inflammation.

IV. A. a) Inflammatory Response:

Inflammation is a natural defense mechanism which offers resistance against various kinds of pathogens recognized by the immune system that are considered as potential threats to the host. 2000 years ago a Roman encyclopaedist Celsus identified inflammation as a constellation of five physical signs: Heat, pain, redness, swelling and loss of function or in classical medical language, "Calor, dolor, rubor, tumor and imobilitate" (Rather, 1971; Cotran; Kumar, 1998; Parakrama Chandrasoma, 2005; Dormandy, 2006; Porth, 2007; Vogel, 2009) (Portrayed in Figure IV-1). These signs are readily visible in the inflammation which accompanies an infected wound or traumatized tissue.

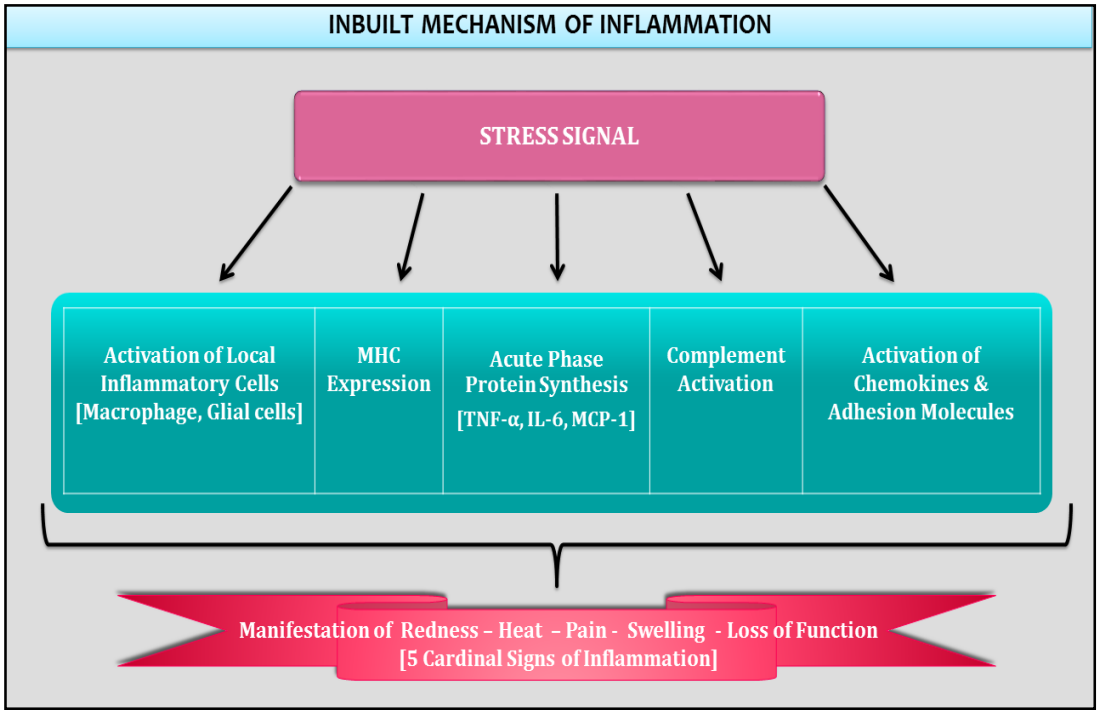


FIGURE IV-1 Inbuilt Mechanism of Inflammation
Image Reference: (Lucas et al., 2006b)

Infection by pathogens, foreign bodies, chemical irritants, toxins, frostbite, stress, trauma and alcohol are the major contributors of inflammation (Schleimer, 1988). Inflammation can be categorized into acute and/or chronic inflammation. Acute inflammation can be diagnosed by the rapid movement of plasma and leukocytes from the blood to the site of injury. Prolonged inflammation leads to chronic inflammation which can be characterized by the rapid and progressive shift of cells at the inflammatory site, followed by the simultaneous destruction and healing of the tissue. Acute inflammation appears within minutes (immediate) and lasts for few days, whereas the onset of chronic inflammation takes longer time (delayed) but sustains in the body for months to years together (Feghali and Wright, 1997).

Host immune cells (monocytes, macrophages, mast cells, lymphocytes, fibroblasts, dendritic cells) plasma derived mediators, (Bradykinin, thrombin, membrane attacking complex - complex of C5b, C6, C7, C8 and multiple units of C9) cell derived mediators (histamines, prostaglandins, TNF-α, IL-8, IL-1, IFN-γ) and blood vessels plays a vital role in the inflammatory process to counteract the initial cause of cell injury and repairs the damaged cell (Larsen and Henson, 1983).

These cells have ligand-specific receptors known as pattern recognition receptors (PRRs) that identifies the molecules associated with groups of pathogens known as pathogen-associated molecular patterns (PAMPs) thereby operates the inflammatory process via the release of inflammatory mediators. Besides the cells and mediators of inflammation, components of complement pathway activated by the bacteria and coagulation and fibrinolysis activated by necrosis runs in parallel along with inflammatory cells to initiate and propagate the inflammatory response further (Rock and Kono, 2008; Gregor and Hotamisligil, 2011; Sun et al., 2012).

Inflammation up to certain extent (clearance of pathogens) will be beneficial to the host system, whereas uncontrolled and prolonged inflammation leads to chronic inflammation and damages the cellular tissues. Besides chronic inflammation, uncontrolled acute inflammation can also lead to the progression of tissue damage. Therefore inflammatory response must be actively terminated when no longer needed to prevent unnecessary damage (Kumar, 1998). This includes: desensitization of receptors, downregulation of pro-inflammatory molecules such as TNF-α, MCP-1 (Eming et al., 2007), upregulation of anti-inflammatory molecules like Adiponectin, IL-10 (Sato et al., 1999), lipoxins such as lipoxinA4 (LXA4) and lipoxinB4 (LXB4) (Serhan, 2008), release of TGFβ growth factors (Ashcroft, 1999; Ashcroft et al., 1999; Werner et al., 2000), apoptosis of pro-inflammatory cells (Greenhalgh, 1998) and having short half-life for inflammatory mediators.

IV. A. b) Neuroinflammation:

Inflammation in CNS (i.e. neuroinflammation) is largely mediated by glial cells in which microglia (Rock et al., 2004b), astrocytes (Zhang and Jiang, 2015) and infiltrating lymphocytes (Lee and Imhof, 2008; Izcue et al., 2009) plays a predominant role. This glial mediated neuroinflammation is characterized by the release of potential neurotoxic mediators such as cytokines (TNF-α, IL-1α and IL-1β) and chemokines (MCP-1, MIP-1α, and MIP-1β) both *in vitro* and *in vivo.* The transcription factor NF-κB plays a prominent role (Chaudhari et al., 2014).

NF-κB is a prototypical pro-inflammatory transcription factor activated in response to cytokines (especially pro-inflammatory cytokines), stress and free radicals (Gilmore, 2006; Lawrence, 2009). NF-κB is expressed by both neuronal and non-neuronal glial cells (microglia and astrocytes). The activity of this factor NF-κB play a major role in the brain's development, synaptic signaling and neuroprotection (Bhakar et al., 2002; Pizzi and Spano, 2006). In addition, NF-κB is necessary to neurogenesis (Zheng et al., 2013). On the one hand, it had been reported that activation of NF-κB in neuronal cells could be beneficial and protective via upregulating the expression of anti-apoptotic proteins such as TNF-α (dual role), Bcl-2 and Bcl-xL in hypoxic or nitric oxide-induced injury conditions (Cheng et al.; Mattson et al., 1997; Tamatani et al., 1999). On the other hand, the activation NF-κB can be detrimental in glial cells (microglia and astrocytes) via the increased expression of pro-inflammatory cytokines such as TNF-α, IL-1β, IL-6 and as well by priming the cells with overwhelmed ROS levels (John et al., 2003b; Kim and de Vellis, 2005; Hsiao et al., 2013).

IV. B. Oxidative stress - OVERLOADED

Oxidative stress is a state where the host system fails to balance reactive oxygen intermediates and system's antioxidant capacity, in which the reactive oxygen species dominates and further contributes to neuronal loss (Gandhi and Abramov, 2012).

Stable molecular oxygen and nitrogen species have their electrons paired up in their outermost orbital and each orbital can accommodate a maximum of two electrons. Each electron of these pair will have an opposite spin, which is essential for the stability of the molecules. On the contrary, a free radical is a molecule with one or more unpaired electrons in its outermost orbital, which makes this species very unstable and tending to react with other molecules to pair up with this unpaired electron in order to attain molecular stability. Free radicals have very short half-life and are therefore difficult to measure. Therefore the most common approach to measure free radicals is to measure the derivatives or end-products of oxidation processes such as lipid peroxidation process (Holley and Cheeseman, 1993). Aerobic cells demand oxygen to meet their energy requirements. When these cells undergo metabolic process they generate some free radicals. The percentage of oxygen in the atmosphere is 21% in dry air (Halliwell et al., 1992; Halliwell and Cross, 1994; Halliwell B, 2007) and during normal metabolic conditions 2–5% of the O_2 consumed by mitochondria is converted to reactive oxygen species (Lopaczynski W, 2007). So the balance of ROS in a host system determines the viability of a cell.

IV. B. a) Sources Of Oxidative Stress:

Free radicals are inevitable and are continuously produced by the body's normal use of oxygen. These are produced by the mitochondria when cells use oxygen to generate energy. The by-products produced are known as reactive oxygen species (ROS) as well as reactive nitrogen species (RNS) (Outlined in Table IV-1) that result from the cellular redox process. All the living beings are exposed to free radicals from exogenous or endogenous sources (Frei et al., 1989; Halliwell, 2007).

Exogenous sources of oxidative stress are: oxygen rich environment, ionizing radiation, U.V light, exposure to air pollution, industrial effluents, chemicals, or solvents (bisphenol A, polychlorinated biphenyl (PCBs), dioxin, alkylphenols, type-2 alkenes, polycyclic aromatic hydrocarbons, and other metals), radiation, anesthetics (Opara, 2006). Toxins exposure mimicking oxidative stress includes exotoxins such as heavy metals like mercury, lead and cadmium. Endotoxin exposure includes the ROS produced from bacteria, yeast, viruses, parasites, stress, allergens, cold, strenuous exercise, cigarette smoking, and alcohol consumption, dietary factors such as excess sugars, saturated fats and deep fried oils (Cadenas and Davies, 2000) (Demonstrated in Figure IV-2).

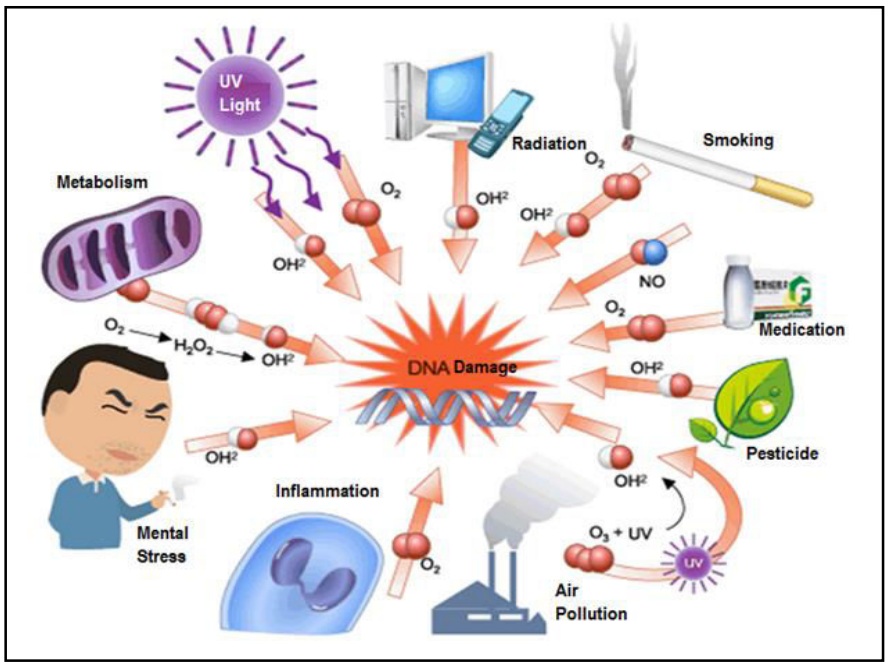


FIGURE IV-2 Sources of Free Radicals and Its Impact on DNA Damage
Image Reference: (Lam Kee, 2014)

By-products formed as a result of aerobic metabolism that takes place primarily in mitochondria (power generator) which is mainly responsible for the production of cell's energy is a major contributor of endogenous ROS. An alternative potential sources for endogenous production of ROS occurs in phagocytic cells (inflammation inducing cells as monocytes, macrophages, mast cells), when these phagocytic cells engulfs foreign particles such as bacteria, virus and other pathogens. This reaction is known as oxidative burst (Opara, 2006; Lau et al., 2007b). Besides this, β-oxidation of fatty acids in peroxisomes, cytochrome

p450 metabolism of xenobiotic compounds also play a contributing role in the production of endogenous ROS (Cadenas and Davies, 2000).

IV. B. b) Affinity Of Free Radicals Towards Biomolecular Degradation:

Disrupting the redox balance in a cell, by environmental stress factors such as U.V exposure, ionizing radiations can cause lethal effects via the production of (ROS), peroxides, unstable oxygen and nitrogen species that degrades the essential biomolecules (nucleic acids, proteins, lipids) (Guetens et al., 2002) eventually progressing to tissue destruction (See Figure IV-3).

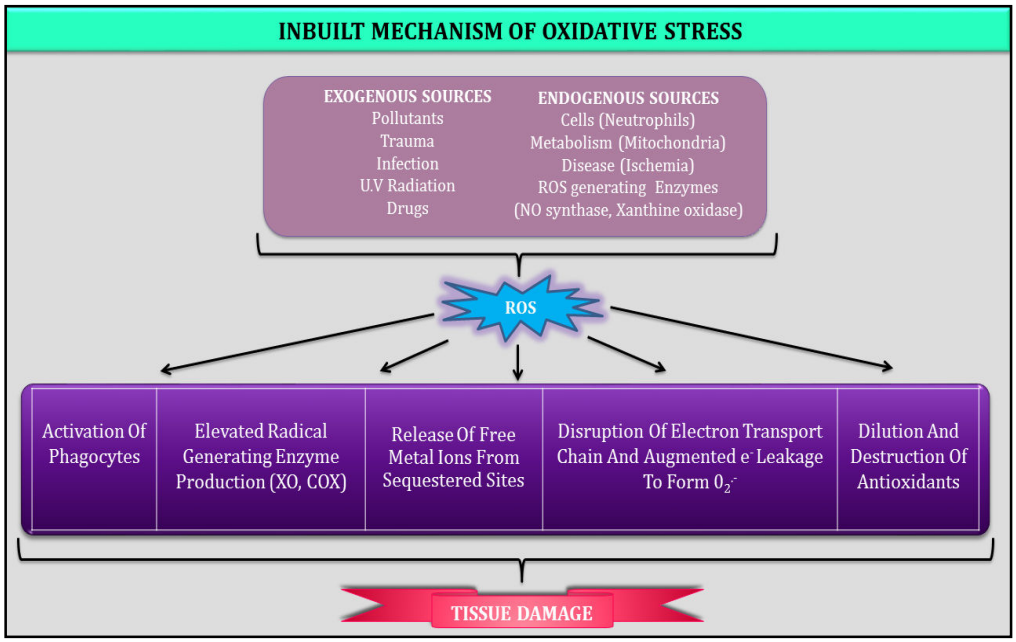


FIGURE IV-3 Inbuilt Mechanism of Oxidative stress
Image Reference: (Halliwell and Cross, 1994)

IV. B. b. i) Mechanism Of DNA Damage By Free Radicals:

Free radicals attacks the DNA directly either at the sugar-phosphate backbone or at the level of purine and pyrimidine bases. Indirect DNA damage by these radicals are mediated via intracellular divalent ca^{2+} ions that might cause structural alterations in DNA such as base pair mutations, rearrangement, unwanted insertions and deletions, sequence amplifications and by the creation of nicks (Rowe et al., 2008). It is estimated that in a given cell, 10^5 oxidative DNA lesions are formed each day (Powell et al., 2005).

IV. B. b. ii) Mechanism Of Protein Damage By Free Radicals:

Free radicals degrade proteins via denaturation and inactivation of proteins containing Sulphur containing amino acid, cysteine, and methionine. They majorly affects the enzymes (calcium ATPase, glucose-6-phosphate dehydrogenase, and glyceraldehyde-3-phosphate dehydrogenase) and membrane ion transporters. These free radicals absorb a proton from these proteins and thereby oxidase the sulphydral moiety ultimately resulting in the generation of newly formed reactive unpaired electrons (Cabiscol et al., 2000).

IV. B. b. iii) Mechanism Of Lipid Damage By Free Radicals:

Free radicals affect the lipids and cause the peroxidation of membrane associated fatty acids and cholesterol that alters the membrane characteristics and permeability finally leading to the extended lipid membrane damage (Betteridge, 2000). This extended chain of lipid peroxidation progressively leads to the formation of Malondialdehyde (MDA), which is now widely used as a marker for free radical mediated lipid damaging reactions (Andrade Júnior et al., 2005) (Figure IV-4).

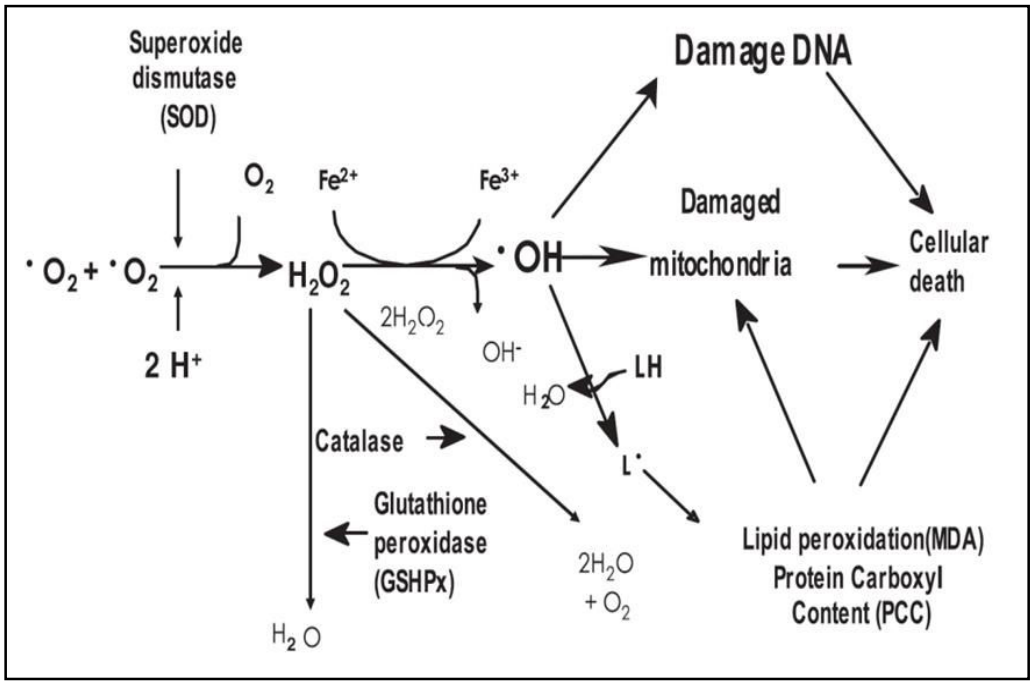


FIGURE IV-4 Mechanisms of Oxidative Cellular Damage: Free radicals are reduced into water with the cooperation of the three main antioxidant enzymes: SOD, Catalase, and GSHPx. The generation of hydroxyl radicals from hydroperoxide produces the development of oxidative cell injury: DNA damage; carboxylation of proteins; and lipid peroxidation, including lipids of mitochondrial membranes. By these pathways, oxidative damage leads to cellular death. **Image Reference:** (Morón and Castilla-Cortázar, 2012)

IV. B. c) Implication Of Oxidative Stress In Disease Progression:

The process of oxidative stress is now strongly believed to be the key regulatory process involved in various kinds of pathologies and disease progression such as Alzheimer's, Parkinson's disease, Huntington's disease, Multiple sclerosis, obesity, atherosclerosis, heart failure, myocardial infarction, cancer and infection in humans (Galli et al., 2005). For instance, patients affected with Alzheimer's disease had shown the evidence of various forms of ROS mediated injury (Outlined in Table IV-1), with lipid peroxidation markers of oxidative stress such as malondyaldehyde and 4-hydroxynonenal or 4-HNE (Portrayed in Figure IV-5) (Keller et al., 1997a) upregulated in brain and CSF fluids when compared to control patients (Lovell et al., 1995). In support to the evidences of human brain, prior to the appearance of amyloid plaques or neurofibrillary tangles, the markers of protein and lipid peroxidation had been found to be increased in the cortex and hippocampus of transgenic animal models of AD (Hensley et al., 1996). The impact of oxidative stress depends upon the dosage and the

concentration levels of the oxidative stress, as well as on the cell's ability to regain into its previous state and functionality. Oxidative stress instils cell injury via two mechanisms, apoptosis and necrosis. For instance, moderate oxidation can trigger apoptosis, and more intense stress response may cause necrosis, whereas severe oxidative stress induced by ROS or RNS can cause cell death by nonphysiological necrosis or by regulated apoptotic pathways (Guetens et al., 2002; Ryter et al., 2007).

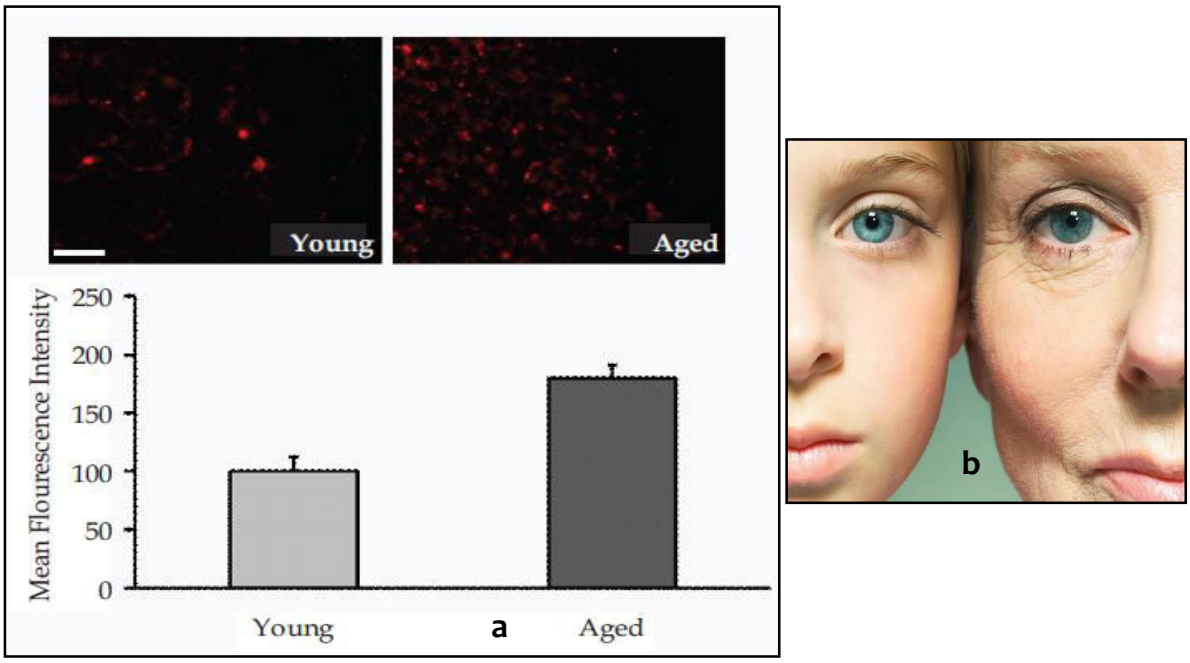


FIGURE IV-5 Relative Impact of Oxidative Stress between Young and Aged Brains: a) Increased oxidative stress production in the normal and aged brain; 3- or 18-months naïve old male mice were sacrificed and 40 µm brain sections were stained for 4-Hydroxynonenal (4-HNE), a marker of lipid peroxidation. Mean Fluorescence intensity assessed by ImageJ program showed that aged animals, overt any pathological condition, had an increased expression of 4-HNE in cortical layers II-III, compared to young mice. 3 sections/animal for 4 animals were analyzed in each condition. Data are represented as Means ± SEM. Scale bar, 100 µm. **b)** Conspicuous mark of oxidative stress between young and old people. **Image Reference:** (Barreto et al., 2011; Befit, 2015)

With respect to age (Ageing) all biological systems usually experiences some structural and functional modifications. In this regard, brain is not an exception. Brain has the well-defined potentiality to deploy its functions even at the late years of life. When the living beings are getting older, the pathways that have been used by the brain in olden days will no longer work. In this scenario brain exhibits appreciable plasticity to perform its functions well even at the late years by the establishing some new pathways as per the current demands of the living being (Stein et al., 2008).

Both oxidative stress and inflammation infuriates the process of ageing especially in neurodegenerative disorders like Alzheimer's and Parkinson's (Cole et al., 2005; Pratico,

2005) in which microglial activation and reactive astrogliosis were found to be cardinal steps involved in such pathology which heightens the process of aging. Apart from oxidative stress and inflammation, ageing is another such and paramount factor that contributes to neuronal loss and neurodegeneration. The mechanisms that are implicated in this aging process which drives to neurodegenerative state remains still elusive and undiscovered. The by-products formed as a result of oxidative damage especially in mitochondria were predicted to the key process of aging and as well in Alzheimer's disease, which is supported by reduced metabolic activity and reduced mitochondrial activity in the Alzheimer's and Parkinson's brain subjects. (Alzheimer's, Parkinson's pathogenesis) (Moreira et al., 2006). In Parkinson's patients, oxidative stress inside the cells were also found to be one of the major factors that damages the dopamine-producing cells in the substantia nigra that have been shown to operate by injuring the mitochondria (See Figure IV-6). These evidences clearly shows that oxidative stress is in fact an important pathological mechanism in neurodegeneration that begins early in the disease process (Pratico, 2002; Smith et al., 2002; Castellani et al., 2006a; Castellani et al., 2006b; Cortical biochemistry in MCI and Alzheimer disease: Lack of correlation with clinical diagnosis.Lau et al., 2007).

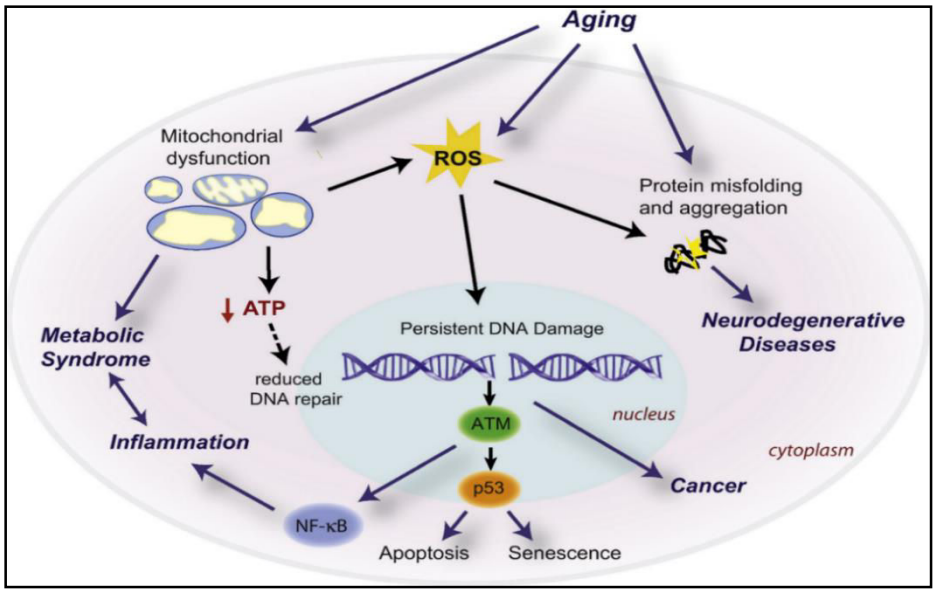


FIGURE IV-6 Age-Related Stress and Disease: Aging is associated with mitochondrial dysfunction, leading to reduced respiratory metabolism and increased generation of reactive oxygen species (ROS). Persistent DNA damage may arise from both increased oxidative damage and reduced efficiency of energy-intensive DNA repair, predisposing to apoptosis, senescence, and inflammation. Aging is also associated with increased protein misfolding and aggregation in the cytoplasm, nucleus, and endoplasmic reticulum. The various sites of age-related cellular damage and the physiological decline that ensues contribute to the pathogenesis of age-related diseases, including metabolic syndrome, inflammatory disorders, cancer, and neurodegenerative diseases. **Image Reference:** (Haigis and Yankner)

Table IV-1 Types of Reactive Oxygen Species			
Reactive Oxygen Species	**Symbol**	**Reactive Nitrogen Species**	**Symbol**
Superoxide	$O_2^{\cdot}$-	**Peroxy nitrate**	$OONO^-$
Hydroxyl	OH^-	**Peroxy nitrous acid**	ONOOH
Hydrogen peroxide	H_2O_2	**Nitric oxide**	$NO^{\cdot}$
Singlet oxygen	O_2^{-1}	**Nitrogen di oxide**	$NO^{\cdot}_2$
Hypochloric acid	HOCl	**Nitrous acid**	HNO_2
Lipid peroxyl	$LOO^{\cdot}$	**Nitryl chloride**	NO_2Cl

Table Reference: (Agarwal and Prabakaran, 2005)

IV. B. d) Potential ROS Generators:

Monoamine oxidase, mitochondrial electron transport chain (ETC) containing complex I (NADH dehydrogenase) and complex III (cytochrome bc1 complex) are the primary producers of ROS within mitochondrial system, where as in the cytosol NADPH oxidase (NOX) and xanthine oxidase (XO) are the large scale producers of ROS. These free radicals target and degrade the permeability transition pore (PTP) and mitochondrial DNA.

IV. B. d. i) NADPH Oxidase (NOX) - Authentic Superoxide Manufacturer: NADPH oxidase a multi-subunit enzyme complex also called NOX2 or phagocytic oxidase (PHOX) belongs to NOX gene family. It contains membrane-bound cytochrome b558 (p22PHOX and the enzymatic subunit, gp91PHOX), several cytosolic proteins (p47PHOX, p67PHOX, and p40PHOX), and the Rac G-protein (Babior et al., 1973; Babior, 2004). NOX is widely expressed in microglia (Colton and Gilbert, 1987), astrocytes and neurons (Noh and Koh, 2000; Abramov et al., 2005). When the cytosolic subunits are phosphorylated, NOX and Rac are activated further leading to the formation of NADPH oxidase - cytochrome b558 active complex. This activated enzyme complex thereby transfers the proton across the membrane that ultimately leads to the formation of superoxides (Babior, 2004).

IV. B. d. ii) Xanthine Oxidase (XO): Xanthine oxidase (XO) the key enzyme responsible for purine catabolism, have two convertible forms of Xanthine oxidoreductase (Xanthine oxidase or xanthine dehydrogenase). It is a molybdoflavoenzyme which is widely distributed and abundantly available in the mammalian milk (Harrison, 2002) and corneal epithelium of normal rabbit eye (Ardan et al., 2004). XO catalyzes the oxidation of substrates and can pass electrons to molecular oxygen to produce uric acid, superoxide, and hydrogen peroxide (Harrison, 2002).

IV. B. d. iii) Mitochondria: Mitochondria being the major responsible organelle that bears electron transport chain (ETC) system is a continuous generator of ROS (Figure IV-7). Respiratory complex-I; NADH dehydrogenase (ubiquinone), complex III; cytochrome bc_1 complex; and mitochondrially located flavoenzymes monoamine oxidase (MAO) are the potential producers of ROS in mitochondria (Gandhi and Abramov, 2012). Besides this, ROS in mitochondria are also produced by the other enzymes aconitase and α-ketoglutarate

dehydrogenase complex. The generation of these ROS solely depends on value of mitochondrial membrane potential (Tretter and Adam-Vizi, 2004; Andreyev et al., 2005). Mitochondria, which harbor the bulk of oxidative pathways, are tightly packed with various redox carriers and centers that can potentially leak single electrons to oxygen and convert it into superoxide anion, a progenitor ROS (Jensen, 1966; Loschen et al., 1971; Andreyev et al., 2005).

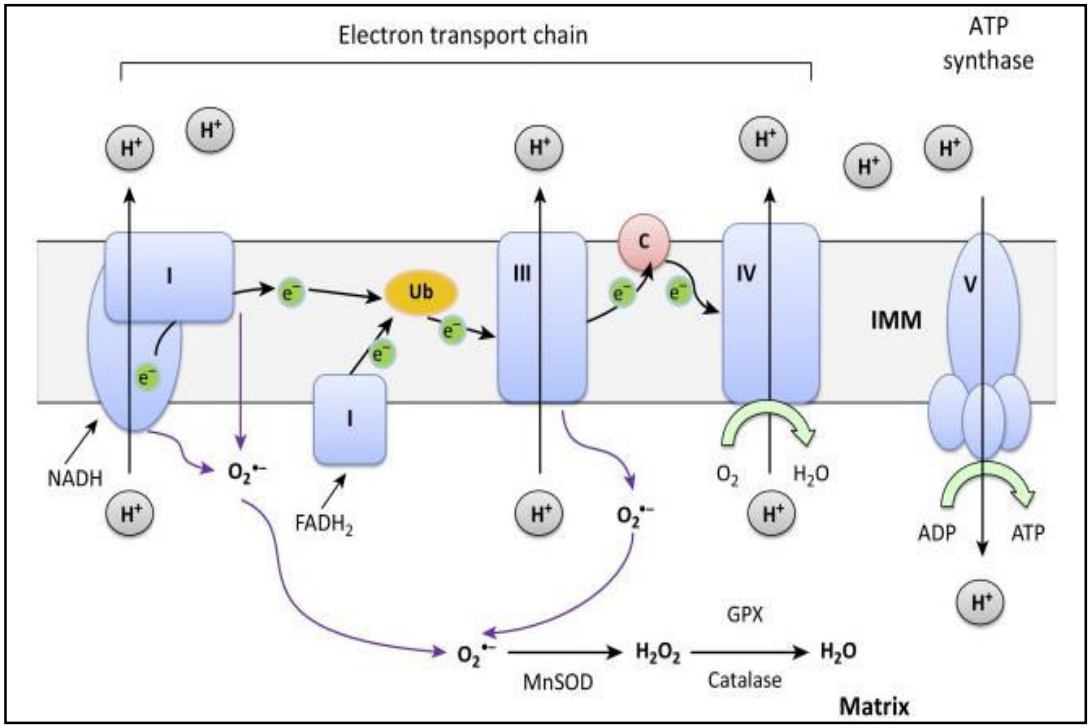


FIGURE IV-7 Mitochondrial Respiratory Chain: The electron transport chain receives electrons (e^-) from NADH and $FADH_2$ and mediates electron transfer from complex I to complex IV, via ubiquinone (Ub) and cytochrome c (C). At complex IV, electrons reduce molecular oxygen to form water. As the electrons are transported, a proton (H^+) gradient is created across the inner mitochondrial membrane (IMM). Complex V (ATP synthase) uses this gradient to convert ADP to ATP. As a byproduct of the respiratory chain, reactive oxygen species (ROS) are generated. Superoxide ($O2^{\bullet-}$) is formed at complexes I and III and is dismutated to hydrogen peroxide (H_2O_2) by matrix manganese superoxide dismutase (MnSOD). H_2O_2 can then be safely reduced to water by catalase or glutathione peroxidase (GPX). **Image Reference:** (Yu and Bennett, 2014)

IV. B. d. iv) Monoamine Oxidase (MAO): Monoamine oxidases are mitochondrially located outer membraned flavoenzymes. In humans there are two types of MAO: MAO-A, MAO-B. MAO-A is an enzyme that degrades amine neurotransmitters such as dopamine, norepinephrine, and serotonin, whereas MAO-B degrades dopamine neurotransmitter. MAO's are widely expressed in the CNS, in which MAO-A is expressed in neurons and both MAO A and B in glial cells (Gandhi and Abramov, 2012). MAO with the help of FAD breakdown monoamines into aldehydes and the FAD-$FADH_2$ cycle further generates hydrogen peroxide (Edmondson et al., 2009).

IV. B. e) Positive Face Of Free Radicals:

Oxidative stress up to certain extent (clearance of pathogens) will be beneficial as it will be used by the immune system, whereas uncontrolled and prolonged oxidative stress damages the cell membrane and further contributes to tissue loss. It is clearly evident from the reports of Gems and Partridge, in which they demonstrated that short-term oxidative stress may also be important parameter to prevent aging by inducing a process known as mitohormesis (Demonstrated in Figure IV-8) (Gems and Partridge, 2008). Low doses result in enhanced function, whereas higher doses result in dysfunction. In addition to this, low concentration of ROS is essential for normal physiological functions like gene expression, cellular growth and defense against infection.

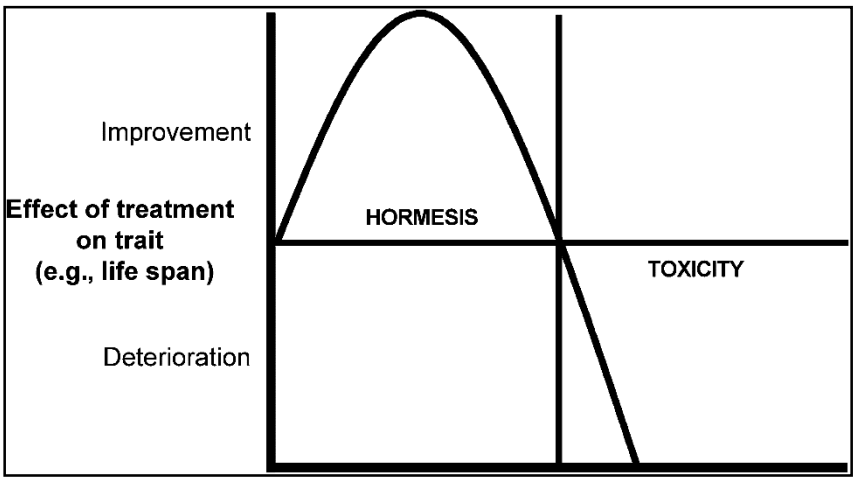


FIGURE IV-8 Dose-Response Curve of Oxidative Stress Treatment with a Hormetic Effect. Image Reference: (Gems and Partridge)

Macrophages and neutrophils are the first line of defense of the innate immune system which generate ROS in order to kill the bacteria and parasites and engulfs them by a process known as phagocytosis (Segal, 2005). Together with this, ROS being majorly credited for the malfunctioning of tissues by attacking the biomolecules of the cell, in a paradox these free radicals plays a key role in the physiological reactions such as catalytic oxidation of endogenous compounds and xenobiotic.

IV. B. f) Metallic Machinery:

Metal ions acting as catalysts are mainly responsible for generating toxicity, in which Cu (II) and Fe (III) plays a contributing role. This has been observed by Fenton in 1876 (Prousek, 2007) and the reaction can be summarized as:

$$Fe^{2+} + H_2O_2 \xrightarrow{\text{Fenton's Reaction}} Fe^{3+} + OH^{\bullet} + OH^{-}$$

Transition metals because of its ability to gain and lose electrons and being in possession of having one un paired electron in its outermost orbital shell renders them to acts as potential free radical generating oxidative stress in the host system. They plays a crucial role in most of the biological processes by activating or inhibiting enzymatic reactions or competing with metalloproteins or other elements for their binding sites (Huang et al., 1999).

IV. B. g) Antioxidant System:

One of the most crucial ways to counteract the destruction of free radicals and oxidative stress is by introducing the compounds that stops oxidation, known as Antioxidants. Antioxidants are large and complex molecules which donate electrons to free radicals to bond with its unpaired electron making them reactively stable. This effectively neutralizes the free radicals. Superoxide dismutase, catalase and glutathione peroxidase are the three vital enzymes that helps in the transformation of ROS into less reactive molecules (Gutteridge and Halliwell, 2010).

IV. B. g. i) Potential ROS Neutralizers - Superoxide Dismutase (SOD):

The composition of SOD exists in diversified isoforms with various metals acting as central metal ions with different amino acid constituency. SOD exists in three different forms in humans, they are cytosolic (Cu, Zn-SOD), mitochondrial (Mn-SOD) and extracellular SOD, whereas Fe-SOD exists in animals but not in humans. Of all the existing forms Mn-SOD have greater scavenging activity of $O^{\bullet -}$ ultimately converting it to hydrogen peroxide and oxygen (Halliwell, 2007).

Cu Zn-SOD and Mn-SOD catalyze the following reaction:

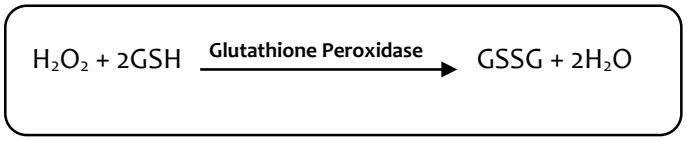
$$2\ O^{\bullet -} + 2\ H^{+} \xrightarrow{\text{Superoxide Dismutase}} H_2O_2 + O_2$$

The generated H_2O_2 is then removed mainly by peroxiredoxins, thioredoxin-dependent peroxidase enzymes (Halliwell, 2007) then catalase neutralizes the hydrogen peroxide (Miao and St Clair, 2009).

IV. B. g. ii) Glutathione Peroxidase (Gpx):

Glutathione is a tripeptide molecule with a free thiol group (-SH group - thioredoxins, cysteine and reduced glutathione) which consists of three amino acids joined together (glutamic acid, cysteine and glycine). It is highly abundant in the cytosol, nuclei and mitochondria (Valko et al., 2006). Thiols are essential for overall protein function (Halliwell et al., 1992; Cengiz et al., 2008). These are nucleophilic (donate electrons) and react avidly with free radicals. Injury causing reactive molecules such as radiation can be neutralized by these thiol groups by donating hydrogen (Navarro et al., 1997). It acts as intracellular antioxidant mediating various physiological reactions including cellular signaling and prevents the protein -SH groups from oxidizing and cross-linking. Defective GSH metabolism leads to hemolysis, neurological disorders and brain damage. Glutathione peroxidase is a selenoprotein which converts reduced form of glutathione (GSH) to oxidized form of glutathione (GSSG). The major biological role of GPx is to protect the host system from oxidative damage generated by

exogenous and endogenous ROS. There are four different isoforms of GPx selenium containing enzymes in mammals (GPx1, GPx2, GPx3, and GPx4). It oxidizes glutathione and reduces lipidic and non-lipidic hydroperoxides to alcohols and hydrogen peroxides (H_2O_2) to water (Michiels et al., 1994).



IV. B. g. iii) Catalase (CAT):

Catalase is a ferriheme-containing enzyme that converts hydrogen peroxide to water (Droge, 2002).

Catalase is an important enzyme which is responsible in protecting the cell from oxidative damage by reactive oxygen species (ROS). It is localized in peroxisomes and may also be found in cytoplasm and mitochondria. Catalase has one of the highest turnover numbers of all enzymes. One catalase molecule can convert approximately 5 million molecules (Glenda Chidrawi, 2008) of hydrogen peroxide to water and oxygen each second (Goodsell, 2004). It has a minor role at low levels of hydrogen peroxide generation but becomes more important at higher levels of hydrogen peroxide production.

IV. B. g. iv) Anti-Oxidative Mechanism:

Exceeded levels of oxygen in the host system will be converted into superoxides. Under these conditions of stress, endogenous enzymes of cells such as Superoxide Dismuatase (SOD) converts superoxide to hydrogen peroxide, which is then detoxified to water either by catalase in the lysosomes or by glutathione peroxidase in the mitochondrial system (Semchyshyn, 2012).

IV. B. g. v) List Of Anti-Oxidative Agents:

Antioxidants shows its potential ROS neutralizing effects via diversified mechanisms (Halliwell, 2007) (Depicted in Table IV-2).

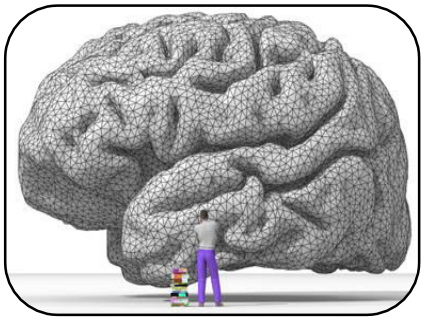
TABLE IV-2 Potential Electron Donating Anti-Oxidative Agents	
Characteristic of Anti-oxidant	**Anti-Oxidant**
Enzymatic Antioxidants	Superoxide Dismutase (SOD), Catalase (CAT), Glutathione Peroxidase (GPx), Glutathione Reductase (GSR)
Non-Enzymatic Antioxidants	Vitamin A, B1, B2, B6, B12, C, E
Antioxidative Proteins	Hemoglobin, Ceruloplasmine, Transferrin, Albumin, Lactoferrin, Protein Sulfhydryl (SH) groups (Thiols)
Trace Elements	Copper, Zinc, Selenium, Bilirubin, Glucose, Ubiquinone Coenzymes Q10 (CoQ10)
Co-factors	Folic Acid, Uric Acid, Albumin, Glutathione, Lipoic Acid and Carotenoids and Flavonoids

Table Reference: (Gutteridge and Halliwell, 2010)

Recently an association between adipose tissue (adipocytokines) and CNS have been equated that are coupled with the cellular process of inflammation and oxidative stress responses suggesting a probable role for neurodegeneration. Many chronic neurodegenerative diseases such as Amyotrophic lateral sclerosis, Alzheimer's and Parkinson's diseases have been associated with inflammation and oxidative stress in the CNS in which microglia and astrocytes were found to be the key players involved in the early and late onset of neuroinflammatory related neurodegenerative process in the CNS. These glial cells triggers the release of multitude of cytokines that plays a key role both in health and diseased conditions of the CNS.

Major part of our research work was focused on these two glial cells (microglia and astrocytes) which were found to be the potential sources of inflammatory and oxidative stress factors in the CNS (Hallmark features of neurodegeneration).

V. BRAIN - THE COMMANDING AND CONTROLLING ORGAN



"Brain is an extremely complex and sophisticated organ which is central for all the information processing that defines and dictates the complete actions of a living being right from birth till death. In fact your brain tells who you are."

V. A. Architecture Of The Brain:

The brain controls both voluntary, involuntary and the entire functions of the body. It receives information through five senses: sight, sense, touch, smell, hearing and taste and interprets the information from the external world. Brain has a defined endocrine system which secretes an array of chemical messengers known as hormones, in which blood is the major transporting medium that acts as a vehicle to transport the hormonal chemical message from one part of the body to another part of the body. The brain is well covered by the hardest skull known as cranium. Inside the skull, brain is covered by three protective layers known as cranial meninges which comprises of outermost dura, middle layered arachnoid and innermost piamatter. From the brain all the nerves, arteries and veins comes out through a slit like opening called Foramen Magnum and protrude throughout the body (from the head to the tip of the toes) which can be compared to all the cables that will be coming out from the back of a computer's desk.

The brain can be divided into three major parts: forebrain, midbrain and hind brain which are developed from embryonic neural tube. Forebrain consists of the cerebrum, cerebral cortex and thalamus, hypothalamus, hippocampus (part of the limbic system), midbrain consists of the tectum and tegmentum, whereas the hindbrain is made of the cerebellum, pons and medulla. Cerebrum is the largest part of the brain which is divided into left and right hemispheres. It contains four lobes; frontal, parietal, occipital and temporal lobes. It operates the learning process, remembering power, reasoning, problem solving, touch, hearing, speech, vision, emotions, control and the fine control of movements. The cranial nerves responsible for perceiving the smell and vision originates in the cerebrum. Sea horse shaped structure present deep inside the temporal lobe is known as hippocampus (HIP). Hippocampus is a part of limbic system which executes essential operations of the brain such as learning, memory and converts short term memory to permanent memory. The hippocampus is also called Cornu Ammonis (CA) which is further differentiated into CA1, CA2 and CA3, CA4 fields. Dentate gyrus is another noteworthy structure innervated in the hippocampus that plays a vital role in the formation of new episodic memories. Dentate gyrus is one of the few regions of the brain where neurogenesis takes place. The surface of the cerebrum is called cortex (COR). It has a folded appearance and looks like a wrinkled blanket. It compared to the crests

and troughs of a wave. The crest is called gyri and the troughs are called sulci. It contains 40% of outer grey matter (neuronal cell bodies, myelinated and unmyelinated axons, dendrites, capillaries and glial cells - astrocytes, oligodendrocytes) and 60% of inner white matter (mostly glial cells and myelinated axons). A number of glial cells will be present in both the tissues that serves as the supporting cells of the CNS but the majority will be in white matter. Simply white matter constitutes of axons and glial cells, whereas grey matter consists of neurons. Cerebellum (CER), also known as the little brain is the smaller structure and is located under the cerebrum. It plays a key role in muscular movements, maintaining postures and balance (Figure V-1, Figure V-2).

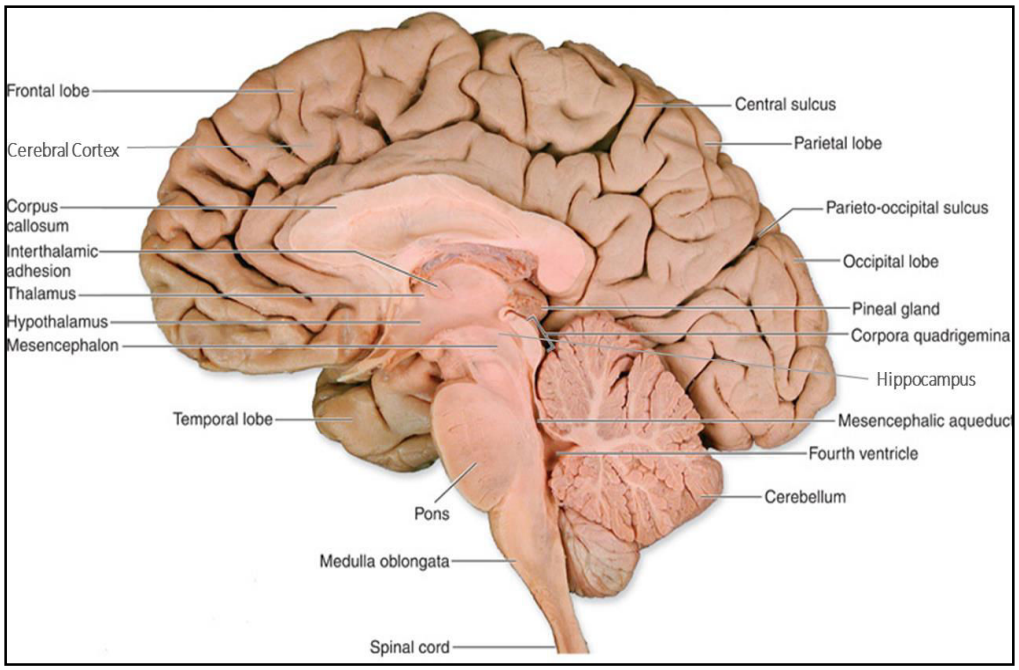


FIGURE V-1 Mid-Sagittal Section of Human Brain.
Image Reference: (Cummings, 2004)

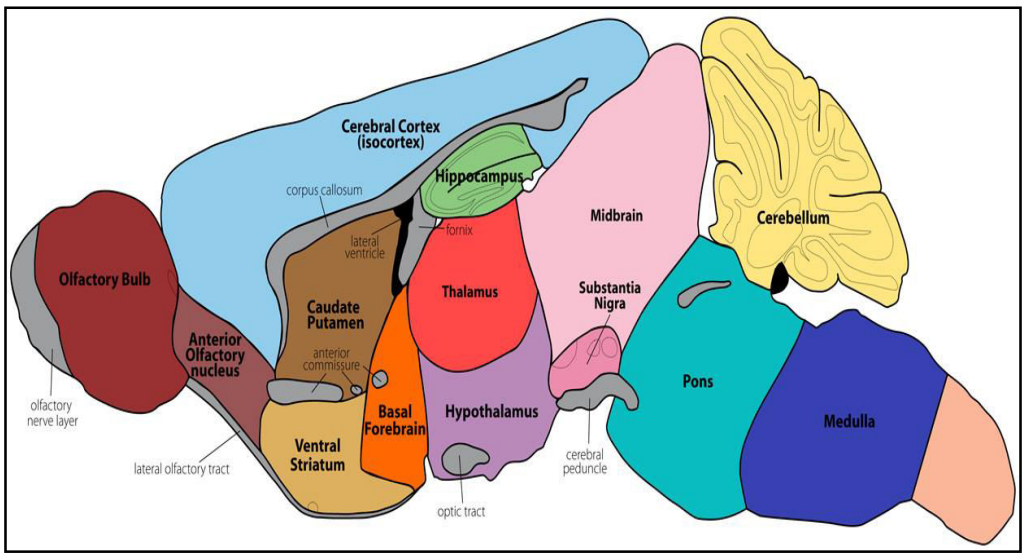


FIGURE V-2 Mid-Sagittal View of Mice Brain.
Image Reference: (Atlas, 2015)

All the cells that are present in the brain will fall under two categories: neuronal and non-neuronal cells (Glial cells). Neurons consists of a cell body, axon and dendrites. It conveys the message from one part of the neuron to another neuron via the release of chemical messengers called neurotransmitters which are stored in the bottom of neuron in a specialized sacs called synaptic knobs. The convey of this chemical messenger exchange occurs at a specified hotspots known as synapse. Dendrites are the projections from the neurons which acts as intracellular messengers that receives the neurotransmitter signal from one neuron and delivers the message to another neuron. As a consequence of this, the delivered neurotransmitter will bind into the specified receptors of another neuron and this is how the neurons will be stimulated and getting activated. Glial cells are the supporting cells of the CNS which provides structural, immunological and nourishment support to the neurons. These are 10 to 50 times greater in number than neurons in density that depicts the importance of these cells in brain. Microglia, astrocytes, oligodendrocytes and ependymal cells are the predominant neuroglial cells exists in the brain. Implication of these glial cells in equilibrating the physiological functions (inflammation and oxidative stress) of the body especially in the CNS, and other essential activities are specifically detailed under microglial and astrocyte sections (Hickey, 1991; Wood and Bunge, 1991; Hirschberg and Schwartz, 1995; Larner et al., 1995; Compston et al., 1997; Harrison et al., 1999; Bailey et al., 2006; Hines, 2013; NINDS-NIH, 2014; Bruno Dubuc, 2015; Central, 2015).

V. B. Developmental Origin Of Brain Cells

V.B. a) Tracking Astrocytes & Microglia to its Origin

Embryonic stem cells (ESC) are self-renewing and pluripotent cells derived from inner cell mass of a blastocyst that are able to differentiate into all derivatives of all three primary germ layers - endoderm, ectoderm and mesoderm (Evans and Kaufman, 1981; Martin, 1981; Brook and Gardner, 1997). When stimulated under defined culture conditions, these embryonic stem cells can differentiate into neural cells including neurons, oligodendrocytes and astrocytes (Faris Q. B. Alenzi, 2011) (Figure V-3)

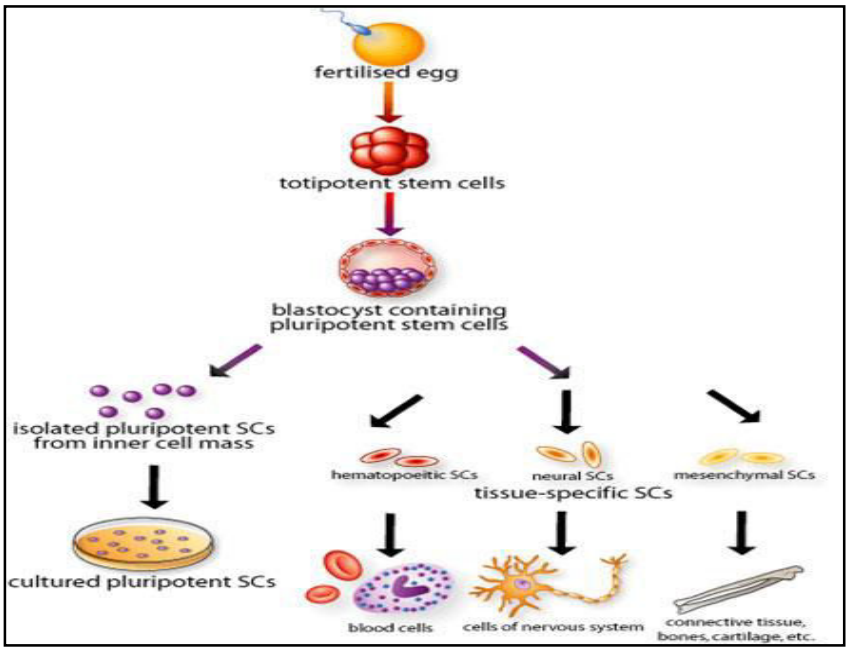


FIGURE V-3 Cellular Ontogeny *in vitro* and *in vivo*: Embryonic stem cells has the potential to develop into any cell type in the body. In respect to this, *in vivo*, the blastocyst containing inner cell mass of ESC flourishes into full fledged hematopietic stem cells that gives rise to blood cells, neuronal stem cells that develops into cells of central nervous system and mesenchymal stem cell that gives birth to adipocyte lineage, bone, cartilage and connective tissues in the body. Whereas *in vitro*, isolated innercell mass from the embryonic blastocyst, with the help of specific differentiators and growth factors (GDNF, CNTF, FGF and EGF) can switch the ESC to take a turn and assists in the development of a full fledged glial phenotype (Microglia and astocytes). **Image Reference:** (Chaudry, 2004)

In this regard, we are curious in understanding the mechanisms underlying the differentiation of microglia and astrocyte lineage that will enable us to understand the neurodegenerative complications.

Recently, it was found that implication of astrocytes and microglia in the brain are very important to determine the optimal functioning and communication of neurons. External or internal factors disturbing the state of these glial cells in the brain may lead to dysfunctioning of microglia and astrocytes (Microglial activation and reactive astrogliosis) that eventually in the course of time engenders neurodegeneration in the brain (Figure V-4).

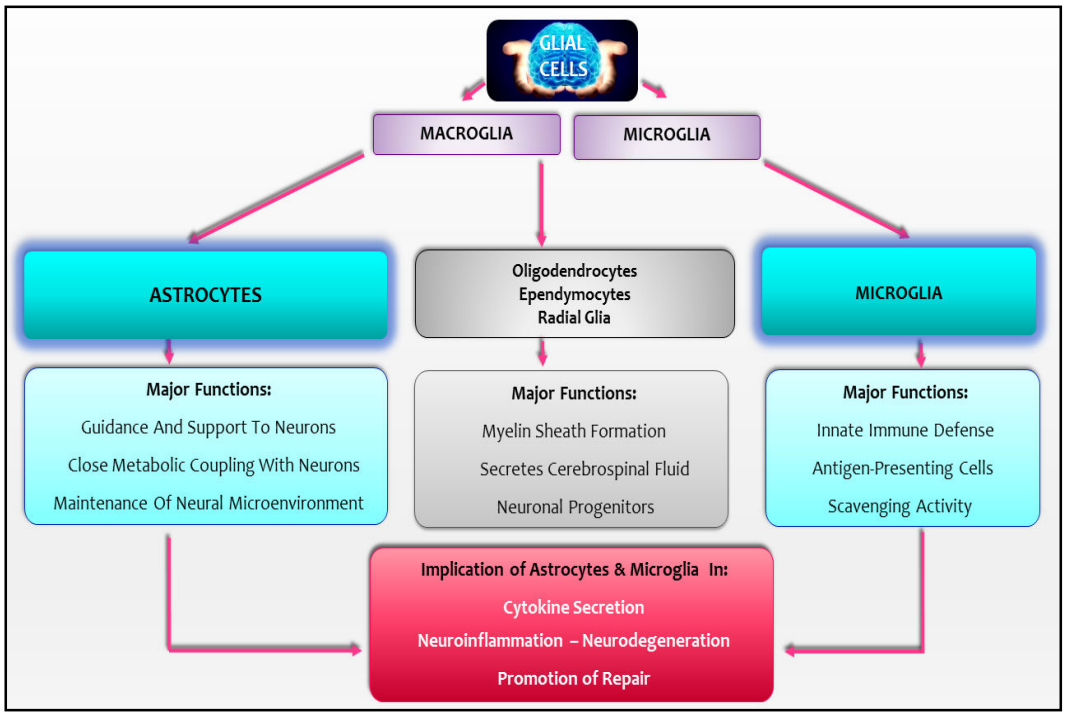


FIGURE V-4 Glial Cells And It's Unmatched Functions

V.B. a. i) Microglia On The Brain Floor:

Microglial cells are scavenger cells of the central nervous system, derived from hematopoietic stem cells (mesodermal in origin), and can be found in all the regions of the brain and spinal cord. Microglia continuously monitors the CNS for the presence of plaques, and infectious agents. In fact, these are the professional macrophages of the brain that performs phagocytic activity by engulfing the foreign infectious agents. They are mobile within the brain and multiply

when the brain is damaged. Microglia activation has been implicated in many neurodegenerative pathologies, such as Alzheimer and Parkinson disease and multiple sclerosis (Gonzalez-Scarano and Baltuch, 1999). During the pathogenesis of various neurodegenerative diseases, the number of microglial cells increases from approximately 2-3% to as high as 12-15% of total brain cells. Microglial activation is always associated with neuronal inflammation and eventually neuronal apoptosis (Emerit et al., 2004). Microglia predominantly express LPA1 and LPA3 (Moller et al., 2001; Tham et al., 2003). Various cellular activities of LPA signaling have been identified, which include enhanced chemokinesis, cell proliferation, membrane hyperpolarization, membrane ruffling, and growth factor upregulation (Schilling et al., 2002; Tham et al., 2003; Schilling et al., 2004; Fujita et al., 2008). It also stimulates the migration of melanoma cells via pertussis toxin-sensitive G protein (Murata et al., 1994). LPA3 is upregulated in LPS-stimulated microglia (Goetzl et al., 1999), suggesting a role for LPA signaling in activated microglia during neuroinflammation.

V.B. a. ii) Astrocytes On The Brain Floor:

Astrocytes have first be considered just as glue and as a provider of physical support to neurons. This simplistic view is now obsolete with a variety of functions attributed to astrocytes. It has been demonstrated that astrocytes have a greater impact in CNS development, homeostasis and CNS vulnerability and pathology (Aschner and Kimelberg, 1991). The complex biology of astrocytes and the reciprocal communicating networks between astrocytes, neurons and other cell types have made these cells the focus for studying the neurodegenerative diseases.

Astrocytes displays a manifold of immune and other assorted functions. In which these star shaped cells plays a critical role in the brain antioxidant defense, it forms a close metabolic coupling with neurons, modulates the neuronal excitability, regulation of K^+ buffering, clearance of glutamate. Major astrocyte functions can be grouped into three categories - guidance and support of neuronal migration during development, maintenance of the neural microenvironment, and modulation of immune reactions by serving as antigen-presenting cells (Montgomery, 1994).

Interestingly, Vaccarino FM et al., reported that, astroglial cells can be the progenitors for the generation of neurons and oligodendrocytes that migrate to the cerebral cortex, replacing the cells that are lost in young mice. These findings were demonstrated in lineage studies first based on retroviruses in the embryonic CNS and then by genetic fate mapping in both the prenatal and postnatal CNS. In response to the perinatal injury, the density of astroglial cells will be highly saturated at the neurogenic niches there by increases the expression of intermediate filaments, the GFAP expression (Vaccarino et al., 2007).

Both astrocytes and microglia express wide range of germline encoded pattern recognition receptors such as toll-like receptors, scavenger receptors, mannose receptor, nucleotide-binding oligomerization domains, double-stranded RNA dependent protein kinase and components of the complement system. They play an important role in neurodevelopmental and neurodegenerative processes. Astrocytes express all LPA receptors (Keller et al., 1997b)

and display a broad spectrum of LPA-induced responses in culture (Rao et al., 2003; Sorensen et al., 2003; Shano et al., 2008). When a stimulus binds these receptors, astrocytes and microglia are activated and secretes a wide variety of soluble mediators such as TNF-α, IL-6, IL-10, CCL2, CXCL2 and others, that will impact on both innate and adaptive immunity, provokes a neuroinflammation that may eventually leads to neurodegeneration (Liu et al.).

V.B. b) Role Of Calcium In Gliogenesis:

Calcium signalling plays a crucial role in the differentiation of ESC into the cells of the CNS (Berridge et al., 2000; Leclerc et al., 2012). So considering the implication of calcium signalling and associated factors that drives the ESC towards the gliogenic switch could be interesting to understand the process of gliogenesis.

Calcium signaling is a ubiquitous intracellular signalling system responsible for controlling numerous cellular processes starting from fertilization through differentiation to organogenesis. In the nervous system, calcium signals plays an important role in maintaining the neural circuits, long term memory and as well majorly involved in the neural induction and differentiation of neural progenitors into neurons and the neuroglial cells (earliest steps of neural development) (Berridge et al., 2000). So the calcium levels should be maintained in substantial proportions to control diversified functions of the CNS such as neuronal proliferation and development. Disrupting the composure of this optimal calcium levels results in abnormal neuronal death via necrosis and apoptosis.

Besides calcium signaling, several signaling systems are also known to play roles in the maintenance and differentiation of neural stem cells. For instance, Bone morphogenic proteins (BMPs) promote neuronal differentiation of cortical ventricular zone precursors. Wnt signaling has been implicated in the expansion of neural precursor cells in the embryo and regulates cell growth during the development of CNS. Ectopically expressed Wnt-1, Wnt-3a, and a constitutive active form of b-catenin, a component of the Wnt signaling pathway, cause expansion of neural precursor cells in the developing brain and spinal cord (Dickinson et al., 1994; Chenn and Walsh, 2002; Megason and McMahon, 2002; Muroyama et al., 2004). Overexpression of Wnt-1 and Wnt-3a, as well as their signaling component, β-catenin, causes an increase in the number of cells undergoing mitosis in the ventricular zone of the spinal cord. This mitogenic effect of Wnt signaling is partly mediated by transcriptional activation of cyclin D1 and cyclin D2. In addition, Gene Disruption Studies indicate that Wnt-1 and Wnt-3a are required for the formation of several regions of the CNS, including the midbrain, diencephalon, hippocampus, and dorsal hindbrain (McMahon et al., 1992; Ikeya et al., 1997; Lee et al., 2000). Yuko Muroyama et al., showed that Wnt-3a proteins enhanced differentiation of neural stem cells into neurons and astroglias at the expense of self-renewal of neural stem cells at least in *in vitro* condition (Muroyama et al., 2004). Thus, Wnt signaling may promote differentiation of neural stem cells *in vivo* at the point when these cells must choose their fates between self-renewing and differentiation. FGF2 and EGF are required for neurosphere (clusters of free floating neural stem cells) formation (McMahon et al., 1992; Parr et al., 1993; Ikeya et al., 1997; Lee et al., 2000). The components of Notch signaling are also

needed for the maintenance of neural stem cells *in vitro* (Ohtsuka et al., 2001; Hitoshi et al., 2002).

V.B. c) Differentiation Program:

Neurons and the glial cells which are typically found in the mature vertebrate CNS conventionally originate from a multipotent embryonic stem cell. Prior to the production of these neuronal and glial cells, ESC produces the neuronal precursors cells at the first which sequentially proceeds towards the engendering of neurons and glial cells in the ventricular zone of fetal brain and spinal cord (Edlund and Jessell, 1999). Canonical signaling factors like Sonic hedgehog (SHH), Fibroblast growth factor (FGFs), WNTs and bone morphogenetic proteins (BMPs), provide positional information to developing macroglial cells through morphogen gradients along the dorsal-ventral, anterior-posterior and medial-lateral axes. This process is similar to neuronal cell specification and its sequential genesis to neurons. Change in the composition of receptor on the cell surface and its responsiveness to growth factors such as Epidermal and Fibroblast growth factor determines the shift more towards astroglial differentiation. Ciliary neurotrophic factor, Bone Morphogenetic Proteins, and Epidermal Growth Factor drives the glial precursors to take a turn more towards the astroglial direction (Muroyama et al., 2005).

It has also been reported in the works of Wiese S et.al. that, however, the early astrocytes influence their environment not only by releasing and responding to diverse soluble factors but also express a wide range of extracellular matrix (ECM) molecules, in particular proteoglycans of the lectican family and tenascins. Lately these ECM molecules have been shown to participate in glial development. In this regard, especially the matrix protein Tenascin C (Tnc) proved to be an important regulator of astrocyte precursor cell proliferation and migration during spinal cord development (Wiese et al.). Before the process of terminal differentiation and after the specification of astrocyte has occurred in the developing CNS, it is believed that astrocyte precursors migrate to their final positions within the nervous system (As seen in Figure V-5) (Hochstim et al., 2008).

V.B. d) Key Aspects Of Glial Cells Differentiation:

1. Neural induction during gastrulation is the intial step towards neural stemcell formation.
2. Formation of neural tube and its expansion into neuroepithelial stem cells and glial cells
3. Formation of mitotically active precursors at ventricular zone.
4. Terminal differentiation of progenitors into mature neuronal, oligodendrocyte and astrocyte phenotype.

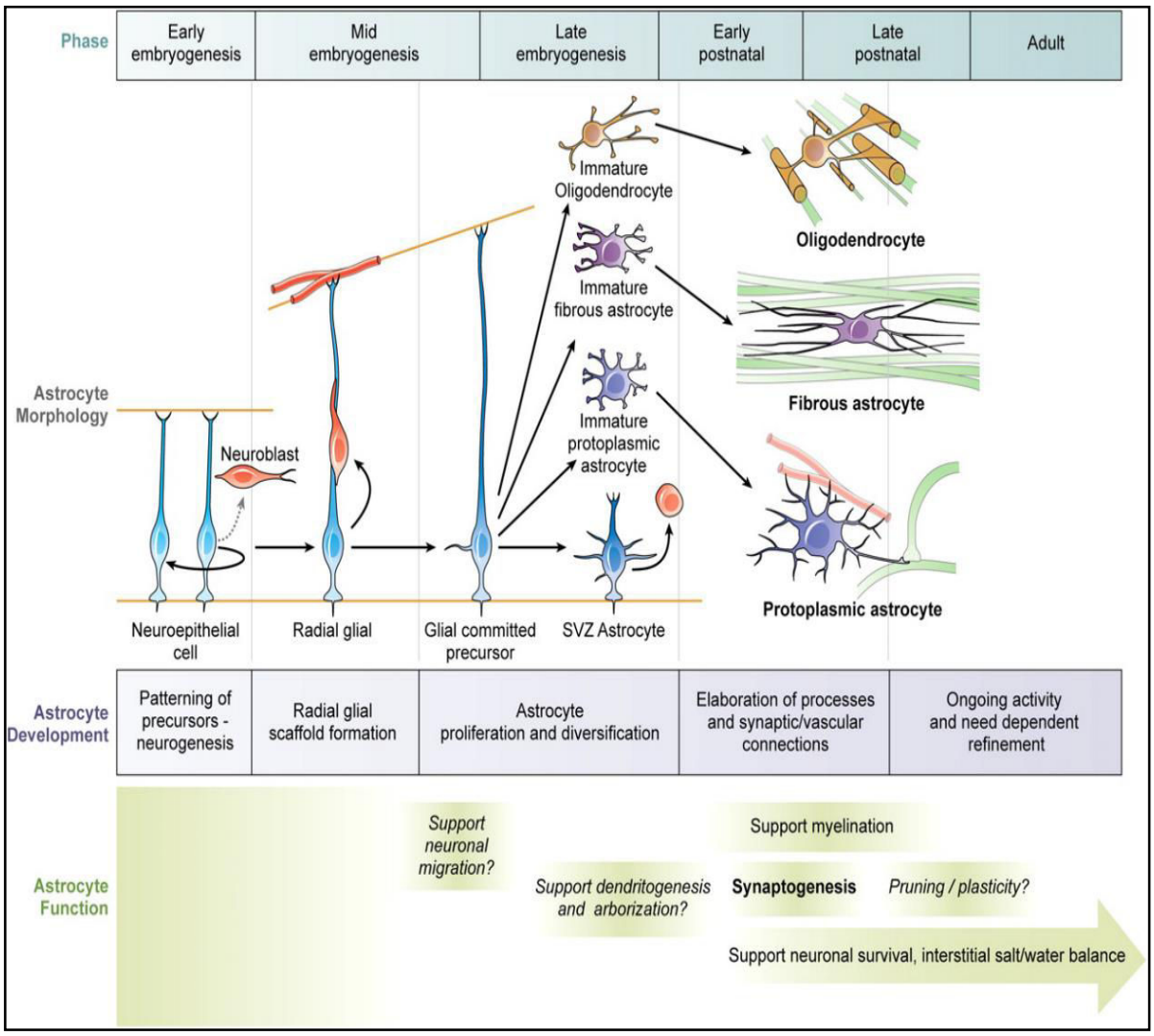
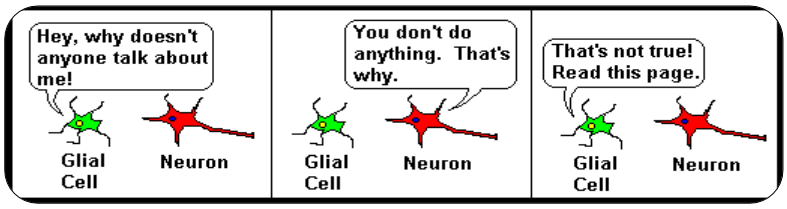


FIGURE V-5 Astrocyte Morphology And Function Changes Across Developmental Time: Neuroepithelial cells give rise to radial glia, which generate first neurons, and then become glial-committed, giving rise to precursors that proliferate and diversify into fibrous and protoplasmic astrocytes, which then go through a protracted stage of postnatal maturation. Astrocyte precursors at these different stages of maturation serve well-established stage-specific roles in assisting myelination and synaptogenesis and may also influence other functions, such as neuronal migration, pruning, and so forth. Well-established adult roles for astrocytes, including supporting neuronal survival and homeostasis, likely develop in parallel.

Image Reference: (Molofsky et al., 2012)

V. C. Neuroglia On The Brain Floor

Candidate Cells: Microglia And Astrocytes



Glial cells: The CNS is composed of interweaved mesh of neuronal and non-neuronal cells (glial cells). Glial cells can be typified into two categories macroglia (astrocytes, oligodendrocytes), and microglia each of them performing defined functions. The principal functions of these cells falls under 4 major categories: **a)** they surround the neurons and assist to withhold them in proper place, **b)** nurture the neurons with nutrients and oxygen supplements for their daily metabolic activities, **c)** provide insulation from one neuron to another in order to protect from external shocks (Shock Absorbers), **d)** act as scavengers to eliminate the pathogenic microbes and cell debris (Montgomery, 1994; Dong and Benveniste, 2001; Maragakis and Rothstein, 2006; Belanger and Magistretti, 2009; Jain et al., 2010; Kimelberg and Nedergaard, 2010; Sofroniew and Vinters, 2010; Gandhi and Abramov, 2012; Ricardo Cabezas, 2013; Allen, 2014; George E. Barreto, December, 2011) (Figure V-6).

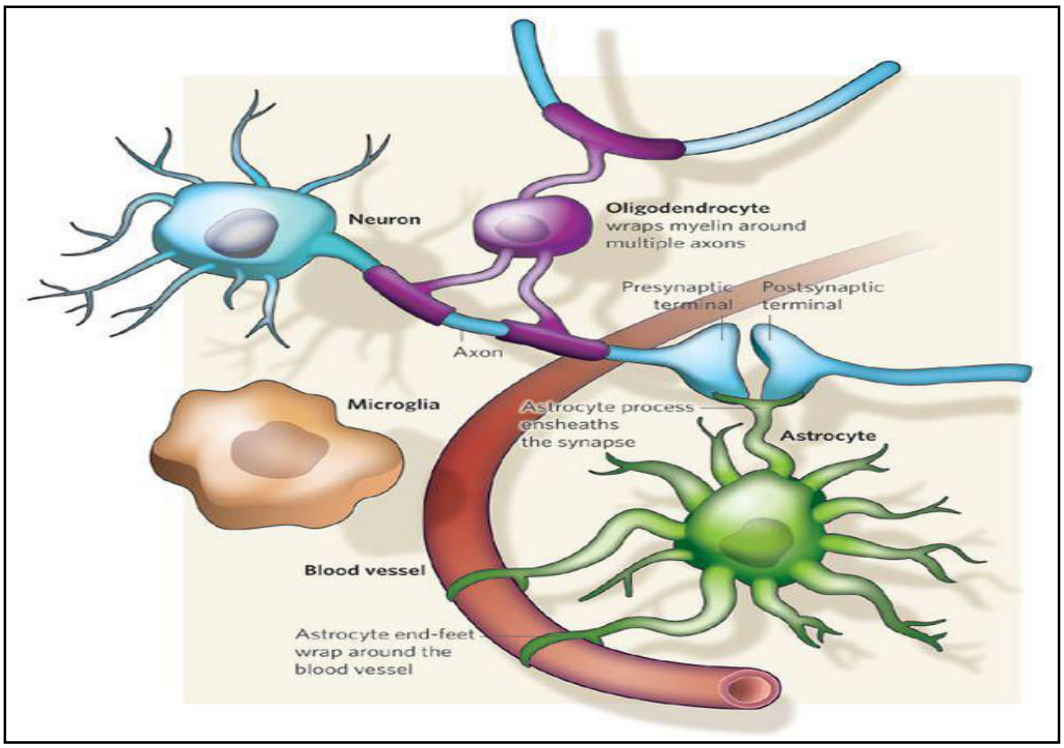


FIGURE V-6 Glia-Neuron Interactions
Image Reference: (Allen and Barres, 2009)

V. D. MICROGLIA or HORTEGA CELLS – The Gatekeepers Of Central Nervous System

Pio del Rio-Hortega a Spanish neuroanatomist was the first to introduce the concept of microglia and is considered as the "father of microglial biology." Besides the discovery of microglia, he was the first to demonstrate that microglia are of mesodermal in origin whereas neurons, astrocytes and oligodendrocytes are of neuroectodermal lineage (Rio-Hortega, 1932a).

TABLE V-1 Salient Features Of Pio Del Rio-Hortega Postulations During 1927

- Microglia are the invading cells that enters the brain during early embryonic development.
- They have amoeboid morphology and are of mesodermal origin.
- They use vessels/vasculature and white matter tracts as guiding structures for migration and enter all brain regions.
- They transform into a branched, ramified morphological phenotype in the more mature brain (known today as the resting microglia).
- In the mature brain, they are found almost evenly dispersed throughout the central nervous system and display little variation.
- Each cell seems to occupy a defined territory.
- After a pathological event, these cells undergo a transformation.
- Transformed cells acquire amoeboid morphology similar to the one observed early in development.
- These cells have the capacity to migrate, proliferate and phagocytose.

Table Reference: (Rio-Hortega, 1919; 1924; 1924, 1925; 1927; 1920.; 1921)

V. D. a) Microglial Backdrop:

Microglia cells are hematopoietic stem cell derived products that differentiate in the bone marrow. During hematopoiesis, some of these stem cells differentiate into monocytes and travel from the bone marrow to the brain, where they settle and further differentiate into microglia (Figure V-7). Microglial cells are the invading cells which penetrates and migrates very quickly into the brain parenchyma during the early periods of postnatal fetal development and are found in every location within the nervous system (Rio-Hortega, 1932a). Over 95% of all microglia are generated after birth and subsequently remains after the formation of the blood–brain barrier (BBB).

Microglia standout significantly from other types of brain macrophages such as meningeal, perivascular macrophages (Polfliet, 2001. ; Nguyen, 2002. ; Polfliet, 2002.) and perivascular cells or pericytes (Thomas, 1999; Williams et al., 2001) by their distinguished parenchymal location and by their defined functions. They are present throughout the CNS including brain and spinal cord. The density and concentration of ramified microglial cells bearing markers such as CD68 and major histocompatibility complex (MHC) class II antigen positive cells are

thickly populated in medulla and white matter than in grey matter (Mittelbronn et al., 2001). Microglia constitutes about 10-15% ranging from 100 to 200 billion cells which largely depends upon health, infected and diseased conditions (Streit, 1995). The proliferation of these cells happens in response to infection, injury and also due to the presence of endogenously produced toxic proteins (Gehrmann, 1996; Aloisi, 2001).

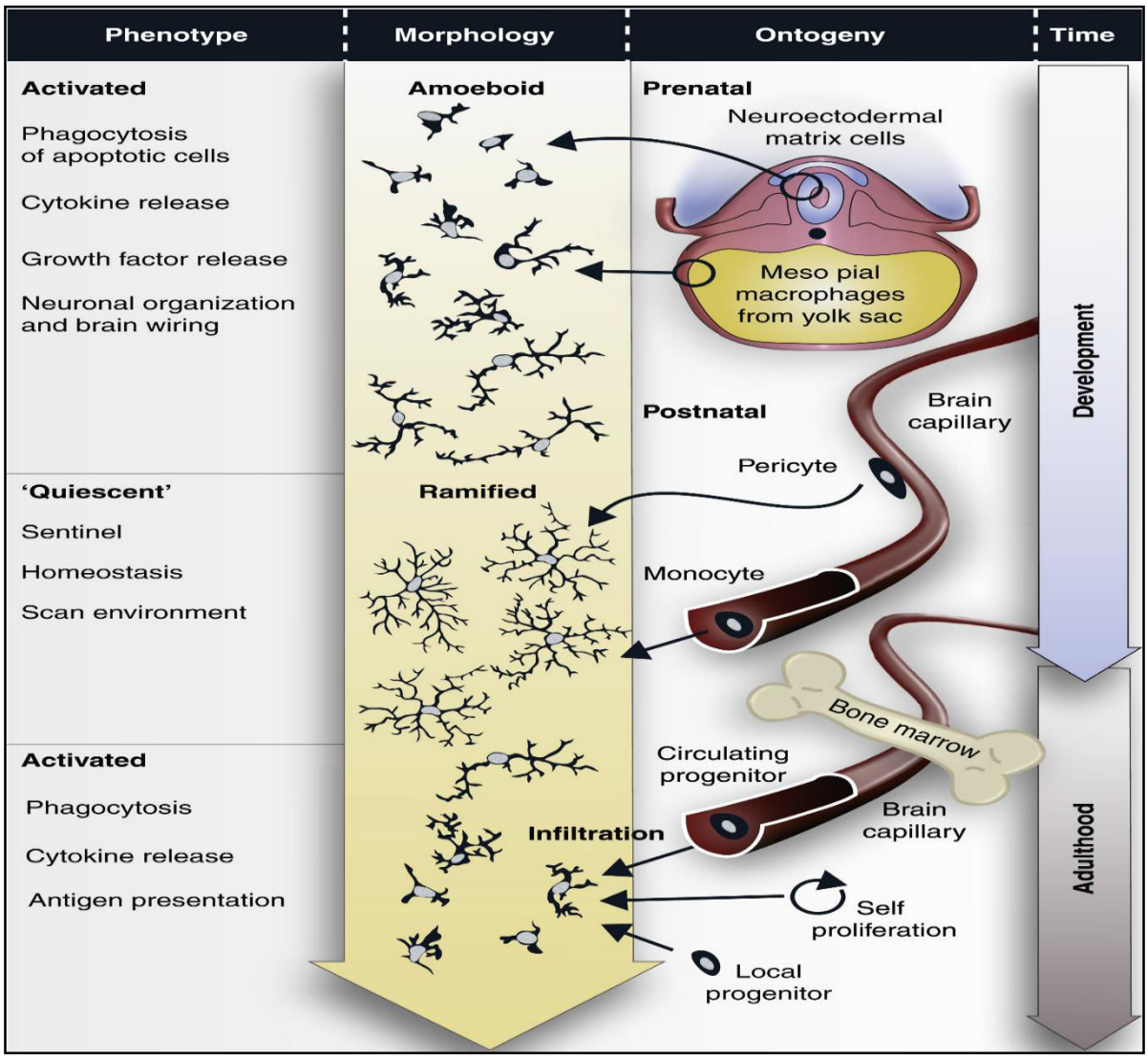


FIGURE V-7 Phenotypes and Morphologies of Microglia During Development And Adulthood And Under Normal And Inflammatory Conditions.
Image Reference: (Soulet and Rivest, 2008b)

V. D. b) Microglial Labelling: Isolectin IB4 conjugate:

Isolectin B4 has been used to label and identify microglial cells which will be elevated during microglial activation. Isolectin IB4 is a glycoprotein isolated from the seeds of the tropical African legume Griffonia simplicifolia, formerly known as Bandeiraea simplicifolia. Isolectin IB4 is cytotoxic to several normal and tumor cell types and has particularly strong affinity for brain microglial cells. Isolectin IB4 has been used effectively for tracing central and peripheral

neuronal pathways following local injections, as well as for labelling stimulated murine microglial macrophages (Murphy and Goldstein, 1977).

V. D. c) Microglial Plasticity:

Microglia are remarkably plastic (Gehrmann et al., 1995). In fact microglia are the first and foremost cells that will be reacting to both extrinsic and intrinsic insults in the CNS. Up on the detection of specific factors produced in response to infection, injury, neighboring glial and neuronal damage, microglia undergoes morphological transformations (resting/ramified, amoeboid, activated and granular gitter morphology) (Depicted in Figure V-8) that quickly reacts via the installation of genetic programs which neutralize and repair the damage occurred there by promoting the homeostasis of the CNS (Rio-Hortega, 1932b; Kreutzberg, 1995).

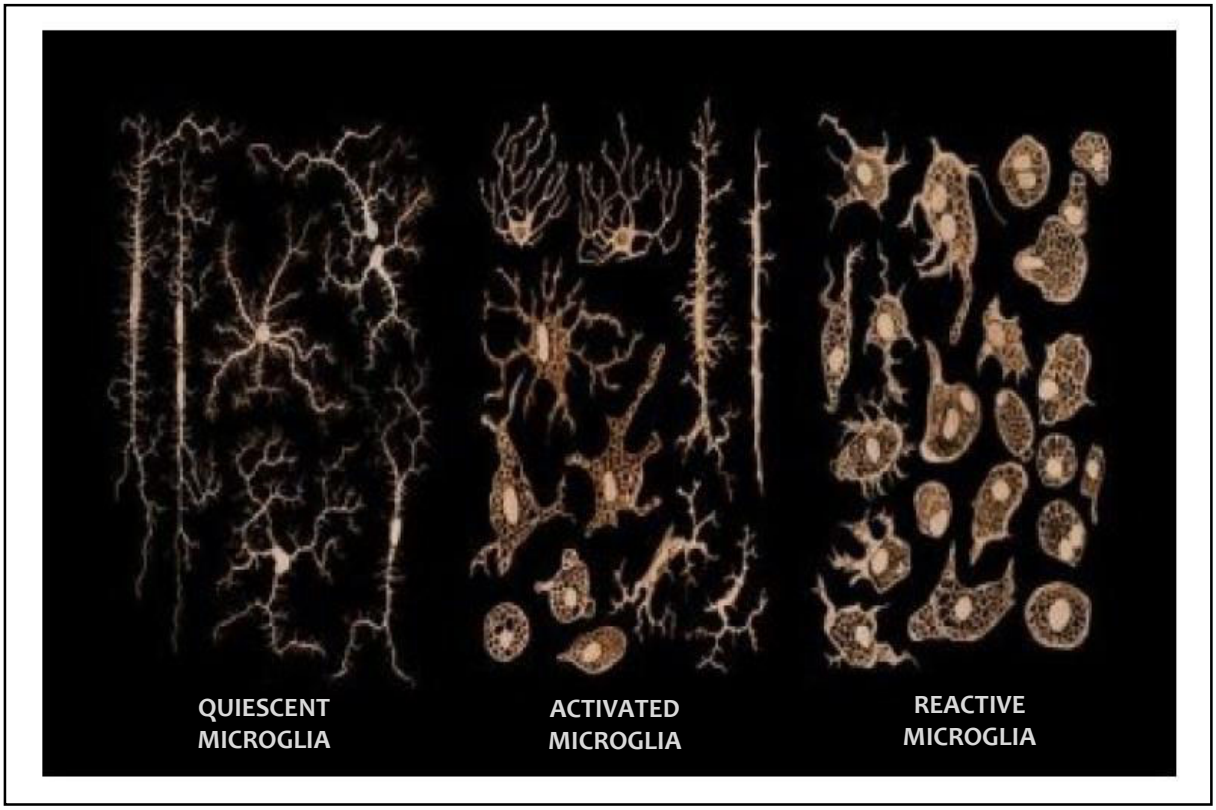


FIGURE V-8 Hortega's Demonstration of Heterogeneous Human Microglial Phenotypes. Image Reference: (Rio-Hortega, 1932b)

This kind of microglial transformations (morphology, phenotype and its functional characteristics) can be observed in most of the neuropathological conditions (Nayak et al., 2014) such as neurodegenerative diseases (Streit, 2006), traumatic brain injury (TBI) (Aloisi, 2001; Streit, 2006), ischemic stroke (IS) (Rock et al., 2004b), infection and as well in the tumor formations in the brain (Gehrmann, 1996).

V. D. d) Microglia Is Active Even At Resting State:

Microglia constantly changes its morphological structures and functional adaptivity to the ever changing surroundings. Rivest et.al reported that *in vivo* two-photon imaging of mouse

neocortex microglial cells are actually highly active even in their resting state without any immune stimulus (Soulet and Rivest, 2008b). During resting and as well in activated phase, microglia will be constantly scavenging the CNS microenvironment for damaged neurons, plaques and infectious agents with their extremely motile processes and ramifications (Soulet and Rivest, 2008a). Suppressing the activity of these activated glial cells leads to compromised natural immune status that leads in turn to the accumulation of toxic chemicals. These cells may then produce inflammatory factors which may be detrimental to the neighboring cells. So the maintenance of these glial cells in a proper steady state is the key to counteract against various neuropathologies. This presents a clear view that microglial cells are never under an inactive state but dynamically patrols the brain environment for the clearance of toxic molecules to keep the brain in healthy and viable state.

V. D. e) Microglial Receptors - Switch Mode Receptor Mediated Signaling:

Cell surface makers pertaining to immune regulation such as MHC class-II molecules are constitutively expressed on ramified microglia in the normal adult brain. Besides the potentiality of the ramified resting microglial cells to transform into reactive migratory microglia during CNS insults, these cells also have the ability to rapidly upregulate a large number of receptor types. Activation of these receptors leads to the secretion of various secretory products which form the basis during the defense against pathogen invasion in the infected brain. A certain population of microglial have similar properties with dendritic cells and are known as dendritic cell-like microglia. These specialized cells appear during infectious and inflammatory conditions and act as antigen presenting cells (APC).They present the antigens to Th1 lymphocytes and $CD4^+$, $CD8^+$ lymphocytes that perform a key role in chronic neuroinflammation and acquired immune response respectively (Fischer and Reichmann, 2001; Nguyen et al., 2002; O'Keefe et al., 2002; Zehntner et al., 2003).

The secretory products of microglia demonstrate both neurodefensive (scavenging) and neurodestructive processes (immunologic and inflammatory) via autocrine, paracrine actions. Microglia exerts its destructive actions via the induction of pro-apoptotic death signals (BAD, Bax, cytochrome C, Smac/DIABLO), directly by the release of toxic mediators such as pro-inflammatory cytokines (TNF-α, IL-1β) and pro-oxidative free radicals (iNOS, COX) or indirectly by attracting activated T cells, monocytes, and neutrophils into the CNS, whereas microglia performs defensive actions via anti-apoptotic (Bcl-2), and by the action of anti-inflammatory (IL-4, IL-10) and anti-oxidative enzymes (SOD, CAT) (Gottschall et al., 1995; Gottschall and Deb, 1996; Anthony et al., 1997; Yoshiyama et al., 1998; Berman et al., 1999; Cross and Woodroofe, 1999; Ghorpade et al., 2001; Mayer et al., 2001; Rosenberg et al., 2001).

V. D. f) Functions Of Microglial Sentinels:

Microgliogenesis and neurogenesis happens synchronously in the developing brain. Microglia share some space with astrocytes and oligodendrocytes in supporting the neurons. Disruption of these interactions severely affects the functioning and development of CNS in a negative manner. Despite the fact that astrocytes are the predominant cells in the CNS, interaction of astrocytes with microglia plays a critical role in microglial cell biology.

V. D. f. i) Surveillance On Neuronal Survival:

Microglia provides a concrete foundation towards the survival of neurons via the release of an array of growth factors. For instance, insulin-like growth factor-1 (IGF-1) promotes the survival of layer V cortical neurons during postnatal development (Yamagata et al., 1995). Besides the secretion of IGF-1, these cells also secretes various trophic factors such as hepatocyte growth factor (HGF), epidermal growth factor (EGF), basic fibroblast growth factor (bFGF), platelet-derived growth factor, nerve growth factor (NGF), and brain-derived neurotrophic factor (BDNF) that plays a prominent role in the formation of neuronal circuits, neuronal development, and promotes their survival (Araujo and Cotman, 1992; Yamagata et al., 1995).

V. D. f. ii) Surveillance On Neuronal Cell Death - (Neuronal Expiry Date):

Faulty neurons defective in the formation of coordinated neural circuits, inability to differentiate and migrate with in the CNS requires elimination via programmed cell death and an active and efficient clean up to remove the resultant cellular debris for the healthy functioning of the brain (Frade and Barde, 1998). This process can be initiated by cell intrinsic factors or by the microglial cells via the release of cell specific factors towards the targeting cells. For instance, neurons in the developing murine hippocampus are induced by microglia to undergo apoptosis via the release of ROS in a CD11b-dependent and DNAX activation protein of 12 kDa (DAP12)-dependent manner that induces neuronal death (Wakselman et al., 2008). In connection with this, blockage of CD11b downregulates the neuronal cell death processivity in the developing hippocampus. This evidence (Microglial positive maintenance of neuronal cell death) is further corroborated by the selective elimination of microglia decreasing the Purkinje neuronal cell death. The reason behind the successful elimination of these cells by the system was due to the fact that these microglial cells release excessive ROS that was in part responsible for the Purkinje neuronal cell death (Marin-Teva et al., 2004).

V. D. f. iii) Scavenging Activity:

Neurons are under continuous vigilance by microglia in the CNS. Maintaining closer proximity between microglial and neuronal membranes are necessary for the proper facilitation of cell to cell signaling interactions and specific signaling occurs between these cells via the diffusion of molecules (cytokines, chemokines, and cell specific factors) and cell surface receptors (Harrison et al., 1998; Nayak et al., 2014). Microglial cells both in amoeboid and ramified resting states regularly surveys (microglial movements in brain) and senses its CNS domains for the presence of foreign material, damaged cells, apoptotic cells, neurofibrillary tangles, DNA fragments, or plaques and act as antigen-presenting cells activating T-cells to prevent fatal damage in the CNS. Presence of unique potassium channels renders these cells to attain this extreme sensitivity (Davis et al., 1994; Aloisi, 2001). Detection of any of these materials will activate the microglia. They will phagocyte the material and thereby help in the clearance of cellular debris. This hypothesis can be justified by the following report. Amoeboid form of microglia is necessary for the active discharge of their macrophagic activity and they exhibited critical scavenging activity by removing the dead cells in the neocortex that die during the normal course of remodeling of the fetal brain (Aloisi, 2001; Rock et al., 2004b).

V. D. f. iv) Microglial Phagocytosis (EAT ME Signalling):

Microglial activation in response to a stimulus will become highly motile secreting an array of inflammatory cytokines. They will reach the area of the lesion and initiate the process of phagocytosis in removing the cellular debris (Neumann et al., 2009). Extracellular nucleotides, such as UDP and UTP, trigger microglial phagocytosis through P2Y6R/ PLC/InsP3 pathway (Demonstrated in Figure V-9). Microglial phagocytosis requires specific receptors expressed on the cell surface to initiate the phagocytic activity such as the family of high affinity microbial pathogen binding toll like receptors (TLR2, TLR4), and the triggering receptor expressed on myeloid cells 2 (TREM-2) recognizes apoptotic cellular substances (Klesney-Tait et al., 2006; Napoli and Neumann, 2010; Veerhuis et al., 2011). An *in vitro* study lead by Paresce et al regarding Alzheimer's disorder reported that, microglial cells exhibited greater phagocytic activity and the cultured microglial cells rapidly uptake the extracellular amyloid beta plaques within 15 minutes. Those vacuoles could reach the acidic endosomes and lysosomes within 1 h and after 4 hours, most of the internalized plaques were completely degraded and released from cells (Paresce et al., 1997; Fu et al., 2014).

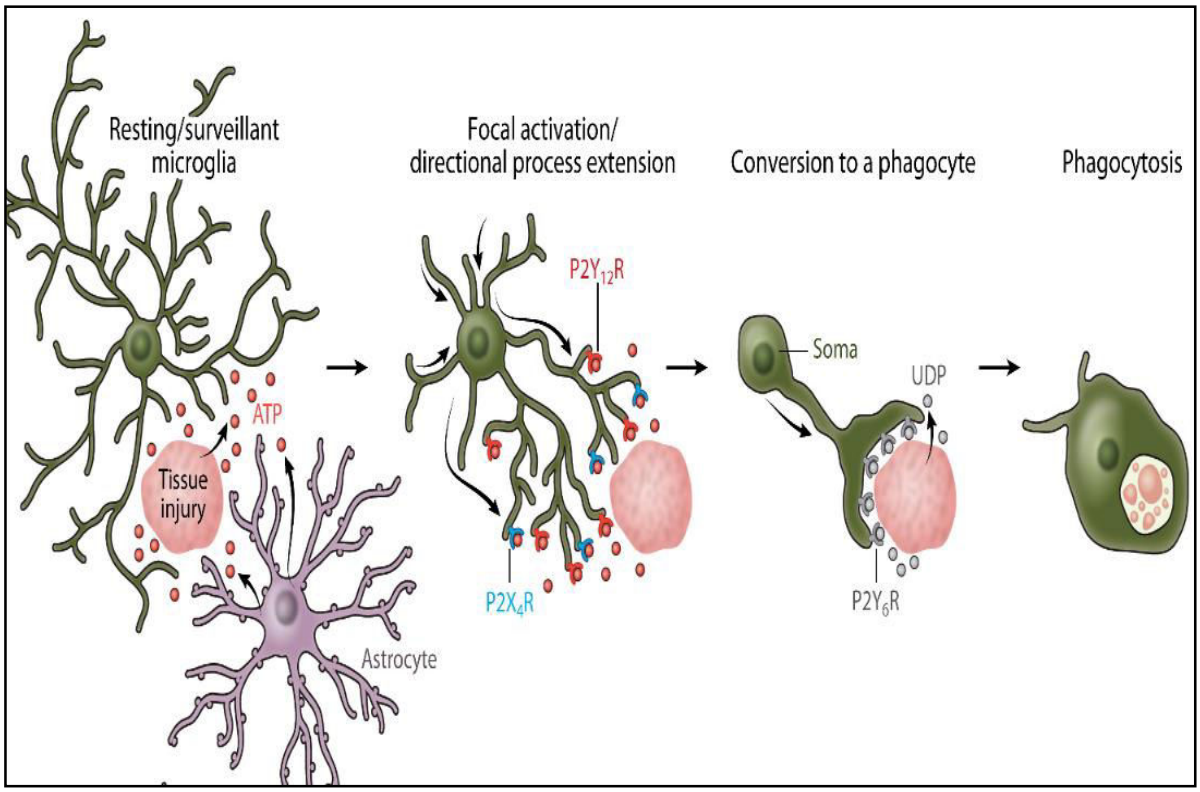


FIGURE V-9 Microglial Activation Following CNS Injury: Microglia in the naive CNS are highly ramified and continuously scan the parenchyma as part of their homeostatic program. Upon focal brain injury that induces necrotic cell death, damaged tissue and surrounding astrocytes release extracellular ATP, which triggers activation of specific purinergic receptors expressed by microglia. For example, microglial detection of ATP via P2Y12R and P2X4R (two purinergic receptors) induces the extension of processes toward the injury epicenter and the concurrent retraction of all other processes. The extent of tissue

injury likely dictates how quickly microglia convert to a phagocyte and participate in lesion cleanup. For example, detection of UDP via P2Y6R causes microglia to invest their cellular material into a single phagocytic process, which is followed by retraction of the soma into this process. Uridine 5'-Diphosphate (UDP - mediator of microglial phagocytosis) induces chemokine expression in microglia and astrocytes through activation of the P2Y6 receptor. The resultant phagocyte then participates in the cleanup of cellular debris.

Image Reference: (Koizumi et al., 2007; Nayak et al., 2014)

V. D. f. v) Microglia In Possession Of Cell Repairing Kit - (Cell Repair):

Post-inflammation, microglia undergo several steps to promote neurogenesis (Gemma and Bachstetter, 2013; Tobin et al., 2014). This process is achieved by the recruitment of neurons, astrocytes at the site of damage, by the secretion of anti-inflammatory cytokines and as well by the formation of gitter cells (cells formed after excessive phagocytic activity). Besides the promotion of repairing activity, microglial cells are also involved in remapping activity (Gehrmann et al., 1995). Absence of these microglial cells towards the promotion of repair would lead to slower regrowth, remapping or impossible vascular systems surrounding the brain and eyes (Gehrmann et al., 1995; Gehrmann, 1996; Ritter et al., 2006).

V. D. g) Microglial Mediated Neuroinflammation:

Unique anatomical niches (meninges, choroid plexus, and perivascular spaces) in the CNS are inhabited by specialized macrophages and dendritic cells acting as resident innate scavengers that orchestrates potent inflammatory responses in the brain (Nayak et al., 2012). Implication of microglia in neuroinflammation and neurodegenerative studies can be well supported and justified by the release of neurotoxic mediators produced in response to various microorganisms and microbial products (lipopolysaccharide). Accumulating evidences on microglia during the past several decades affirms that, microglial activation triggered in response to infectious agents have been implicated in various neuropathogenesis of inflammatory and neurodegenerative diseases such as multiple sclerosis (Merrill, 1992; Chiang et al., 1996; Greenlee and Rose, 2000; Smith, 2001; Nelson et al., 2002; Filipovic et al., 2003; Klesney-Tait et al., 2006), Alzheimer disease (Xia et al., 1998; Xia and Hyman, 1999; Halliday et al., 2000; Bamberger and Landreth, 2001; Benveniste et al., 2001; Cagnin et al., 2001; in t' Veld et al., 2001; EkDahl et al., 2003; Rosenthal and Khotianov, 2003; Solito and Sastre, 2012a; Li et al., 2014; Mosher and Wyss-Coray, 2014; Johansson et al., 2015), Parkinson disease (Le et al., 2001; Gao et al., 2002; Koutsilieri et al., 2002; Gao et al., 2003; Liu and Hong, 2003) amyotrophic lateral sclerosis (Koutsilieri et al., 2002), Huntington disease (Sapp et al., 2001) brain injury due to ischemia and trauma (Giulian et al., 1993; Ivacko et al., 1996) as well as on neural stem cells (EkDahl et al., 2003; Monje et al., 2003), that plays an important role in new memory formations.

V. D. g. i) Microglial Activation Under Inflammatory Settings:

Inflammation and microglial activation are the interweaved conceptual terms that one will encounter while studying neurodegenerative disorders in the CNS context. Claims are now being made that microglial activation is one of the major and hallmark features of

neuroinflammation that resulted from brain damage during neurodegenerative disease and suppression of microglial activation is supposed to prevent the progression of neurodegeneration. Widespread microglial activation reflects widespread neuronal damage and synaptic alterations in neurodegenerative disorders (Demonstrated in Figure V-10).

The proposition of monoclonal antibodies and lectins during 1980s stirred up the concept in detecting microglial activation in the damaged CNS. The discovery and detection of MHC molecules as markers for activated microglia in neurodegenerative disorders (Alzheimer's and Parkinson's diseases) from the McGeer group became the benchmark in microglial research during late 1980s. Histopathological observations of various neuropathologists had described the concept of microglial activation for almost over a century (Streit, 1995; Kreutzberg, 1996; Streit and Sparks, 1997).

TABLE V-2 **Unprecedented Features of Microglial Activation**

- Exhibits cellular hypertrophy.
- They have characteristic bushy appearance because of extended cytoplasmic processes.
- Displays increased phagocytic activity.
- Exhibits remarkable morphological and phenotypic changes.
- Upregulates thee expression of inflammatory and oxidative stress factors.
- Post inflammation, they helps in the promotion of neural repair and neurogenesis.

Table Reference: (Rio-Hortega, 1932b)

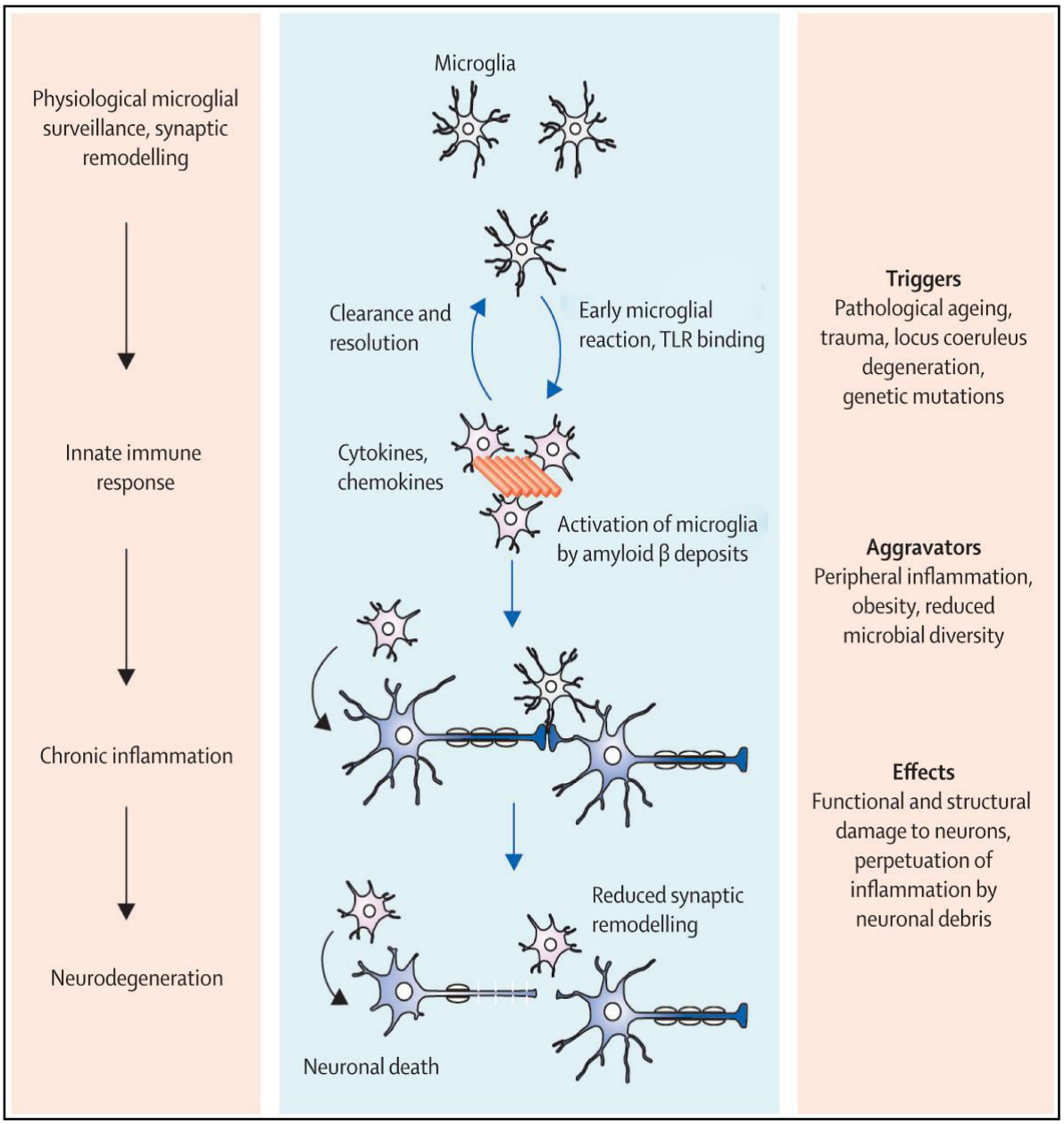


FIGURE V-10 Pathomechanistic Sequelae of Microglia Activation: Physiological functions of microglia, including tissue surveillance and synaptic remodeling, are compromised when microglia sense pathological amyloid β accumulations. Initially, the acute inflammatory response is thought to aid clearance and restore tissue homoeostasis. Triggers and aggravators promote sustained exposure and immune activation, which ultimately leads to chronic neuroinflammation. Perpetuation of microglia activation, persistent exposure to pro-inflammatory cytokines, and microglial process retraction cause functional and structural changes that result in neuronal degeneration. **Image Reference:** (Heneka et al., 2015)

These microglial activities are not only observed in neurodegenerative diseases, trauma and toxic lesions but also happens in the aging brain which serves as an important index to monitor the neuronal damage in the brain (Streit and Sparks, 1997).

V. D. g. ii) Dual Natured Microglia:

Besides the tendency of exhibiting the positive face of microglia in CNS, a number of studies have also revealed the negative characteristics of microglia towards the brain damage. It is reported that conditioned media derived from primary microglial cultures are neurotoxic to cultured neurons which revealed the presence of nitric oxide, glutamate, and a small-molecular weight microglial neurotoxin are responsible for causing neuronal cell death (Piani et al., 1991; Boje and Arora, 1992; Chao et al., 1992; Giulian et al., 1993).

In a contradictory way *in vitro* studies demonstrated by Mallat et al disclosed that, microglial conditioned media producing neurotrophic factors promote the survival of cultured neurons (See Figure V-11). This paradoxical behavior of microglia accounts for conflicting findings. One possible explanation could be that, neurons maintained in cell cultures are more susceptible to neurotoxic effects of conditioned media than neurons that are protected in the brain. And this activity might be due to the activity of microglial cells within the surroundings of neurons (neuronal vicinity) (Mallat et al., 1989; Nagata et al., 1993; Elkabes et al., 1996; Miwa et al., 1997). In a contradictory way *in vitro* studies demonstrated by Mallat et al disclosed that, microglial conditioned media producing neurotrophic factors promote the survival of cultured neurons (See Figure V-11).

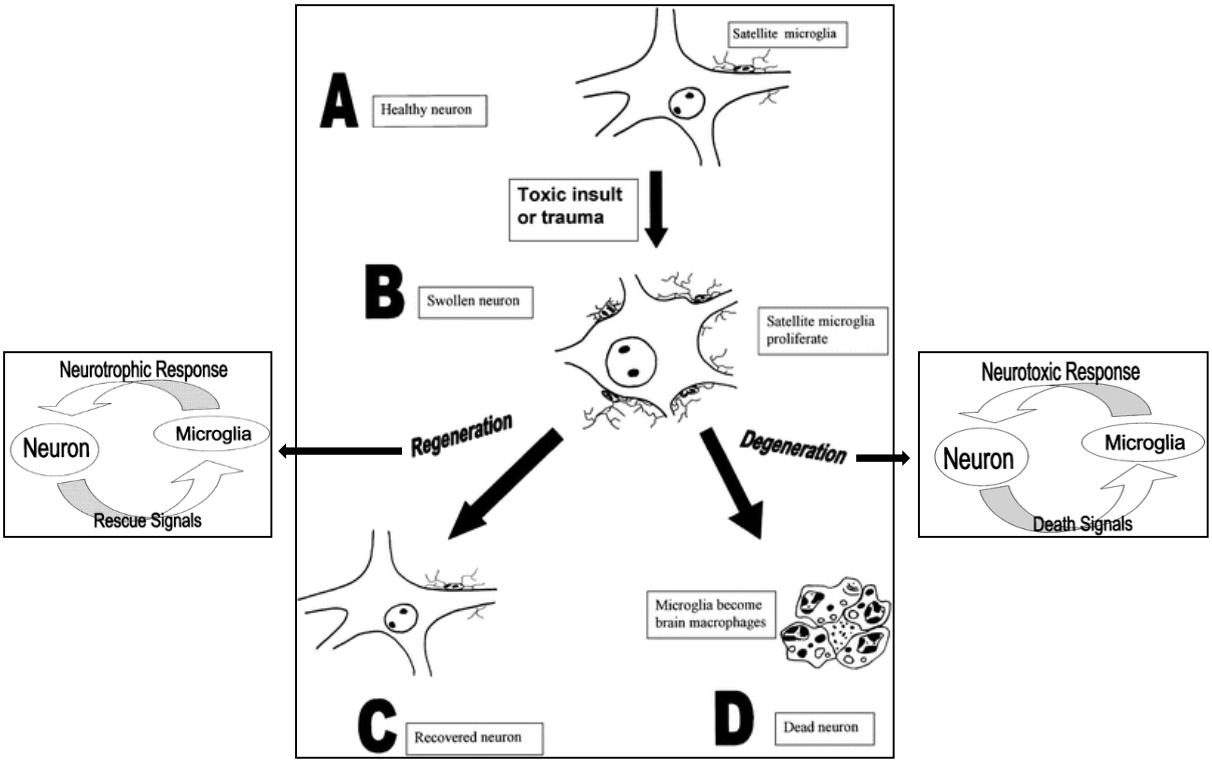


FIGURE V-11 Fate of a healthy neuron (A). After toxin exposure or trauma. In the early stages after an insult, the neuron may swell and attract numerous satellite microglia, which cover the neuronal surface with their cytoplasmic processes **(B).** The nature and severity of the primary insult largely determines whether a neuron is able to recover from it or not **(C, D).** In addition, perineuronal satellite microglia can sense whether a neuron is destined

to regenerate or degenerate, and they consequently produce either trophic or toxic factors to promote regeneration or degeneration, respectively. **Image Reference:** (Aschner et al., 1999)

V. D. g. iii) Alternative Definition For Microglial Activation:

Another explanation for this dual natured response of microglia can be corroborated from the reports from Aschner.M et al. In this study they considered interleukin-1 (IL-1) mRNA expression as one of the notable marker for microglial activation. They tested and compared the IL-1 mRNA levels between *in vivo* and *in vitro* microglia. This study had revealed that IL-1 mRNA levels in cultured, unstimulated (no lipopolysaccharides) microglia expressed approximately 1000-fold higher gene expression than in microglia of the normal CNS (Mallat et al., 1989; Aschner et al., 1999). The probable outcome of this experimentation further postulated that, once microglia are isolated and maintained *in vitro*, the cells attains and exists in a permanent state of near-maximal activation which can be characterized by the profused secretory activity of factors such as cytokines, neurotrophins, nitric oxide, and other potential neurotoxins. As a consequence of this processivity, these cells will achieve a higher microglial activation state (Detailed under Table V-2) through the exposure to stress (LPS, Hydrogen peroxide or other stimulants) and further become super active (See Figure V-12). So depending on the kind of experimental setup used (*in vitro* or *in vivo*), the definition and behavior of microglia activation will takes a different turn.

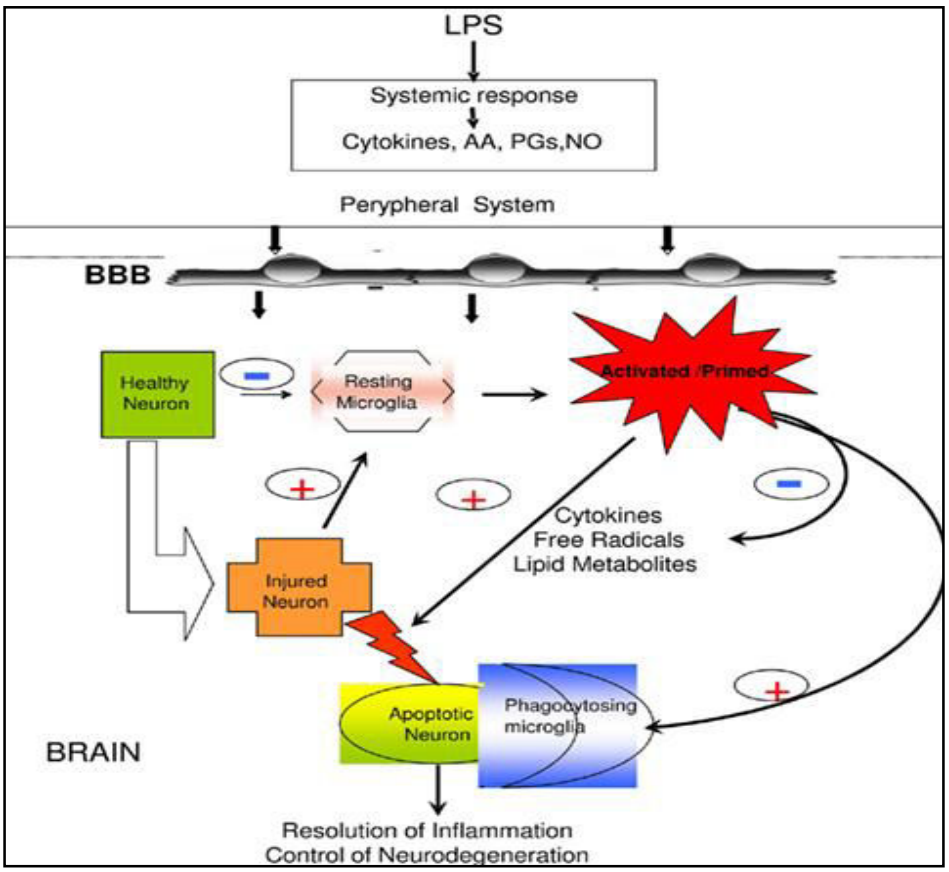


FIGURE V-12 LPS Induced Inflammatory Cascade: Peripheral infection/inflammation causes the release of pro-inflammatory mediators, including cytokines (TNF-α, IL-1β, IL-6), arachidonic acid (AA), prostaglandins (PGs), and nitric oxide (NO) synthesis. The brain also mounts an inflammatory response to systemic inflammation, as well as to local injury (neurodegeneration, trauma, stroke), with the microglial cells responding soonest and with production of the greatest amounts of pro-inflammatory mediators. However, the central response appears to be under tighter control than the peripheral response in that it is delayed and more modest, probably in order to avoid the dire consequences of a full-blown inflammatory response within the confines of the skull. **Image Reference:** (Solito and Sastre, 2012b)

V. D. h) Microglial Mediated Oxidative Stress:

Microglia are the resident macrophages of the brain and plays a critical role in the development and maintenance of the neural environment (Kraft and Harry, 2011). Although microglia continually survey the surrounding tissue, they remain in essentially quiescent state under tight regulation until they become activated in response to perturbations in the brain's microenvironment or changes in the neuronal structure (Hanisch, 2002; Kraft and Harry, 2011).

Up to certain extent free radicals (ROS, RNS) generated by the microglial cells can be protective and serves as an important defensive mechanism to fight against pathogens and toxic molecules released into the extracellular medium. Now it is a well-accepted that uncontrolled microglial mediated oxidative stress leads to the degeneration of neurons which is evidenced in the animal models Alzheimer's (Solito and Sastre, 2012a) and Parkinson's disease (Yoo et al., 2003; Peterson and Flood, 2012).

NADPH oxidase (NOX) a key oxidative stress regulatory enzyme performs a major role in oxidative stress mediated neurodegeneration. This enzyme activity determines the fate of microglial mediated oxidative stress in case of PD (Peterson and Flood, 2012). This reaction in microglial cells can be mimicked by pro-inflammatory stimuli and are mediated via the activation of ERK signaling pathway leading to the phosphorylation and translocation of the p47phox and p67phox cytosolic subunits, the activation of membrane-bound PHOX, and the production of ROS. Phosphorylation of Ser345 on p47phox is a prerequisite to the degeneration of DA neurons. Significantly decreased DA neurotoxicity is seen in both PHOX−/− mice (*in vivo*) and PHOX−/− midbrain neuron/glia cultures (*in vitro*) compared to PHOX+/+ controls (Qin et al., 2004).

V. D. h. i) Dichotomous Roles Of Microglia:

Microglial activation is tightly regulated. Pathogens, structurally and genetically altered proteins and changes in the neuronal structure can activate the microglia and generate a pro-inflammatory response towards the neighboring target cells. After a certain period of time microglia will mount a series of responses (oxidative stress response) and generates mediators that helps in the clearance of pro-inflammatory signalling (Hanisch, 2002; Kraft and Harry, 2011). This activated microglia will perform dichotomous roles and exhibits both pro-

oxidative and anti-oxidative status. When the pro-oxidative stimulus is removed, microglia can help in the facilitation of neurogenesis via the release of neurotrophins and anti-inflammatory cytokines that promote neuroregeneration and wound healing within the striatum (Heese et al., 1998a; Heese et al., 1998b; Qin et al., 2004; Whitney et al., 2009; Kraft and Harry, 2011).

V. D. h. ii) Microglial Activation Under Oxidative Stress Settings:

Microglial cells are the key contributors of oxidative stress in the CNS in which microglial activation is the key step towards the production of ROS and NO in the damaged tissues (Colton et al., 2000). This activated glial mediated stress response can be operated via the release of an array of cytokine factors that destructs the dopamine producing DA neural tissue in case of PD (Liu et al., 2003a; Peterson and Flood, 2012). Reactive microgliosis or microglial activation in the mice models of PD can be artificially mimicked by LPS (Bronstein et al., 1995; Araki et al., 2001; Liu et al., 2002), nitrated-α-synuclein (Zhang et al., 2005) and several neurotoxins such as 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and 6-hydroxydopamine (6-OHDA) can activate microglia through a process called reactive microgliosis that promotes DA neurotoxicity (Czlonkowska et al., 1996; Du et al., 2001; Cicchetti et al., 2002).

Once activated, microglial cells produce a wide variety of inflammatory mediators such as ROS and NO that mediate innate immune response. NF-κB and MAP-kinase are the key signaling pathways that controls the gene expression of many of these pro-inflammatory cytokines, chemokines, and enzymes that produce these secondary inflammatory mediators. This statement is supported by the post mortem detection of upregulated levels of ROS and NO as in case of PD patients which affirms the implication of microglial mediated ROS and NO involvement in the chronic neurodegenerative process (Baldwin, 1996; Chandrasekar and Freeman, 1997; Baldwin, 2001).

Neurons and endothelial cells within the CNS expressing CD200 and its interaction with the receptor CD200R is the key step towards the suppression of the reactive signals. Interaction between neurons and microglia containing CD200 and its CD200R receptor helps to keep the microglia in inactivated state (Neumann, 2001; Barclay et al., 2002; Lyons et al., 2007). Mice lacking the expression of CD200 exhibited heightened and upregulated the activation of microglial markers CD11b and CD45 that leads to the elevated levels of pro-inflammatory mediators such as TNFα, ROS, and NO subsequently contributing to compromised immune status (Hoek et al., 2000).

V. D. i) Microglia As A Pharmacological Target:

Penetration of the drug, optimal dose, perfect timing of drug administration, selective permeability, and many other biological considerations are the primary constrains that needs to be carefully controlled while using microglial cells as therapeutic targets for a pharmacological action. Inflammatory mediators generated by the microglial cells having debilitative role in the pneumococcal meningitis (van de Beek et al., 2003) and tuberculous meningitis (Byrd and Zinser, 2001) can be effectively prevented by the use of glucocorticoids

and dexamethasone. In addition to these molecules, drugs such as minocycline (Tikka and Koistinaho, 2001; EkDahl et al., 2003) dextromethorphan (Liu et al., 2003b) and agents that up-regulate glutamate receptors (Gras et al., 2003; Taylor et al., 2003) have neuroprotective properties through their effects on microglial cells.

V. D. j) Neurodegenerative Therapies:

TABLE V-3 Drugs Halting Neurodegenerative Disease Processing

Drug	Characteristic	Specific Blocking Site
Dexamethasone	Steroidal anti-inflammatory drugs (SAIDs)	Halts LPS induced neurotoxicity and dopaminergic neurodegeneration
Aspirin and Ibuprofen	Nonsteroidal anti-inflammatory drugs (NSAIDS)	Reduces inflammation by inhibiting COX activity
IL-10 or TGF-β1	Endogenous anti-inflammatory cytokines	Halts the inflammatory process
DPI (Diphenyliodonium)	NADPH oxidase inhibitor (PHOX)	Blocks Oxidative stress process
L-DOPA (L-3,4-dihydroxyphenylalanine)	NADPH oxidase inhibitor (PHOX)	Blocks Oxidative stress process
DM (Dextromethorphan)	Morphinan-related compounds	Exhibits anti-inflammatory properties and inhibitory function towards microglia activation.
Sinomenine	Morphinan-related compounds	Inhibits microglia activation.
Other compounds		Agents that block pro-inflammatory transcription factors (NF-κB, IKK) and by activating the peroxisome proliferator activated receptor-γ (PPARγ)

V. E. ASTROCYTES: Key Player In Neuroinflammation Induced Neurodegeneration

V. E. a) Astrocyte Backdrop:

Mihaly von Lenhossek in 1895 proposed the name Astrocyte for the first time to identify a specialized group of cells and suggested that the term neuroglia be used to encompass all supporting cells in the CNS (Tower DB, 1988). Astrocytes are the macroglial cells of the brain which constitutes about 50-60% and are ten times more numerous than neurons in the mammalian CNS (Hansson, 1988; A., 1991; Oberheim et al., 2006). These are neuroectodermal in origin (Richardson, 1997).

V. E. b) Glial Fibrillary Acidic Protein Expression - An Astrocyte Biomarker Sketch:

Astrocytes activation is mainly indicated by the increases in GFAP immunoreactivity, which is often paralleled by increased GFAP mRNA synthesis. Glial fibrillary acidic protein (GFAP) is the most abundant intermediate filamentous and cytoskeletal protein expressed in mature astrocytes that balances the astrocyte motility and helps in stabilizing the astrocyte extensions. Formation of glial scar otherwise known as reactive astrogliosis is the predominant characteristic of astrocytes in response to brain insults (Eng et al., 1971; Eng et al., 2000). Expression of this filamentous glial fibrillary acidic protein have been found to be upregulated in various neurodegenerative disorders, traumatic brain injury and stroke (Rosengren et al., 1994; Rosengren et al., 1995; Herrmann et al., 2000; Nylen et al., 2002; Vos et al., 2004).

V. E. c) Heterogeneous Population Of Astrocytes:

Immunohistological studies of astrocytes during 18th century (Virchow, 1858) revealed the diversified forms of astrocytes which includes: protoplasmic astrocytes, fibrous astrocytes, radial glia, bergmann glia, muller cells, velate astrocytes, tanycytes, pituicytes, interlaminar astrocytes, perivascular and marginal astrocytes (Cajal, 1897.; WL., 1893).

V. E. c. i) **Fibrous astrocytes** are located within the white matter. They exhibit long unbranched cellular processes and cellular extensions (perinodal processes) that contact axons at nodes of Ranvier. This form of astrocytes establish vascular, perivascular or subpial endfeet that maintains closer proximity with the outside capillary walls (Kettenmann and Verkhratsky, 2011).

V. E. c. ii) **Radial glia** are the second biggest group of astrocytes. These are bipolar cells which have ovoid cell body and elongated processes. They have two main processes, one of them forming an endfeet at the ventricular wall and the other at the pial surface. They are buried deeply with in the gray matter. Radial glia plays a critical role in the developmental process and these are the first cells to be formed from neuronal progenitors. Once matured, radial glial cells disappear from many regions of the brain that takes a transformation phase and modifies into stellate astrocytes (Schmechel and Rakic, 1979; Misson et al., 1988). In exceptional cases such as in lower vertebrates these radial glial even after maturation will

remain in the retina as Mueller astrocytes which act as progenitors for both astrocytes and oligodendrocytes (Kettenmann and Verkhratsky, 2011).

V. E. c. iii) **Protoplasmic glia** are extensively found within the grey matter and possess a greater quantity of organelles. They exhibit short and highly branched tertiary processes (Levison and Goldman, 1993; Zerlin et al., 1995).

V. E. c. iv) **Mueller cells** have characteristic extending longitudinal morphology along the rods and cones of retina. They are present within the cerebellar cortex. Mueller cells and Bergmann glia with an exception will be present even in adulthood (Bhattacharjee and Sanyal, 1975). Mueller cells occupy an overall volume of about 20 percent in the human retina and a single Mueller cell supports about 16 neurons in human retina and up to 30 neurons in rodents (Kettenmann and Verkhratsky, 2011) and helps in the formation of synapses and synaptogenesis (Bhattacharjee and Sanyal, 1975).

V. E. c. v) **Bergmann glia** are the semi radial epithelial glial cells or Golgi epithelial cells. They are well distributed within the cerebellum and extend from the Purkinje cell layer to the pial surface (Riquelme et al., 2002; Yamada and Watanabe, 2002). 8 Bergmann glial cells in rodents surrounds a single Purkinje neuron and their extended processes form an ensheathment towards the Purkinje cell dendrites. Early in development these cells have contacts with true radial glial cells and behaves as such, but during the developmental process soon they lose the morphology of radial glial cells and attain a characteristic Bergmann glial granular morphology. These are required for the addition of synapses and provides coverage up to 8000 synapses (Kettenmann and Verkhratsky, 2011).

V. E. c. vi) **Ancillary Astrocyte populations:**

Pituicytes are the astrocytes present with in the neurohypophysis that surrounds neurosecretory axons and axonal endings under resting conditions (Hatton, 1988). **Tanycytes** are the astrocytes present in the hyphophysis and raphe part of spinal cord (periventricular organs) (Langlet et al., 2013). **Velate astrocytes** are found in the cerebellum. They forms a sheath that surrounds the granular neurons. **Interlaminar astrocytes** are profoundly located in cerebral cortex. They extends from the soma located within the supragranular layer to cortical layer IV. **Perivascular and marginal astrocytes** form numerous endfeet with blood vessels. They can be found very close to the pia matter (Kettenmann and Verkhratsky, 2011).

V. E. d) **Functions Of Astrocytes:**

The functions of astrocytes are multiple. Major astrocyte functions can be grouped into three categories-guidance and support of neuronal migration during development, maintenance of the neural microenvironment, and modulation of immune reactions by serving as antigen-presenting cells (Montgomery, 1994). Interestingly, Vaccarino FM et al., reported that, astroglial cells can be the progenitors for the generation of neurons and oligodendrocytes that migrate to the cerebral cortex, replacing the cells that are lost in young mice. Astrocytes

localized in the neurogenic niches retains the properties of stem cells throughout their life span even in the adult stage that facilitates the promotion of neurogenesis and gliogenic activity (Vaccarino et al., 2007). Astrocytes together with microglia and other glial cells share some functional space in supporting the electrically excitable cells of the CNS (neurons), and performs vital functionalities including synaptic transmissions, uptake of neurotransmitters, recycling of neurotransmitters, detoxifying actions, supply of energy substrates to neurons, phagocytosis and many more.

V. E. d. i) Synaptic Transmission:

Neurons are surrounded by glial cells (astrocytes). More than just providing the structural support for neurons, it displays a manifold of reactions in which synaptic transmission is one of the key activity performed by the astrocytes towards neurons. Specialized chemical junctions which helps in the facilitation of communication from one neuron to another are called synapses (Waites et al., 2005). It is estimated that in adult hippocampus, a single astrocyte can contact up to 140,000-160,000 synapses (Bushong et al., 2002) from different neurons thereby it senses and integrate information from neighboring neurons. Astrocytes senses neurons via its fine bushy process. Traditional labelling of Astrocytes with GFAP antibody reveals the presence of only the cytoskeleton intermediate filaments which might not be sufficient enough to monitor the astrocyte-neuronal interaction of synaptic transmission. In order to rule out this approach, Shigetomi et al. in 2013, labelled astrocytes with cytosolic calcium indicator GCaMP3, which labels even the fine and bushy processes of the cells and are conspicuously visualized (Shigetomi et al., 2013). This synaptic neurotransmission involves the two key process such as uptake of neurotransmitters and recycling of neurotransmitters.

V. E. d. i-a) Uptake Of Neurotransmitters:

Neurotransmitters will be released at a very higher concentrations into the synaptic cleft during neurotransmission. Synaptic transmission termination requires uptake of these neurotransmitter molecules from the synaptic cleft. Astrocytes are rich in the enzyme glutamine synthetase which converts glutamate to glutamine in an ATP-requiring reaction (Figure V-13). Glutamine is transported to nearby presynaptic terminals where it is converted to glutamate for synaptic release. Finally astrocytes recapture the released glutamate via high affinity glutamate uptake system. Although glutamate transporters are present in neurons, astrocytes are the most active in removing glutamate. In the absence of the normal transmembrane Na^+ gradient, maintained by the ATP-dependent Na^+pump, the glutamate transporter ceases to remove glutamate and can run in reverse so that it pumps glutamate into the extracellular space (ECS). The probable mechanism to halt this neurotransmitter signaling process is the diffusion of transmitters into the synaptic cleft. Rapid removal of neurotransmitter is the key to maintain the spatial and temporal encoding of synaptic transmission. Illustration supporting this hypothesis were justified from the reports of Thomas et al. in 2011 (Thomas et al., 2011). The major pathway for glutamate clearance in the first postnatal week of developing hippocampus is via diffusion process. Later on after this time, due to the decrease in extracellular space, astrocytes upregulate the expression of glutamate

transporters (GLT-1 and GLAST) which facilitates the dominant mechanism of glutamate removal via transporter uptake mechanism (Lopez-Bayghen and Ortega, 2011). This synaptic activity is directly proportional to the transporter levels (Benediktsson et al., 2012). This astrocyte mediated neurotransmitter transporters (Glutamate, GABA, and glycine) expression will be located in the astrocytic process situated just next to synapse (Lopez-Bayghen and Ortega, 2011).

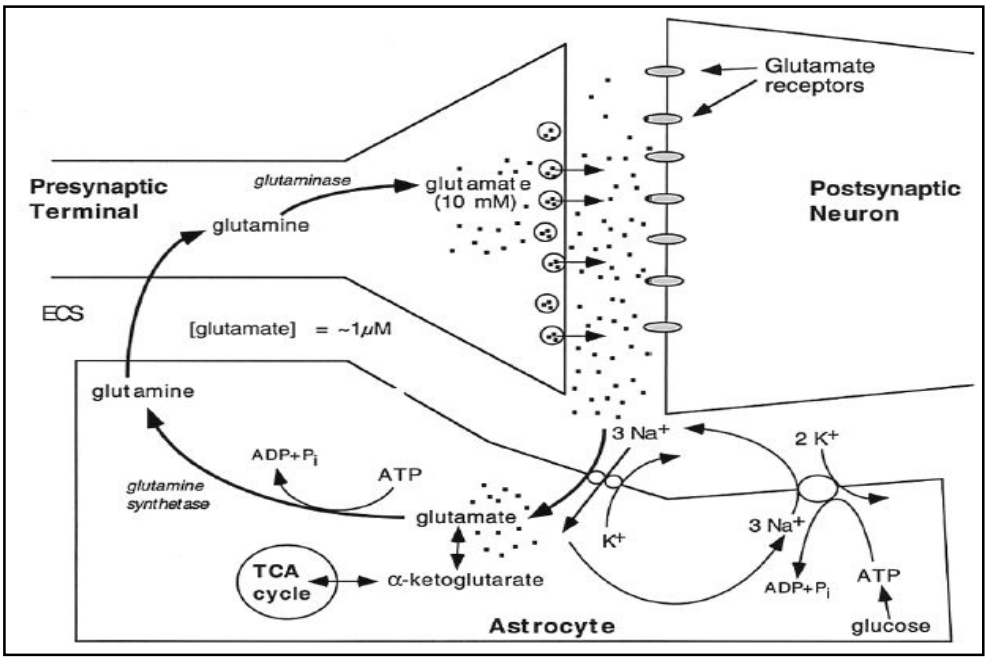


FIGURE V-13 Astrocytes in Glutamate Metabolism and Uptake
Image Reference: (Pierre J. Magistretti, 2002)

V. E. d. i-b) Astrocytes As Neurotransmitter Recycling Centers:

Neurotransmitters can be reused or metabolized (Allen, 2014). Astrocytes besides having the potential to terminate synaptic transmission, also play a key role in recycling neurotransmitters. This mechanism can be achieved via glutamate-glutamine cycle (Uwechue et al., 2012). Glutamate can be converted to glutamine with the help of the enzyme glutamine synthetase which is localized in astrocytes and oligodendrocytes (Montgomery, 1994). This is released back into the extracellular space which is further taken up by the adjacent neurons at neuronal synapses (glutamatergic and GABAergic synapses) which prevents the demand for the synthesis of new neurotransmitters from peripherally located precursors (Liang et al., 2006; Uwechue et al., 2012; Tani et al., 2014) (Figure V-14).

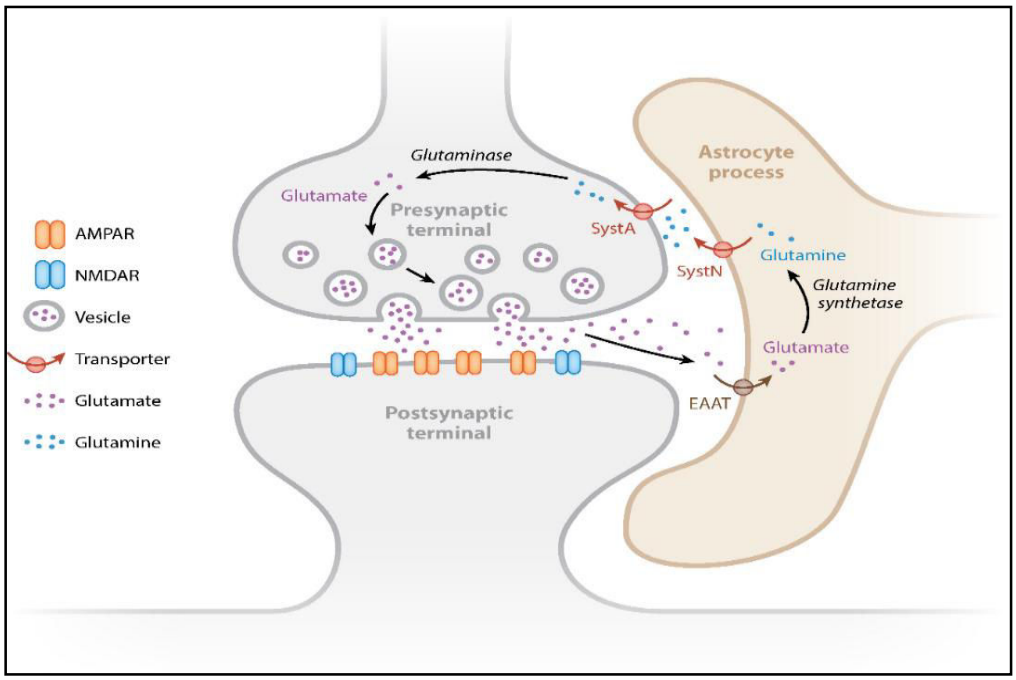


FIGURE V-14 Astrocytes Recycle the Neurotransmitter Glutamate via the Glutamate-Glutamine Cycle: The neurotransmitter glutamate is stored in vesicles ready for release from presynaptic terminals. After glutamate is released, its action is terminated by uptake into neighboring astrocyte processes via glutamate transporters (EAATs). Within the astrocyte, glutamate is converted to glutamine by glutamine synthetase. Glutamine is exported from astrocytes by system N (SystN) glutamine transporters and taken up into neighboring neuronal presynaptic terminals by system A (SystA) glutamine transporters. Within the neuron, glutamine is converted back to glutamate by phosphate-activated glutaminase and repackaged into vesicles by vesicular glutamate transporters, ready to be used again.

Image Reference: (Allen, 2014)

V. E. d. ii) Power Suppliers:

Astrocytes are positioned in such a way in the brain, that it will have the direct access to blood vessels to take up the nutrients via its astrocytic endfeet and supply it to neurons via its fine bushy processes. Astrocytes produce lactate via glycolysis and shuttle it to neurons for further use in oxidative phosphorylation. This generates ATP molecules and facilitates synaptic transmission and the neuronal membrane potential (Harris et al., 2012). This glycolytic process power up the uptake of glutamate and simultaneous conversion into glutamine by astrocytes during synaptic transmission (Pellerin and Magistretti, 1994; Harris et al., 2012).

V. E. d. iii) Neuronal Survival:

Astrocytic presence is essential for neuronal survival. Removal of astrocytes in the mouse brain lead to neurodegeneration. The probable reasons accounting for this neuronal cell death were, removal of astrocytes lead to the excessive stimulation of neurons (excitotoxicity) caused by the failure to remove the excitatory neurotransmitter glutamate and by excessive activation of glutamate receptors (Montgomery, 1994).

V. E. d. iv) Detoxification Process:

Astrocytes deploy it detoxifying actions via the uptake and sequestering of heavy metals such as lead. It also prevents the accumulation of potentially neurotoxic amino acids like glutamate via glutamine synthetase. Astrocytes contain specialized metal binding proteins known as Metallothionein. These metallothionein proteins are involved in metal metabolism which plays a critical role in the sequestration of specific metals (lead) and facilitates the prevention of metal toxicity augmentation in the brain (Montgomery, 1994; Kimelberg and Nedergaard, 2010).

V. E. d. v) Astrocytes As APC Performing Phagocytosis:

Expression of class II major histocompatibility complex (MHC) molecules renders the astrocytes to function like professional phagocytes of the CNS which are capable of performing phagocytic activity and acts as antigen presenting cells (APC). When an astrocyte senses a piece of neuronal debris via its astrocytic process (Astrocytic Arms), it pushes themselves towards the debris. As a result of this it eventually engulfs and digests the debris in order to prevent neighboring cells from further damage (Dong and Benveniste, 2001; Sokolowski and Mandell, 2011). Potential inducers of class II MHC molecules include IFN- γ and TNF-α and dibutyryl CAMP. These are known to elicit strong immune mediated response that triggers the activation of astrocytes (Reactive astrogliosis), whereas the TGF-b, IFN-β, IL-1, IL-4, IL-10, norepinephrine, glutamate, vasoactive intestinal peptide, nitric oxide are the inhibitors of class II MHC (Dong and Benveniste, 2001).

The expression of class II MHC molecules acts like a switch on the astrocytes to trigger immune reactions. MHC class II molecules present the processed antigens to $CD4^+$ T-helper cells and triggers the immune response. Class II MHC molecules are normally expressed in professional antigen presenting cells such as macrophages, dendritic cells, B cells, but astrocytes do not normally express class II MHC, or else if expressed it will be at a very low level (Montgomery, 1994). Overexpression of class II MHC molecules resulted in neurodegenerative diseases such as Multiple sclerosis, and inflammatory diseases such as rheumatoid arthritis, whereas under expression of class II MHC molecules in the hereditary bare lymphocyte syndrome (BLS) lead to patient death due to viral and bacterial infections (Grusby and Glimcher, 1995). Therefore the expression of class II MHC molecules should be carefully balanced in a host system.

Antigen Presentation Process Reaction Mechanism: The primary contact between APCs and $CD4^+$ T-cells are mediated by transient interaction between adhesion molecules such as intercellular adhesion molecule (ICAM) expressed on APC and Integrins on T-cells. Interaction between the T-cell receptor (TCR) and class II MHC molecules happens together with CD4 on the T-cell and establishes a specialized region known as immunological synapse (Monks et al., 1998; Grakoui et al., 1999).

TCR interacts with Class II MHC and enhances the expression of CD40 on APC (Primary signal). This inductive interactions sequentially promotes the expression CD40 ligand

(CD40L) on the surface of T-cells which stimulates the APC to synthesize and express B7 proteins (B7-1, B7-2) on the cell surface and express an array of cytokines, chemokines and other factors (Secondary signal) (Castle et al., 1993; Leveille et al., 1999). The B7 proteins thus produced will then reacts with constitutively expressed CD28 on the T cells delivering the tertiary signal, thereby finally completes the antigen presentation process (Lenschow et al., 1996; Lanzavecchia, 1997).

V. E. e) Astrocytes Mounting Neuroinflammatory Response:

Besides microglia, inflammation in the CNS are also mediated by astrocytes. Astrocyte activation (Detailed under Table V-4 and demonstrated under Figure V-15) has become a pathological hallmark of CNS structural lesions (Ridet et al., 1997; Pekny and Nilsson, 2005).

TABLE V-4	Phenomenal Features Of Reactive Astrogliosis

- Spectrum of morphological, molecular, cellular and functional changes.
- Cellular proliferation, scar formation and cellular hypertrophic reactions.
- Intracellular and intercellular molecular signaling changes.
- Both protective and destructive actions towards the adjacent neuronal and glial cells

Table Reference: (Sofroniew, 2014)

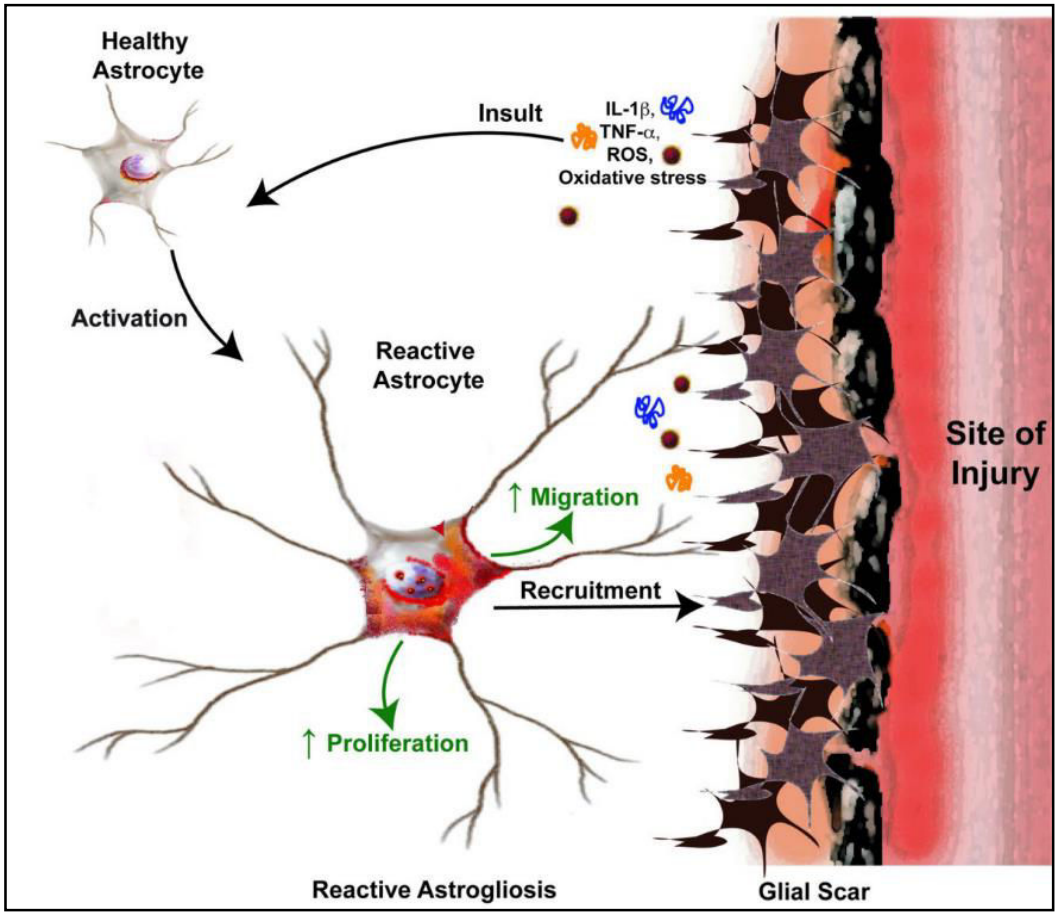


FIGURE V-15 Reactive Astrogliosis: Central nervous system (CNS) insults ranging from mild cellular disturbances to severe tissue damage and cell death lead to release of molecular mediators of reactive astrogliosis such as inflammatory cytokines interleukin-1β (IL-1β), tumor necrosis factor-α (TNF-α) and molecules of oxidative stress such as reactive oxygen species (ROS). These mediators in turn activate the local healthy astrocyte population by inducing a spectrum of changes in the microenvironment and intracellular signaling pathways resulting into reactive astrogliosis. **Image Reference:** (Neha and Anuja, 2012)

TABLE V-5	**Neuroprotective Actions Of Astrocytes**

Mediated via:

- Uptake of excitotoxic glutamate neurotransmitter.
- Production of glutathione that prevents cells from oxidative stress
- Degradation of amyloid beta peptides and by the release of adenosine.
- Blood brain barrier repair.
- Protection from NH_4^+ toxicity.
- Mitigates vascular swelling after trauma and stroke.
- Maintaining extracellular fluid stability and ionic balance.
- Limiting the wide spread range of inflammatory cells, infectious and damaged products from the site of injury to the site of healthy cellular parenchyma.

Table Reference: (Sofroniew and Vinters, 2010)

V. E. e. i) Astrocyte Mediated Neuroinflammation:

Most of the neurodegenerative disorders in the CNS shares common pathophysiological pathways involving inflammation, oxidative stress (Niranjan, 2014), neurotransmitter dysregulation, metabolic failure and excitotoxicity (Allen, 2014), in which astrocytes counteract to neutralize the CNS damage. Neuroinflammation leading to reactive astrogliosis in the CNS can be mediated by a range of inflammatory factors (Table V-6) in which infection, injury, ischemia, cellular debris and misfolded protein aggregates (β-amyloid, tau, and α-synuclein) play a contributing role (Mosley et al., 2012). It has both protective and destructive effects in the CNS (Sofroniew, 2009; Sofroniew and Vinters, 2010).

TABLE V-6 Molecular Triggers Of Astrocyte Activation	
Molecular Triggers	**Archetype**
Cytokines	TNF-α, IL-6, IL-10, IFN-γ
Growth Factors	CNTF, TGFb, FGF2.
Innate Immunity Mediators	Lipopolysaccharide and TLR ligands
Neurotransmitters	Glutamate, Noradrenaline
Purines	ATP
ROS Generators	NO, singlet oxygen, superoxides
Neurodegenerative Products	β-amyloid, α-synuclein, Parkin, TNF-α
Toxic Metabolites	Ammonium ($NH4^{+}$)
Cell Proliferation Regulators	Endothelin-1, EGF, FGF
Other Factors	Hypoxia and glucose deprivation

Table Reference: (Khandelwal et al., 2011; Mosley et al., 2012)

Besides microglia, astrocytes owing to the presence of class II MHC molecules also acts as immunocompetent cells in regulating the brain inflammation and as well in the participation of phagocytosis process (Montgomery, 1994; Dong and Benveniste, 2001). Activated astrocytes in response to stimulants produces repertoire of inflammatory mediators such as cytokines (IL-1β, TNF-α, IL-6, IL-10, IL-15, TGF-β), chemokines (MCP, MIP, RANTES) and growth factors (CNTF, BDNF, GDNF, EGF, FGF) that exhibits both neuroprotective and neurodestructive actions (Farina et al., 2007). Besides the production of inflammatory mediators, astrocytes are also capable of generating ROS that inhibits the neurite outgrowth and neurogenesis (Sofroniew, 2005). The balance between the production of these pro and anti-inflammatory cytokines in the CNS is tightly controlled and dysregulation of this equilibrium may lead to progressive neurodegenerative disorders.

Cytokines produced by glial cells have pleiotropic effects (Nicola, 1994). Astrocytes operates this inflammatory process by expressing several soluble cytokine receptors and the cytokines produced acts via autocrine, paracrine and endocrine mechanisms (John et al., 2003a). The function of soluble receptor is to enhance or diminish the biological activity of the cytokine. Characterizing the precise effect of these cytokines in glial cells especially in astrocytes and microglia are extremely challenging, because of complex cellular and molecular interactions. Cytokines act either antagonistically or synergistically (John et al., 2003a; Trendelenburg and Dirnagl, 2005). This is the reason behind the execution of either beneficial or detrimental or both the effects. For instance upregulation of IL-1β both *in vivo* and *in vitro* accelerates neuronal damage (neurodestructive) (Relton and Rothwell, 1992; Lawrence et al., 1998; Thornton et al., 2006), at the same time IL-1β in a contradictory way had been involved in neuroprotective actions such as blood-brain barrier repair (Herx and Yong, 2001), remyelination (Mason et al., 2001), ischemic tolerance (Strijbos and Rothwell, 1995) and neurotrophic factor production (DeKosky et al., 1996; Ohtsuki et al., 1996; Herx et al., 2000; Juric and Carman-Krzan, 2001). Hence the complex interplay between these numerous pro and anti-

inflammatory cytokines released by the astrocytes, microglia and neighbouring target cells, the cytokine receptors expression, severity of the damage, time, concentration levels and various other parameters determines the fate of the CNS.

V. E. e. ii) Astrocyte - Microglial Brotherly Hood:

Astrocytes are the predominant cell type within the CNS, in which astroglia-microglia interactions appear to play an important role in glial cell biology towards the safeguarding target of neurons (Depicted in Figure V-16) (Milligan and Watkins, 2009; Shinozaki et al., 2014). Astrocytes, in addition to their role in controlling neuroinflammation, also have a great impact on microglial cells. Astrocyte tends to participate in the suppression of microglial activation through negative feedback loops. Indeed, astrocytes presence together with the microglial cells, or use of astrocyte conditioned media on microglial cells downregulated the microglial activation and pro-inflammatory factors production and promotes the maintenance of neuronal survival and neuronal synaptic transmission (Vincent et al., 1997; Hailer et al., 2001; Min et al., 2006; Block et al., 2007). This suppressive effects of astrocytes on the microglial activation could be at least partially explain by the involvement of TGF-β as suggested by experiments in animal models of excitotoxicity as well as in ischemic stroke (Prehn et al., 1993; Henrich-Noack et al., 1996; Ruocco et al., 1999).

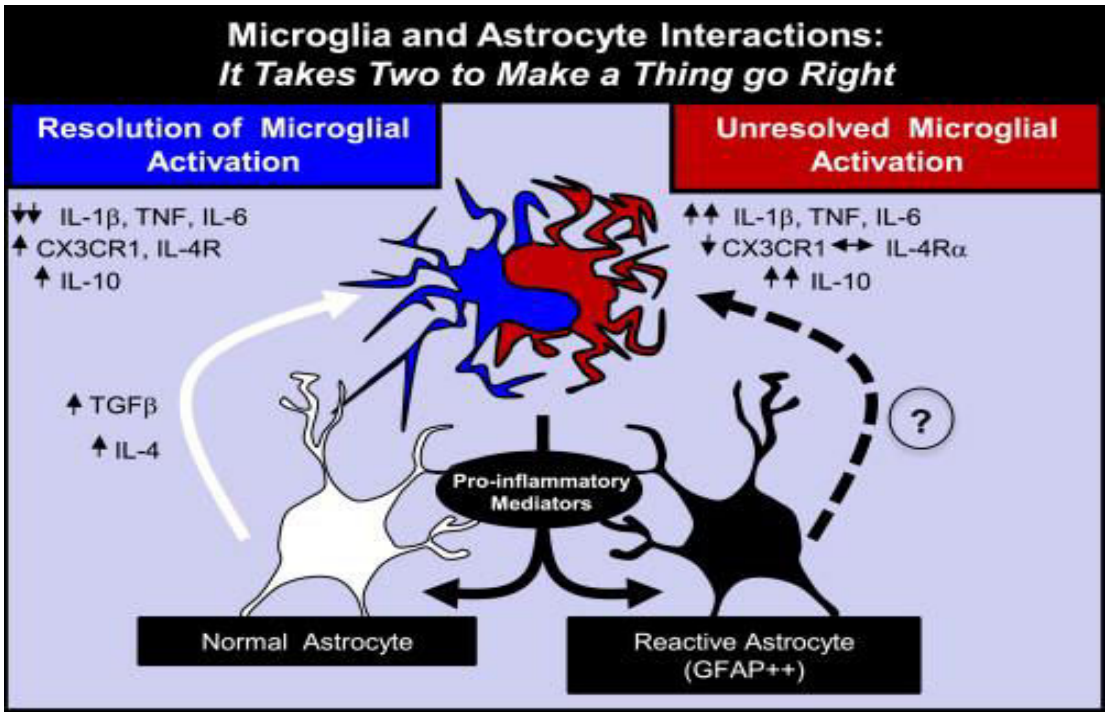


FIGURE V-16 Primed Glia And Impaired Regulation Of Active Microglia By Reactive Astrocytes. Astrocytes also have a more reactive profile with higher GFAP expression after traumatic brain injury (TBI) or in neurodegenerative disease. The long-term consequence of this reactive astrocyte profile in the brain is not well understood. One idea that this these altered profiles of astrocytes affects the dynamic interaction with active microglia. In this scenario, astrocytes help to regulate microglia activation. Thus it takes the appropriate interactions between these two glia cells types to make things go right.

Image Reference: (Norden et al., 2014)

In addition to this, several other *in vitro* studies demonstrated that amoeboid microglial cells when layered on astrocytes have the potency to develop ramified branching process, which suggests the astrocytic induction of microglial morphological branching process even under resting microglial conditions (Rock et al., 2004a). Despite of its differential variation in structural, functional and morphological appearances, astrocytes and microglia, act in coherence for the fine tuning of intrinsic immune system of the CNS.

V. E. f) Astrocytes Mounting Neurooxidative Stress Response:

In conjunction with inflammation, oxidative stress is another substantial and decisive factor involved in the progression of neurodegenerative disorders, traumatic brain injury, and stroke which depicts the vulnerability of CNS towards oxidative stress injury (Slemmer et al., 2008). Billions of cells in the mammalian brain uses 20 to 25% of blood carrying oxygen which is sufficient to run almost all the activities and the metabolic activity and is remarkably constant over time (Raichle and Gusnard, 2002; Jain et al., 2010). This clearly represents the demand of oxygen for energy metabolism required by the brain which inevitably generates ROS with a lower anti-oxidative capabilities. Oxidative stress induced CNS damage is regarded as the hallmark feature of neuroinflammation and neurodegenerative disorders such as Alzheimer's and Parkinsons disease (Luth et al., 2002; Pratico, 2008).

Neurons share some functional relationships with astrocytes in order to protect themselves from oxidative stress (Wilson, 1997; Milligan and Watkins, 2009; Weber and Barros, 2015). This has been demonstrated by *in vitro* studies which showed that neurons cultured in the presence of astrocytes are more resistant to toxic materials such as nitric oxide (Tanaka et al., 1999; Gegg et al., 2003), H_2O_2 (Langeveld et al., 1995; Desagher et al., 1996; Fujita et al., 2009), superoxide radicals and iron metals (Lucius and Sievers, 1996; Tanaka et al., 1999).

Mitochondria are central for astrocyte and neuronal survival. Dysfunctioning of mitochondrial activities eventually lead to neuronal and glial cell death (Wilson, 1997; Chen and Swanson, 2003). Oxidative stress generated reactive oxygen and nitrogen species (ROS, RNS) progressively deteriorates the nucleic acids, proteins, lipids and the power houses of the cell (mitochondria) which causes cell death in astrocytes and neurons (Motori et al.; Volobouéva et al., 2007).

Astrocytes plays a pivotal role sequestering metals such as iron, preventing the brain from metal induced neurotoxicity, which can be accomplished by the expression of metallothioneins and ceruloplasmin known to have effects on metal ion binding and iron trafficking (Montgomery, 1994; Tiffany-Castiglion and Qian, 2001).

V. E. f. i) Destructive Face Of Astrocytes:

Astrocytic deposition of insoluble α-synuclein proteins in the substantia nigra of the brain resulted in the process of phagocytic microglial recruitment that leads to the nigral neuronal cell death (Figure V-17). The consequence of this insoluble protein deposits in the astrocytes towards the neuronal cell death clearly depicts the impact of astrocytes towards the neurodegenerative disease progression (Martin et al., 2006; Devi et al., 2008). Once astrocytes

are activated (reactive astrocytes) they release a spectrum of inflammatory cytokines that participates in aggravating neuronal cell death by activating apoptotic mechanisms via a series of cytokines like TNF-α, IL-1β, IL-6, IFN-γ. They also activate proapoptotic mechanisms through the activation of caspase 3, caspase 8, and cytochrome c and also generate various ROS such as nitric oxide (NO) production by using iNOS (Chao et al., 1996; Hu et al., 1998; Calingasan et al., 1999; Akiyama et al., 2000; Hensley et al., 2000; Hirsch et al., 2003). This generation of oxidative stress affects neurons by breaking the DNA strands, induces lipid peroxidation and causes mitochondrial dysfunction (Hirsch et al., 2003; Halliday and Stevens, 2011; Vives-Bauza and Przedborski, 2011).

Additionally, deficiencies in astrocyte mitochondrial complex I in the substantia nigra of Parkinson's patients led to exaggerated production of ROS that augmented lipid peroxidation (4-hydroxynonenal or 4-HNE), abnormal protein cross linkage and protein fragmentation suggesting a probable role for astrocytes mediated oxidative stress that culminates and finally ends up in neurodegeneration in the substantia nigra and adjacent parts of the brain (Chinta and Andersen, 2008).

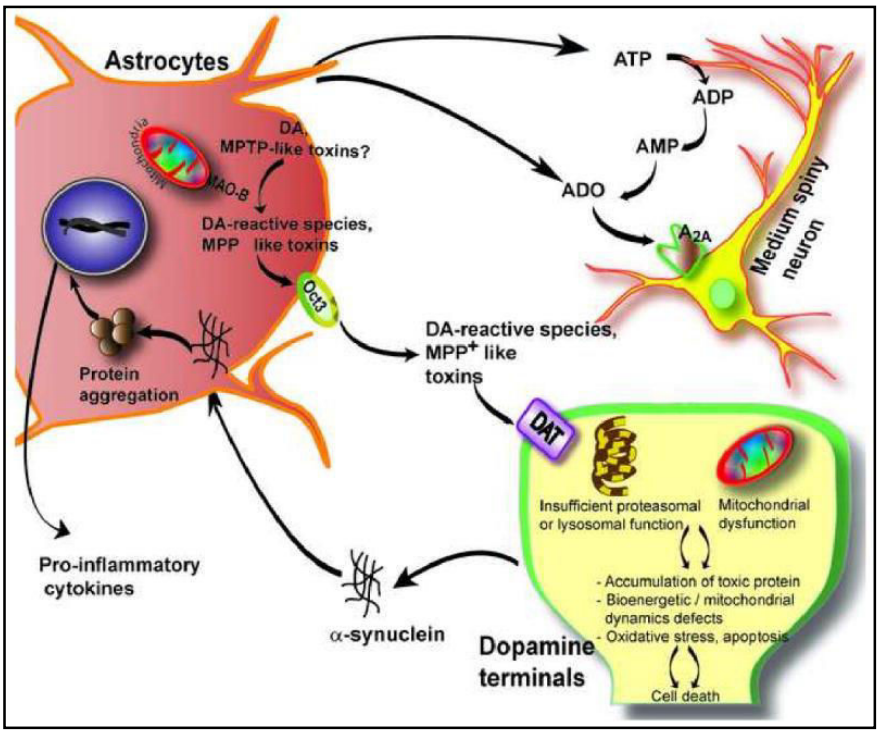


FIGURE V-17 Potential Neurotoxic Pathways Of Astrocytes: Astrocytes may also adversely affect the survival and function of dopaminergic neurons through the following mechanisms: **1)** Release of pro-inflammatory cytokines under pathological conditions such as accumulation of aggregated α-synuclein; **2)** Monoamine oxidase-B (MAO-B) mediated release of cytotoxic molecules such as dopamine-related oxidants and MPP+-like organic cations through the organic cation transporter (Oct3) into the extracellular space where they are subsequently transported into DA neurons through the dopamine transporter (DAT); **3)** Astrocytes can also release adenosine (ADO) directly or indirectly via ATP. As discussed

in the text, ADO may increase movement disorders in patients with PD through the A2A receptors in striatal medium spiny neurons. **Image Reference:** (Rappold and Tieu, 2010b)

V. E. f. ii) Protective Face Of Astrocytes:

At the same time it's been clearly evident from the subjects of PD patients that astrocytic expression of various antioxidant molecules such as glutathione peroxidase, SOD, and catalase had suppressed the amplitude of dopaminergic neuronal cell death (Ramaswamy and Kordower, 2009; Rappold and Tieu, 2010a; Yasuda and Mochizuki, 2010). When compared to neurons, astrocytes possess highly effective functioning machineries to counteract ROS. This includes variety of antioxidant molecules such as glutathione (GSH), recycling of ascorbate, and vitamin E and express greater ROS-detoxifying enzymes such as glutathione S-transferase, glutathione peroxidase, and catalase (Ricardo Cabezas, 2013) (Figure V-18).

Of all these antioxidant compounds, thiol group containing glutathione acts as an predominant electron donor and donates electrons to the freely moving unstabilized free radicals to get paired up in order to make them reactively stable. Astrocytes possess a greater concentration of glutathione (3,8 mmol/L) than neurons (2.5 mmol). This higher levels of glutathione expression in astrocytes are solely mediated by the presence of astrocytic enzyme y-glutamylcysteine synthethase (Rappold and Tieu, 2010a). Glutathione demonstrates great anti-oxidative capacity that maintains redox homeostasis and defends the insulted CNS (Dringen, 2000; Bambrick et al., 2004). Both neurons and astrocytes secrete this glutathione (GSH) tripeptide (L-glutamyl-Lcysteinylglycine) via glutamate cysteine ligase and glutathione Synthetase (Valko et al., 2006). Even though neurons have a moderate ability to secrete, they greatly depend on astrocytes for the production of GSH tripeptide. Indeed, when neurons are cultured in the presence of astrocytes, neurons exhibit greater GSH levels than when cultured alone. This is due to the fact that astrocytes release GSH tripeptide into extracellular space, which will be taken up by the neurons thereby ultimately improving the GSH protein production levels in the neurons (Aquilano et al., 2014). This demonstrates the significance of astrocytes antioxidant defense towards neurons (Maier and Chan, 2002; Slemmer et al., 2008; Giordano et al., 2009). In a similar context, increased glutathione peroxidase (GPx) levels led to decreased neuronal population death as observed in patients of PD which suggests the implication of glutathione peroxidase is considered as the critical factor responsible for the protective actions of astrocytes under oxidative stress conditions in PD patients (Damier et al., 1993).

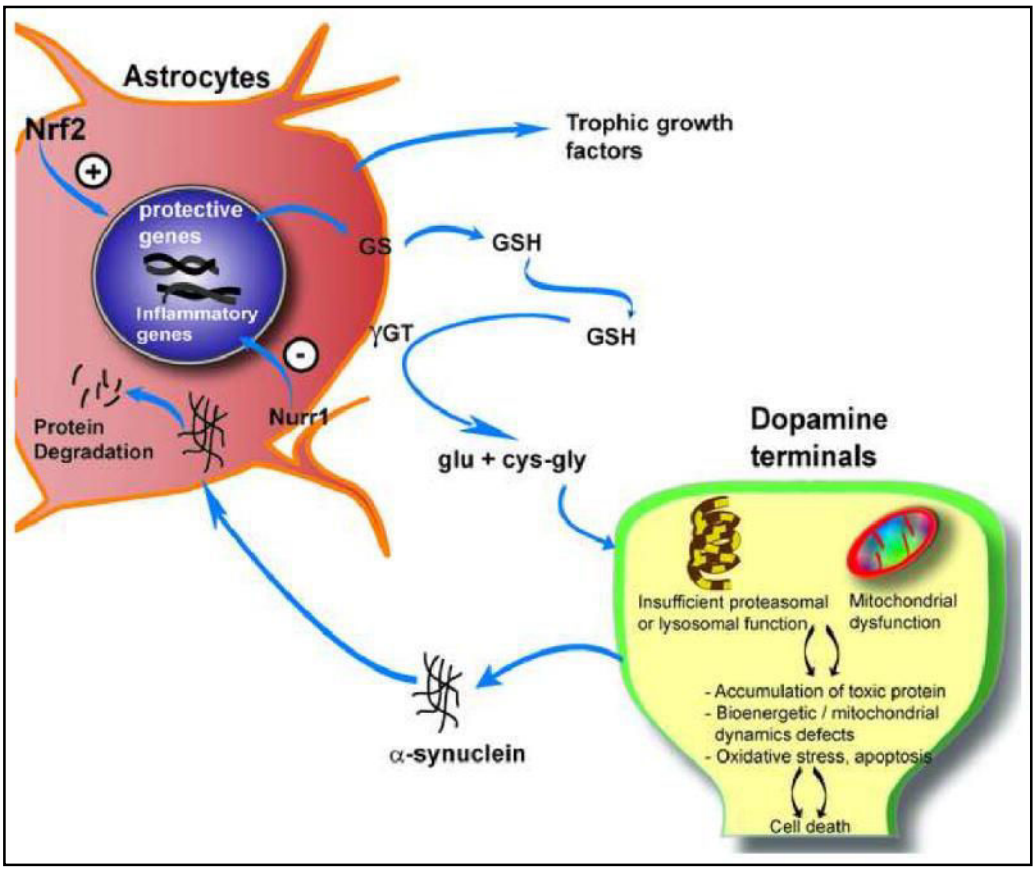


FIGURE V-18 Potential Neuroprotective Pathways of Astrocytes: Genetic mutations, environmental toxicants or a combination of both may induce nigral dopaminergic neurotoxicity through mechanisms such as mitochondrial dysfunction and insufficient degradation of misfolded proteins. Astrocytes may mediate neuroprotection through the following pathways: **1)** Release of trophic growth factors such as bFGF, GDNF and MANF); **2)** Release of glutathione (GSH) which is then cleaved by γ-glutamyltranspeptidase on astrocytic plasma membrane to generate glutamate and cysteinylglycine, which serves as precursors for neuronal GSH synthesis; **3)** Activation of the transcription factor Nrf2 leads to expression of genes containing the antioxidant response element (ARE), including γ-glutamylcysteine synthetase (GS) which is involved in GSH synthesis; **4)** Activation of the transcription factor Nurr1 which suppresses the production of inflammatory cytokines; **5)** Removal and degradation of cytotoxic molecules such as α-synuclein.

Image Reference: (Rappold and Tieu, 2010b)

V. E. g) Astrocytes As A Pharmacological Target:

Astrocyte dysfunctioning or the process of reactive astrogliosis is a potential mechanism that might contribute to the CNS disorders. Practical involvement of astrocytes in animal models of neurodegenerative Alzheimer's, Parkinson's disorders and as well as in clinical trials led some remarkable results in treating neurodegeneration. For instance, over the last years much research has focused on specific molecules produced by astrocytes that exert neuroprotection during brain injuries and diseases including PD, both through the reuptake of glutamate, or by producing gliotransmitters, antioxidant enzymes such as SODs, growth

factors and peptide hormones (Bambrick et al., 2004; Ouyang et al., 2011). For example, glutathione tripeptide a beneficial astrocyte produced antioxidant in the brain helps in the conversion of toxic methylglyoxal into non-toxic d-lactate by glyoxalase I (Yasuda and Mochizuki, 2010).

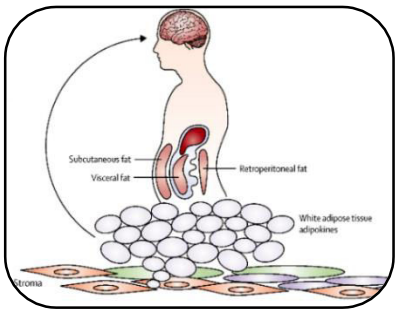
Reactive astrocytes can exert both beneficial and detrimental effects in a context-dependent manner determined by specific molecular signaling cascades. Reactive astrocytes turned on in response to various insults exerts both protective and destructive functions which will be specifically regulated by bio-signalling pathways. So the use of astrocytes as potential therapeutic agents should be targeted in such a way, so as to accelerate the beneficial functionalities of reactive astrocytes at the same time decelerating or blocking the harmful activities. But the overall blockage of this complete reactive astrogliosis could render the treatment to be highly ineffective, besides rather it creates potential lethality than the beneficial aspects on adjacent cells towards their healthy existence.

Conclusively, inhibition of exaggerated astrocyte activation (reactive astrogliosis) towards neuronal damage and suppressing the pro-inflammatory response via the inactivation of the transcription factor NFkB generated in these astroglial cells could be considered as promising therapies to heal inflammatory and oxidative stress implicated neurodegenerative disorders like Alzheimer's and Parkinsons disorders.

Accumulating scientific reports on the relation between influences of obesity on neurodegeneration strongly affirms that systemic inflammation is associated with obesity and obesity is a risk factor for neurodegenerative disorders.

In this regard, we are curious in understanding and studying the anti-inflammatory properties of factors produced by the fat tissue and their potential involvement in neurodegeneration. This will enable us to understand the neurodegenerative disease progression in the CNS.

VI. SECRET TALK BETWEEN ADIPOSE TISSUE AND CENTRAL NERVOUS SYSTEM



VI. A. Adipocytokines and Targets In The Brain

Adipocytokines, as the combination between adipocytes and cytokines, are secreted by the adipose tissue and acting through receptors. It is also worth noticing that these adipocytokines and its soluble receptors are not only secreted inside the fat tissue, but as well expressed inside other system including the mammalian central nervous system.

VI. A. a) Leptin is one of the most important adipose-derived hormones. It has weight-reducing effects (Friedman and Halaas, 1998; Gorska et al., 2010) by acting on hypothalamic arcuate nucleus. In homeostatic conditions, leptin inhibits food intake and in extra-hypothalamic sites leptin act on neurogenesis, synaptogenesis, neuronal excitability and neuroprotection (Arnoldussen et al., 2014; Bouret, 2010; Paz-Filho et al., 2010b). In the CNS, Leptin and its receptors (OB-R or LepR) are also locally produced within the brain, notably in the cerebellum, the cortex, choroid plexus and the hypothalamus (Morash et al., 1999; Wilkinson et al., 2000; Brown et al., 2007; Brown et al., 2008). Leptin negatively correlated with the development of Alzheimer's disease in lean humans (Paz-Filho et al., 2010b; a) and leptin signaling seems to be dysregulated in Alzheimer's disease brains (Bonda et al., 2014).

VI. A. b) Adiponectin is one of the most important adipocyte-derived hormones considering it's abundance in plasma relative to many other hormones (Matsuzawa, 2005; Thundyil et al., 2012b). It modulates a wide range of metabolic processes such as body-weight regulation, glucose regulation, insulin sensitivity, lipid catabolism (fatty acid oxidation), modulation of endothelial function and also anti-atherogenic (Berg et al., 2002; Okamoto et al., 2002; Stefan and Stumvoll, 2002; Whitehead et al., 2006; Thundyil et al., 2012b). Adiponectin is well expressed at the mRNA and/or protein level by the placenta, the liver, epithelial cells, osteoblasts, myocytes and also by pituitary cells (Wilkinson et al., 2007; Psilopanagioti et al., 2009; Thundyil et al., 2012b). In the pituitary, adiponectin could have a role in the release of somatotrophs and gonadotrophs (Thundyil et al., 2012). Adiponectin receptors appear to be widely expressed in the mammalian brain and their actions are mediated by three different receptor types: adiponectin receptor 1 (ADIPOR1), adiponectin receptor 2 (ADIPOR2) and T-cadherin (CDH13). Recently, adiponectin receptor 1 and 2 expression was described in primary human astrocytes (Wan et al., 2014). It also appears that adiponectin induces a pro-inflammatory response in human astrocytes, increasing notably IL-6 and MCP-1 through NF-κB, p38MAPK and ERK1/2 pathways (Wan et al., 2014). In contrast, adiponectin was described to inhibit pro-inflammatory signal, notably by

suppressing IL-6 release from blood brain barrier endothelial cells (Spranger et al., 2006). It results that adiponectin indirectly modulates inflammatory signaling across the blood brain barrier by negatively modulating Il-6 and TNF-α release. Adiponectin has also been shown to be expressed in the brain of mouse, rat, human and also pork in different brain structures such as the pituitary, the hypothalamus, in cortical and subcortical neurons (Degawa-Yamauchi et al., 2003; Yamauchi et al., 2003b; Fry et al., 2006; Hoyda et al., 2007; Psilopanagioti et al., 2009; Repunte-Canonigo et al., 2010; Thundyil et al., 2010; Thundyil et al., 2012b). Interestingly, some studies documented adiponectin transcript expression in the brain. Thus, adiponectin was detected by RT-PCR and Northern analysis in the anterior pituitary gland and the diencephalon in chicken (Maddineni et al., 2005; Wilkinson et al., 2007), in the human pituitary (Psilopanagioti et al., 2009).

VI. A. c) LysoPhosphatidic Acid (LPA) and Autotaxin: ATX is a multifunctional phosphodiesterase that converts lysophospholipids (LPLs) into LPA through its lysophospholipase D activity. LPA is detected in several biological fluids and tissues including the brain (Tokumura, 2004). To date, LPA effects are mediated through five G protein coupled receptors. However, additional receptors have been identified for their potential responsiveness to LPA (Noguchi et al., 2003; Kotarsky et al., 2006; Noguchi et al., 2009). In the nervous system, neural progenitor cells, neurons, oligodendrocytes, Schwann cells, astrocytes and microglia have been documented for expressing different subsets of LPA receptors (Noguchi et al., 2009). This partially explains why LPA exerts a wide variety of effects on these different cell-types. LPA also displays effect on cell morphology and neurite formation in both neural progenitors cells and neurons (Noguchi et al., 2009). LPA also exerts various effects on glial and microglial cells, by modulating intracellular calcium levels in oligodendrocytes, astrocytes and microglia (Noguchi et al., 2009). It notably favors astrocytes and microglia proliferation *in vitro* (Keller et al., 1997b; Moller et al., 2001). Interestingly, in human post-mortem brains LPA receptors 1-3 and autotaxin are only weakly expressed while LPAR2 is increased and autotoxin transcripts are decreased following brain injury. Such data also reinforce the fact that LPA signaling is involved in neurotrauma (Frugier et al., 2011).

VI. B. Interplay Between Microglia, Astrocytes And Adipocytes

Adipose tissue in addition to its storage capacity, also serves the purpose of bodily needs by acting as endocrine tissue producing a number of different factors including inflammatory-related factors such as chemokines (MCP-1, MIP-1, RANTES), cytokines (adipocytokines - Leptin, Adiponectin) and others factors (Autotaxin / LPA) acting at a physiological level that affects the whole-body energy metabolism. Control of energy homeostasis requires communication between the brain and adipose tissue (Turtzo and Lane, 2002). Some of these adipocytokines act locally as paracrine factors, and others such as leptin and adiponectin have long-range effects that act on the feeding centers of the central nervous system notably in hypothalamus and hippocampus (Waki and Tontonoz, 2007a).

Here in this context we are coining a new term for the first time - "**Astradipo / Micrastradipo**" in relating this adipocytokine and neurodegenerative research to have a better perception that fits this adipocytokine arena.

© **Astradipo** - Interaction between astrocytes and adipocytokines.
© **Micrastradipo** - Interaction between microglia, astrocytes and adipocytokines (Parimisetty et al., 2015b).

It is now well accepted that adipose tissue is a key player in the development of inflammation (Weisberg et al., 2003). Excess fat tissue in the obese environment contributes to a low-grade chronic inflammation (Greenberg and Obin, 2006) with elevated production of pro-inflammatory cytokines, such as tumor necrosis factor alpha (TNF-α), MCP-1, Interleukin -6 (IL-6) and IL-1 (Tilg and Moschen, 2006; Moschen et al., 2007). Increased TNF-α, MCP-1, CCL2, or other chemotactic factor expression in obese adipose tissue may trigger the recruitment of macrophages (ATMs). Macrophages infiltrate into adipose tissue from the circulation in obesity (As seen in Figure VI-1). MCP-1 acts by binding to its receptor CCR2 on monocytes. Macrophages are a source of inflammatory factors such as TNF-α, IKKβ, iNOS, IL-6, and MCP-1 whose secretion may be elevated in obese status and might therefore contribute to the pathophysiological consequences of obesity such as insulin resistance, type 2 diabetes (Lumeng et al., 2007) and obesity induced neurodegeneration (Gustafson et al., 2004; Debette et al., 2010). Apoptosis of adipocytes in obese adipose tissue may also play a role in the recruitment of macrophages.

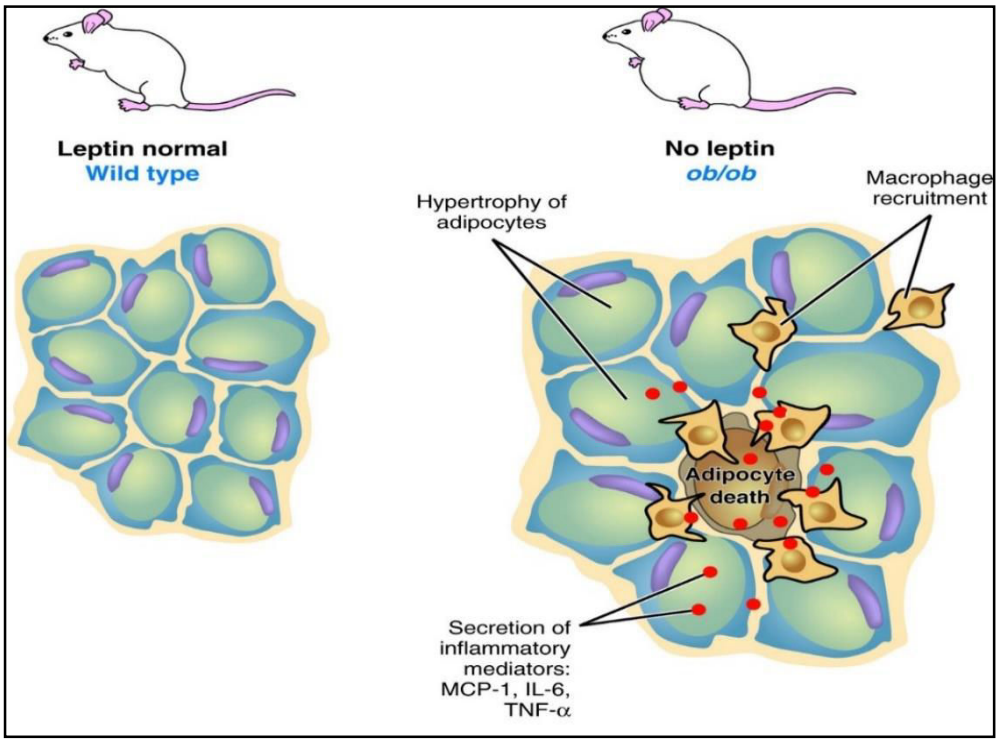


FIGURE VI-1 Macrophage recruitment in adipose tissue under obese settings
Image Reference: (Waki and Tontonoz, 2007a)

As previously stated, WAT can produce an array of inflammatory-related factors, whose expression levels may be modified in obesity (Figure VI-2).

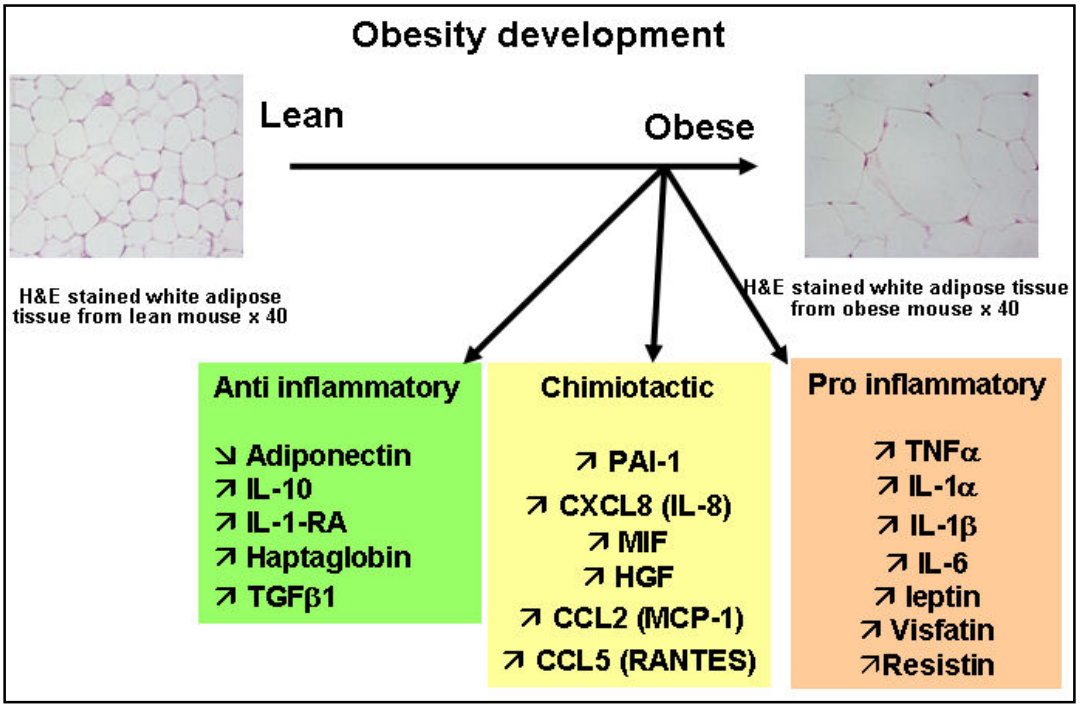


FIGURE VI-2 Inflammatory factors produced by WAT in obese situations
Image Reference: (Awada et al., 2013a)

It has been proposed that an obesity-related chronic low-grade inflammation can serve to change the environment and prime the brain for subsequent insults leading to a heightened inflammatory response and possibly exacerbation of the damage. While the causal nature of these processes to neurodegeneration has not been definitively established, it is widely accepted that neuroinflammation and oxidative stress responses occur with clinical manifestation of the disease. The mechanisms that initiate and trigger these processes are not yet totally elucidated, but different hypothesis have been proposed. A more recent association between obesity and neurological function is based upon correlations with biological processes of oxidative stress and inflammation (Awada et al., 2013a).

VI. B. a) Adipocytokines And Neurodegeneration:

It is only relatively recently that the concept that obesity could have an effect on the brain has been emerging. Additional consideration has been raised that obesity may be linked to various progressive and aging-related neurodegenerative diseases such as Parkinson's disease, Alzheimer's disease (AD), and autoimmune nervous system diseases like multiple sclerosis. Given the recent reports of adipocytokines within the body fat and the elevation of these inflammatory factors with stimulation, a more direct linkage between obesity and various human diseases, including neurodegenerative disease, has been hypothesized (Gustafson et al., 2003; Gustafson, 2006) (Demonstrated in Figure VI-3, Figure VI-5, Figure VI-6).

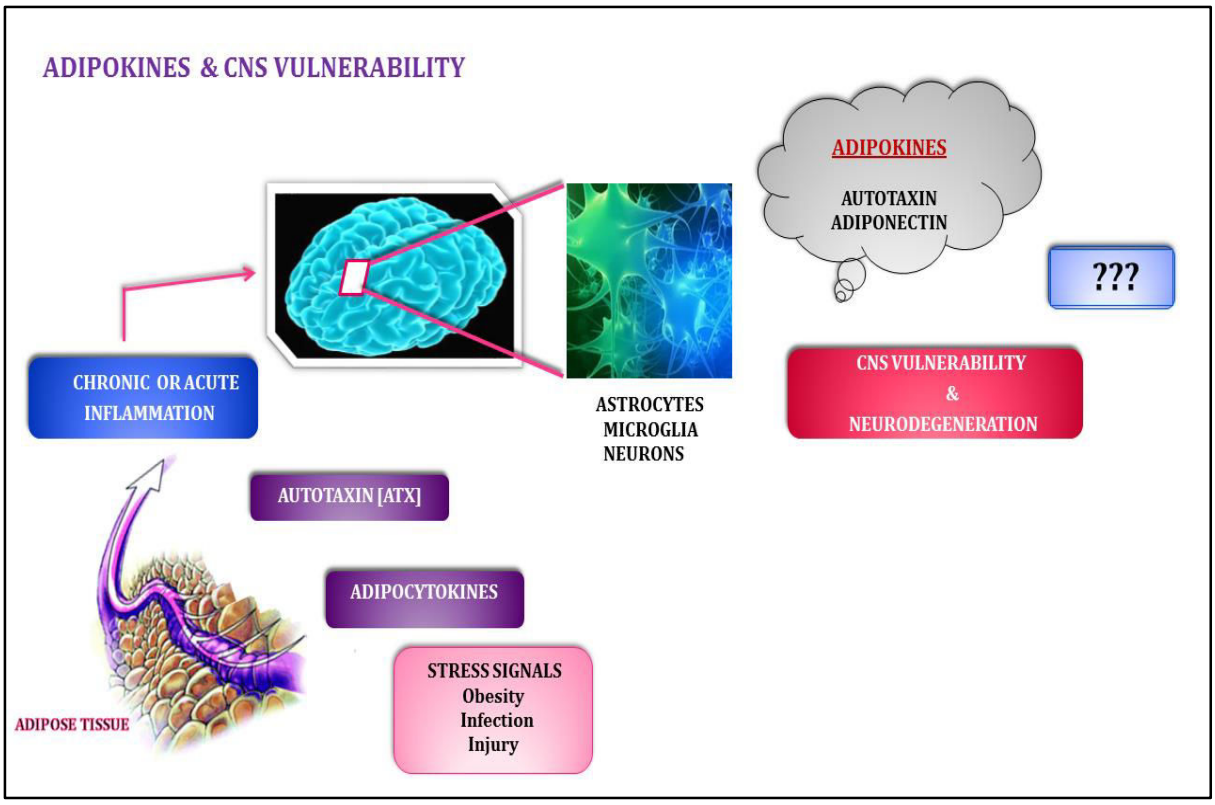


FIGURE VI-3 Adipocytokines and CNS Vulnerability: It is a well-admitted fact that systemic inflammation is associated with obesity and obesity is a risk factor for neurodegenerative disorders. Adipose tissue in response to the stress signals (high fat diet, infection, injury), triggers the release of diversified range of factors popularly known as adipocytokines. Depending on the cytokine (pro-inflammatory adipocytokines) produced it eventually leads to chronic or acute inflammation in and around the site of stress. Adipose tissue has now been regarded as a specialized endocrine organ. Because of its endocrine properties, the cytokines produced inside the fat tissue travels all along the blood and may reach the brain. The receptors of these factors produced by adipose tissue are present in the central nervous system (CNS) as well, including in resident microglia and astrocytes, further contributing to neuroinflammation and neurodegenerative status in the brain.

VI. B. b) Evidences Supporting Obesity Induced Neurodegeneration:

Emerging evidences from multiple clinical studies reveals the devastating effects of adiposity on cognitive dysfunctioning more particular on neuronal disorders such as Alzheimer's and Parkinsons disorders (Gustafson; Gustafson et al., 2003; Kivipelto et al., 2005; Hayden et al., 2006; Lafortuna et al., 2006; Whitmer et al., 2007; Whitmer et al., 2008; Fitzpatrick et al., 2009; Gustafson et al., 2009; Lee, 2011; Bluher, 2013; Kiliaan et al., 2014).

The first and foremost scientific report on the nexus between adiposity (higher BMI) and Alzheimer's disease were published by Deborah Gustafson on 2003 in which a cohort of 392 non-demented patients were followed up from the age of 70 to 88 for a period of 18 years by using neuropsychiatric, anthropometric and body mass index to examine whether overweight is a risk factor for dementia and Alzheimer's disorders. This report affirmed that

"*overweight at high ages is a risk factor for dementia, particularly Alzheimer's disease, in women*" (Gustafson et al., 2003). In relation to this cohort study, several other reports corroborated the linkage between adiposity and the dysfunctioning of the brain (neurodegeneration disorders) which demonstrates that people with higher BMI levels or adiposity have a two times higher risk of dementia in later life (Kivipelto et al., 2005; Whitmer et al., 2007; Whitmer et al., 2008; Gustafson et al., 2009).

Alzheimer's type of amyloid beta and tau protein expression (neuropathological change) were found to be significantly expressed in hippocampus from morbidly obese individuals with a BMI >45 (Mrak, 2009; Naderali et al., 2009). Besides this, when compared to non-obese patients, obese individuals exhibited lower dopamine D2 receptor availability in the striatum. Obese people are less active than non-obese individuals and lowered physical activities is one of the potential risk for developing Parkinson' neurodegenerative disorder (Chen et al., 2014). High susceptibility to environmental toxins and accelerated pathological effects of PD are directly proportional to the exposure of high-fat diet treatment and as well with overweight and obesity (Bousquet et al., 2012; van der Marck et al., 2012; Chen et al., 2014).

In addition to these supporting evidences, within a cohort study of obese women it has been reported that there is a correlation between increase in total body fat irrespective of its body distribution, and a negative destructive activity on cognitive functioning of motor neurons (Lafortuna et al., 2006).

Besides this, initial studies demonstrated a higher BMI and/or waist-to-hip ratio in middle-aged individuals associated with a reduction in whole brain volume. Over the last decade, a number of magnetic resonance imaging (MRI) and computed tomography (CT) studies have reported alterations in brain morphology of overweight/obese individuals (Figure VI-4) (Ward et al., 2005; Taki et al., 2008; Bruce-Keller et al., 2009). In the past decade, a linkage has been demonstrated between being overweight in middle age and increased risk for Alzheimer's disease and other forms of dementia (Gustafson et al., 2003; Gustafson, 2006). A similar association was observed with temporal lobe atrophy in elderly women with additional evidence of hippocampal atrophy (Gustafson et al., 2004). Debette et al. reported a link between abdominal fat and reduced brain volume in otherwise healthy middle-aged adults (Debette et al., 2010). In a cross-sectional study of normal elderly individuals showing no sign of cognitive deficit, tensor-based morphometry unveiled atrophy in the white and gray matter of the frontal lobes, anterior cingulate gyrus, hippocampus, and thalamus in both male and female subjects with a high BMI (BMI > 30) as compared to individuals with a normal BMI (18.5–25) (Raji et al.). Upon further investigation, the brain volume reduction in gray and white matter was found to be associated with a common variant of the fat mass and obesity associated (*FTO*) gene (Ho et al.).

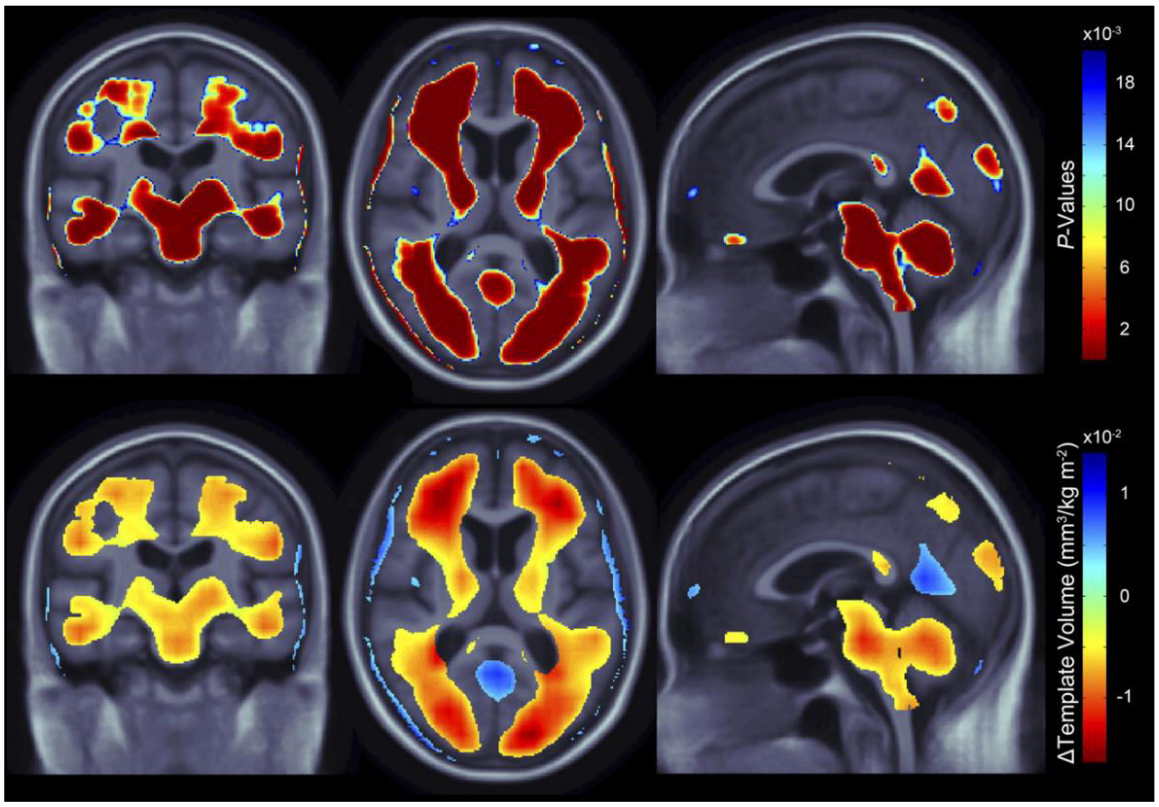


FIGURE VI-4 Obesity and Brain Atrophy: Radiological Image of distinct regions of the brain associated with BMI in healthy elderly individual (top) and obesity linked FTO allele carrier individual (bottom). **Image Reference:** (Ho et al., 2010b)

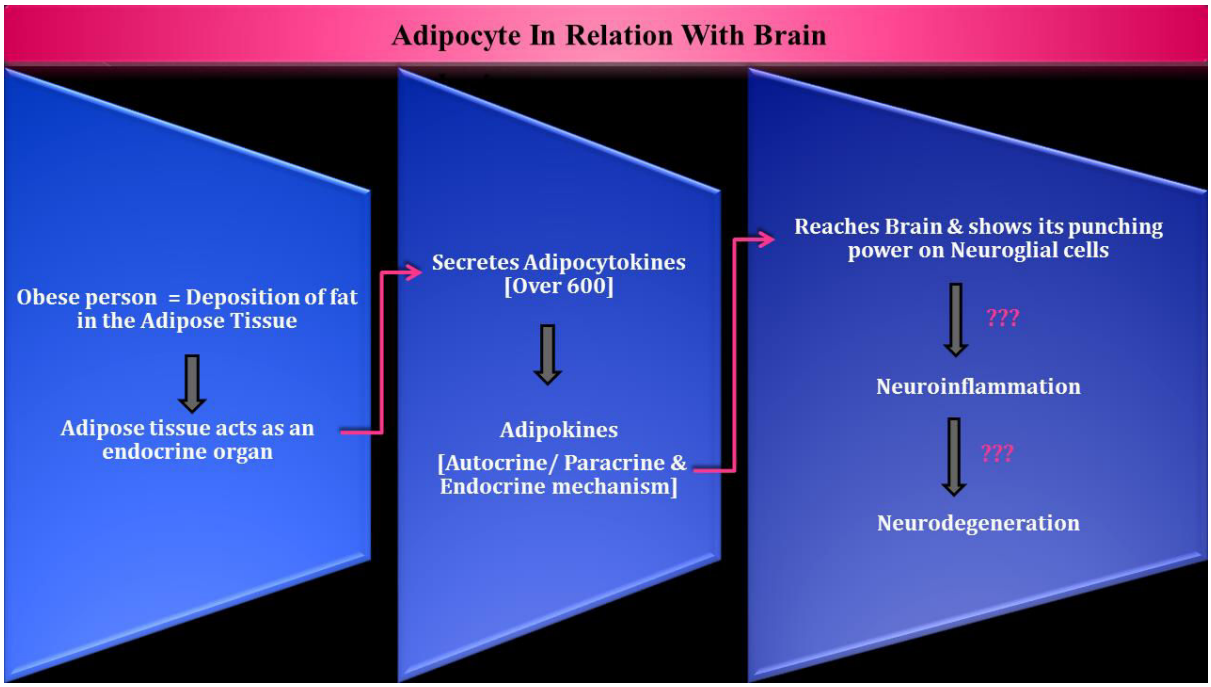
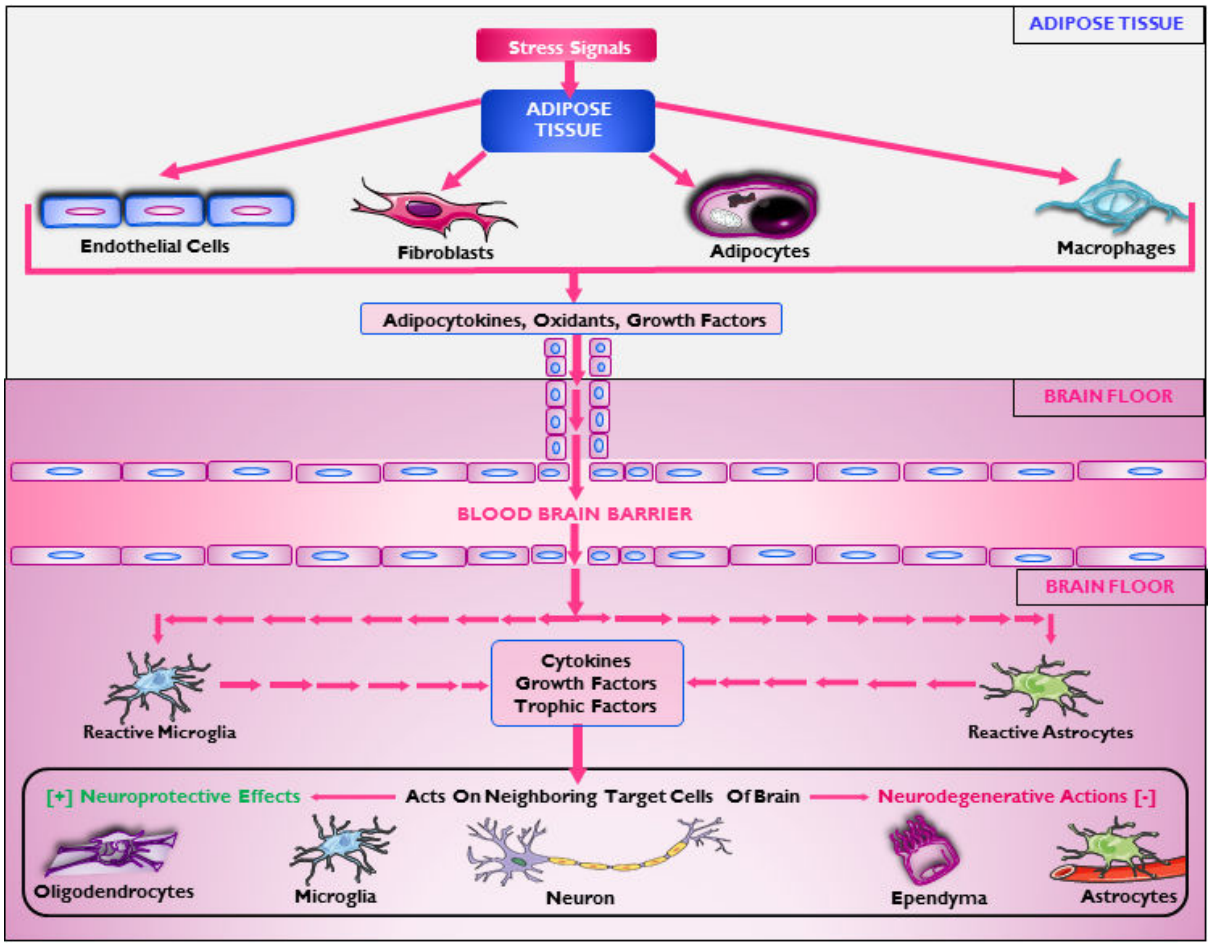


FIGURE VI-5 Adipocyte in Relation with Brain: Consumption of energy dense food rich in saturated fats, sugars, sedentary life styles and reduced physical activity contributes to accumulation of fat in the adipose tissue (obesity). It is a renowned fact that adipose tissue acts as an endocrine organ and secretes adipocytokines (over 600 in number) which have

diversified range of actions inside the cell. Secreted adipocytokines just because of their autocrine, paracrine and endocrine properties, make use of blood as a transporting vehicle to reach other systems. This includes, the brain and shows their potential effects on the neuroglial cells thereby elicits neuroinflammatory response that may eventually progress to life threatening neurodegenerative disorders (Alzheimer's and Parkinson's disease) (**?** – symbolizes some uncertainty).



Sequence: Stress Signal/Adipose Tissue/Adipocytokines/BBB/Glia/Adipocytokines/Target Cells [+/-]

FIGURE VI-6 Relation between Adipocytokines and Glial Cells: Most probably, there are numerous factors that aggravates inflammatory response. Among them infections, trauma, aging, high fat diet, palmitate, exogenous factors (metals, dioxins, and radiations), endogenous factors (excess ROS formed due to aerobic metabolism, oxidative burst) and other factors plays a contributing role in generating stress to cells. Activated adipose tissue (Adipocytes, macrophages, fibroblasts, endothelial cells) in response to exogenous or endogenous stimulus triggers the release of array of factors including adipocytokines (Leptin, ATX, IL-1β), oxidants (NO, iNOS) and growth factors (TGF- β, HGF, IGF, VEGF). The factors thus secreted by fat tissue, just because of its endocrine properties it may reach the brain and further acts up on the brain resident microglia and astrocytes. It is worth noticing that these adipocytokines and its soluble receptors are not only expressed inside the adipose tissue but as well inside the central nervous system.

Endothelial cells present in the BBB are very stringent and permits the access to only few molecules to pass through the brain. The factors released by the adipocytes in response to the stimulus under compromised conditions crosses the blood brain barrier and reaches inside the brain. When the stimulus in the form of cytokine signalling reaches the brain, ramified resting microglia and brain resident astrocytes transforms into reactive migratory microglia (microglial activation) and activated astrocytes (reactive astrogliosis) which accelerates the recruitment of macrophages further mounts an inflammatory response as a result of peripheral inflammation (fat tissue). This reaction is further amplified or carried over by the glial cells and the same cytokines, nerve growth factors and trophic factors will be generated within selected regions of the brain. The factors thus produced can be pro or anti (inflammatory/oxidative) and further acts up on the neighboring target cells like microglia, oligodendrocytes, neurons, ependymal cells, astrocytes and blood derived macrophages. If the produced cytokines are anti-inflammatory, it will confer some neuroprotective effects such as glutamate uptake, neurotropic release, and trophic factors release. To the contrary, if the cytokines produced are pro-inflammatory they will have neurodegenerative actions on neighboring target cells such as neuroinflammation, neurodegeneration (Alzheimer's, Parkinson's), dementia (mental behavior) and central nervous system vulnerability.

Image Reference: (Parimisetty et al., 2015a)

Therefore, understanding the inter-related mechanisms of inflammation and oxidative stress coupled with glial cells (microglia, astrocytes) and adipose tissue and its potential response towards the central nervous system are of great interest to find out the basic roots of neurodegenerative disorders.

EXPERIMENTAL HYPOTHESIS

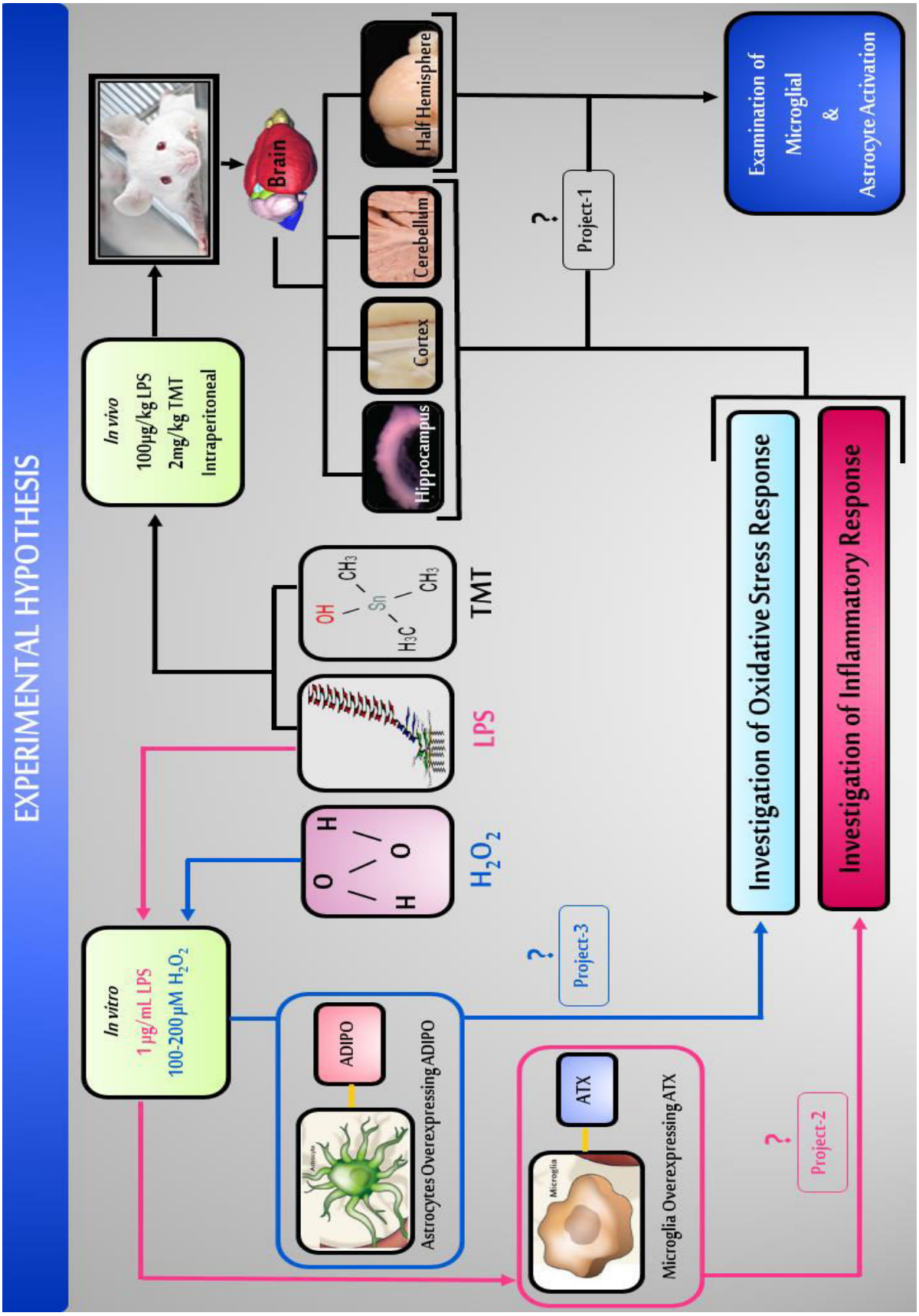


FIGURE EXP-1 Bird's Eye View On The Overall Project Design

Experimental Hypothesis

Now it is clearly evident from the scientific literature that "Obesity is one of the crucial factors in chronic inflammation that plays a potential role towards the negative modulation of glial cells in CNS (Neurodegeneration) via the release of adipocyte secreted factors (Adipocytokines) acting in an endocrine fashion." Therefore, our global aim of the research is to investigate the inflammatory properties of the factors produced by the adipose tissue (Autotaxin and Adiponectin) and its potential implication in neuroinflammation and neurodegeneration. The potential of these two factors will be evaluated, both *in vitro* in immortalized cell cultures and *in vivo* in neuroinflammatory, acute hippocampal neurodegenerative murine mice models.

In vivo

PROJECT-1:

Does peripheral inflammation induce CNS vulnerability in the mice brain?

Peripheral infection or inflammation could greatly affect the CNS in a negative context which are largely mediated by the upregulation of pro-inflammatory cytokine production. Here, we propose to use two distinct inflammatory stimuli (LPS and TMT) to characterize the expression of inflammatory factors (ATX and ADIPO) and to investigate glial cell activation (microglia and astrocyte) in mouse CNS. Acute intraperitoneal (ip) injection of lipopolysaccharide (LPS) (100 µg/Kg bwt) mimics gram negative bacterial infection, while acute ip injection of organometal trimethyltin (TMT) (2mg/kg bwt), induces hippocampal neurodegeneration.

In vitro

PROJECT-2:

Does overexpression of ATX downregulate LPS induced microglial activation?

Microglial cells are the major source of inflammatory factors in the brain and to investigate the role of ATX on these cells in inflammatory condition, we generated stable over-expressing transfectant in murine microglia BV2 cells for Autotaxin. BV2 and stably transfected, overexpressing clones were treated with LPS (1 µg/mL) and investigated for the presence of inflammatory response.

PROJECT-3:

Does overexpression of ADIPO protect astrocytes against H_2O_2 induced oxidative stress?

Astrocytes are a potential sources in generating oxidative stress factors in the brain and to investigate the role of ADIPO on these cells in oxidative stress condition, we generated stable over-expressing transfectant in murine Astrocyte CLTT cells for Adiponectin. CLTT and stably transfected, overexpressing clones were treated with H_2O_2 (100-200µM) and investigated for the presence of oxidative stress response.

Henceforth, comprehending the inter-related mechanisms between factors secreted by the adipose tissue (adipocytokines) and its potential response towards the central nervous system especially on glial cells (microglia and astrocytes) coupled with inflammation and oxidative stress physiological process are of great interest to find out the basic roots of neurodegenerative disorders and to fight against them.

RESULTS

PROJECT-1

Adiponectin, Resistin and Autotaxin Expression in Neuroinflammation and Neurodegeneration

Project-1: Article Introductory Preface

Adiponectin, Resistin and Autotaxin Expression in Neuroinflammation and Neurodegeneration

Aim: Does peripheral inflammation induce CNS vulnerability in the mice brain?

Experimental Design:

- Mice were handled, and sacrificed in accordance with the European Union regulations and strict efforts were taken concerning the protection of experimenting animals.
- Housed mice were subjected to mimic neuroinflammation and neurodegeneration with LPS and TMT at a concentration of 100µg/kg and 2mg/kg bwt respectively.
- Brains were sagittally sectioned and half of the hemisphere were used for immunohistochemistry and from the rest of the hemisphere hippocampus, cortex and cerebellum tissues were collected to study the gene expression of factors produced in response to LPS induced peripheral inflammation.
- Gene expression of TNF-α, iNOS, ADIPO, RES and ATX mRNA levels were quantified by using quantitative real time PCR (qRT-PCR) approach.
- LPS stimulated microglial activation and reactive astrogliosis were examined by immunohistology.

Principal Findings:

- ✓ Inflammatory mediators are expressed in the brain in response to i.p LPS and TMT peripheral stimulations.
- ✓ Peripheral inflammation could induce a transient neuroinflammatory response in three distinct regions of the brain (HIP-COR-CER) that involved inflammation and oxidative stress physiological processes.
- ✓ Peripheral inflammation induced by infection will not induce neurodegeneration unless a massive infection, but could prime the glial cells and make them more responsive to the next stimulation.

Biological Sciences - Neuroscience

ADIPONECTIN, RESISTIN AND AUTOTAXIN EXPRESSION IN NEUROINFLAMMATION AND NEURODEGENERATION

Avinash Parimisetty [1], Nicolas Diotel [1], Cynthia Planesse[1], Aurelie Catan [1], Dorothée Girard[1], Christian Lefebvre d'Hellencourt[1]*

[1] ***INSERM UMR 1188 Diabète athérothrombose Thérapies Réunion Océan Indien (DéTROI), Sainte-Clotilde, F-97490, France***

2 Université de La Réunion, UMR 1188, Sainte-Clotilde, F-97490, France - Groupe d'Etude sur l'Inflammation Chronique et l'Obésité (GEICO), Plateforme CYROI- Fédération Environnement Biodiversité Santé, Université de La Réunion- 15, Avenue René Cassin - BP 7151- Cedex 9 97715 Saint Denis Messag - France

Address all correspondence: Prof. Christian Lefebvre d'Hellencourt
UMR 1188 Diabète athérothrombose Thérapies Réunion Océan Indien (DéTROI), Sainte-Clotilde, F-97490, France

Tel: (+262) 262 93 82 01 - Fax: (+262) 262 93 82 37

E-Mail: Christian.Lefebvre-d-Hellencourt@univ-reunion.fr

Grant Sponsor: Region La Reunion, Europe (CPER/FEDER)

ABBREVIATIONS

ADIPO	Adiponectin
ANOVA	Analysis of Variance
AP-1	Activator Protein-1
ATX	Autotaxin
BBB	Blood Brain Barrier
BSA	Bovine Serum Albumin
CER	Cerebellum
CNS	Central Nervous System
COR	Cortex
COX	Cyclooxygenase
CREB	cAMP Response Element-Binding Protein
DEPC	Diethylpyrocarbonate
FFA	Free Fatty Acids
GAPDH	Glyceraldehyde-3-Phosphate Dehydrogenase
GFAP	Glial Fibrillary Acidic Protein
GOI	Gene of Interest
HIP	Hippocampus
IL-1	Interleukin-1
IL-6	Interleukin-6
i.p	Intraperitoneal
iNOS	inducible Nitric Oxide Synthase
LPA	Lysophosphatidic Acid
LPC	Lysophosphatidyl Choline
LPS	Lipopolysaccharide
MCP-1	Macrophage Inflammatory Protein-1
NF-kβ	Nuclear Factor-Kappa β
NO	Nitric Oxide
Nrf2/ NFE2L2	Nuclear Factor Erythroid-Derived 2-Like 2
PBS	Phosphate Buffered Saline
PG	Prostaglandin
RES	Resistin
TMT	Trimethyltin Hydroxide
TNF-α	Tumor Necrosis Factor-α

ABSTRACT

Background: Peripheral immune system activation stimulated in response to inflammation induces neuroinflammation in the central nervous system (CNS) with an elevated cytokine production. In spite of the accumulating data suggesting the involvement of cytokines in regulating neuroinflammation, the precise pathways responsible for the onset of these inflammatory changes in the CNS remains uncertain.

Objective: We aimed at characterizing gene expression of inflammatory potential mediators generated in response to intraperitoneal lipopolysaccharide (LPS) and trimethyltin (TMT) stimulations. In parallel, we also examined microglial activation and reactive astrogliosis

Methodology: Here, we propose to use two distinct stimuli to characterize the expression of potential inflammatory mediators on LPS and TMT challenged mouse brains via intraperitoneal injections, and examined the onset of inflammatory response and glial cell activation in different regions of the brain (hippocampus, cortex, and cerebellum). Acute intraperitoneal (i.p) injection of LPS (100 µg/Kg bwt) mimics gram negative bacterial infection, while acute ip injection of organometal trimethyltin TMT (2mg/kg bwt), induces hippocampal neurodegeneration. Gene expression mRNA levels of different factors (TNF-α, iNOS, Autotaxin (ATX), Adiponectin (ADIPO) and Resistin (RES)) on distinct regions of the brain including hippocampus (HIP), cortex (COR) and cerebellum (CER) were determined by qRT-PCR, whereas microglial activation and reactive astrogliosis on the hemispheres of the brain were assessed by immunohistological approach.

Results: LPS-induced peripheral inflammation resulted in an early response of TNF-α and iNOS in the hippocampus, cortex and cerebellum with a peak at 2-4 hours, while ADIPO showed its expression peak at 6, 120, 24 hours in hippocampus, cortex and cerebellum respectively. Whereas RES was significantly upregulated at 6h only in hippocampus, while no significant changes were observed for ATX. We confirmed that acute i.p injection of TMT heightened TNF-α and iNOS mRNA levels at 24 and 4 hours respectively, while elevated ATX and ADIPO mRNA levels in the hippocampus were demonstrated at 5 and 8 days respectively. Following i.p LPS injections, no microglial or astrocytes activation was observed by immunohistology.

Conclusion: Taken together from our experimentational outputs our results confirm that inflammatory mediators are upregulated *in vivo* in the brain in response to i.p LPS and TMT injections and suggests that adipocytokines may play a role in the regulation of these induced neuroinflammation. The precise functions and mechanisms associated with these factors remain to be elucidated.

Key words: Peripheral Inflammation, LPS, Cytokines, Neuroinflammation, TMT, Neurodegeneration.

INTRODUCTION

Over the decades ago brain was considered as an immune privileged organ that depicted reduced inflammatory immune response. But the recent advances in neuroimmunology research unmasks the fact that, brain as well can exert the hall mark features of inflammation such as generation of inflammatory cytokines including the production of pro-inflammatory cytokines and free radicals by the resident cells of the CNS. This in turn induces the recruitment and local invasion of circulating immune cells and further leads to glial cell activation (Lucas et al., 2006a; Medzhitov, 2008). Brain is capable of influencing immune response via cytokine signalling at the same time immunological responses are capable of controlling brain (Wilson et al., 2002).

Growing body of evidences suggests interaction between the peripheral immune system and the central nervous system mediated via cytokine signalling (Stitt, 1986; Saper and Breder, 1992; Dantzer et al., 2000). Cytokines are the chemical messengers between immune cells and play a crucial role in mediating inflammatory and immune responses. They act as neuromodulators within the brain and have a potential impact both on normal and pathological conditions (Deverman and Patterson, 2009; Qi et al., 2009). Numerous studies have provided evidences that peripheral inflammation triggered acute phase reaction in the CNS which are largely mediated by pro-inflammatory cytokines such as TNF-α, IL-1β, IL-6 and MCP-1. The peripheral cytokines generated in response to inflammation under compromised conditions can cross the blood brain barrier, reaches the brain parenchyma and can signal the CNS via four possible mechanisms: a) passive transport of cytokines into the brain at the sites having leaky or lacking blood-brain barrier system, b) binding of cytokines into the cerebral vascular endothelium, thereby inducing the generation of secondary messengers such as prostaglandins (PG) and nitric oxide (NO), c) carrier-mediated transport of cytokines into the brain, across the blood-brain barrier, and d) activation by cytokines of peripheral afferent nerve terminals at the site where cytokines are released (Freidin et al., 1992; Kronfol and Remick, 2000).

Inflammation up to certain extent will be beneficial to the host system, it will help to the clearance of pathogens. However, uncontrolled and or prolonged inflammation may damage the tissues. Therefore, inflammatory response must be actively terminated when no longer needed to prevent unnecessary damage (Collins et al., 1998). Many chronic neurodegenerative diseases such as Alzheimer's, Parkinson's and amyotrophic lateral sclerosis have been associated with inflammation in the central nervous system. Inflammation in the CNS are

largely mediated by glial cells in which microglia and astrocytes play a dedicated role. They execute essential operations such as cytokine secretion, promotion of cell repair and performing scavenging activities in cleansing the brain in order to maintain a healthy and viable brain environment that should be free from cellular debris and toxic materials (Amor et al., 2010; Lull and Block, 2010).

Lipopolysaccharide is the outermost layer of gram negative bacteria and induces a strong innate immune response. It acts as an endotoxin which promotes the secretion of pro-inflammatory cytokine production and reactive oxygen species generation (nitric oxide and superoxide) (Abbas and Abul, 2006). Acute exposure of mice to TMT results in extensive damage to dentate granule cells (Reuhl et al., 1983), accompanied by early activation of glial cells (Bruccoleri et al., 1998; Eskes et al., 2003). It is widely accepted that intraperitoneal administration of gram negative bacterial endotoxin (LPS) generates the peripheral production of pro-inflammatory cytokines which then crosses the BBB and reach the CNS. Once this inflammatory stimulus/mediators reaches the CNS, this reaction is further amplified and carried over by the glial cells and the same cytokines will be generated within selected regions of the brain. (Kronfol and Remick, 2000). A study led by Kozak et al., demonstrated that injecting high doses of LPS (500µg/kg) resulted in severe systemic inflammation and development of fever which reflects the symptoms of septic shock in mice (Kozak et al., 1997). Besides this, it is also reported that animals and humans typically encounters infectious pathogens that replicate in vivo and are exposed to lower concentrations of LPS over a more prolonged period of time (Teeling et al., 2007).

Adipose tissue is highly active metabolic and endocrine organ. It produces an assortment of factors (also known as Adipocytokines) which includes hormones, inflammatory mediators such as cytokines (TNF-α, IL-6, Leptin, Adiponectin), chemokines (MCP-1, MIP-1, RANTES) or adipolipokines (LPA via Autotaxin) (Blüher and Mantzoros, 2015). These adipocytokines have versatile biological activities in specified host system (Gale et al., 2004; Sahin-Efe et al., 2012; Bluher and Mantzoros, 2015) such as neuroendocrine regulation, inflammation and functions of the immune system (Van Gaal et al., 2006; Catalan et al., 2009; Bluher, 2012; Sahin-Efe et al., 2012; Bluher, 2014) in the target organs including the brain and immune system. For example, Adiponectin exhibits direct anti-inflammatory and anti-diabetic, effects (Yamauchi et al., 2001). Autotaxin is a secreted enzyme well known for its roles in cell motility, cell migratory and proliferation, notably in tumorigenesis (Nishimasu et al., 2011b), but the inflammatory properties of this particular adipocytokine in the CNS remains to be determined.

TNF-α is a known pro-inflammatory adipocytokine that facilitates the dysregulation of insulin sensitivity, and in a contradictory way TNF-α promotes the elevation of acute phase reactants (IL-1, IL-1β, IL-6). Resistin is an insulin resistance aggravating factor largely produced by the adipose tissue with direct pro-inflammatory and pro-diabetic effects (GS et al., 1994). Besides these adipocytokines, iNOS is a pro-oxidative signalling factor which is well-known for its potential oxidative stress mimicking actions. (Green et al., 1994)

It is also worth noticing that these cytokines and their corresponding soluble receptors are not only produced by the adipose tissue but as well expressed in the CNS (Kronfol and Remick, 2000). For example, there are substantial evidences showing that adiponectin receptors (AdipoR1 and AdipoR2) are widely expressed in the regions of the mouse hypothalamus, brainstem, cortical neurons, endothelial cells, pituitary extracts and as well as in whole brain (Thundyil et al., 2012a), whose (AdipoR) expression levels will be diminished during inflammatory status. In addition to this, apart from adipocytes and cancerous cells, central nervous system is another major hotspot for the Autotaxin's produced lysophosphatidic acid (LPA) receptor expression (Contos et al., 2000; Fukushima et al., 2001; Ishii et al., 2001) regulates the activity of various neural cell types, such as neural cell lines, neural progenitors, primary neurons, oligodendrocytes, Schwann cells, astrocytes, and microglia (Choi et al., 2010), and their whose (ATX) expression levels will be upregulated during pathological conditions.

In spite of the extensive research and the growing body of evidences accumulating on this neuroimmunological pathways, the precise mechanisms responsible in establishing the link between the immune system activation and CNS inflammation via peripheral infection still remains questionable. In our current study, potential neuroinflammatory response in the mice were induced by using LPS. To mimic neurodegenerative effects, we employed a chemical-induced model of hippocampal damage using the prototypic neurotoxicant, trimethyltin (TMT). Therefore we aimed to characterize the gene expression of inflammatory mediators generated in response to activated peripheral immune system as a result of defined intraperitoneal LPS and TMT stimulations. In addition to this, we also examined microglial activation and reactive astrogliosis (glial cell activation) with respect to LPS stimulus. The results thus obtained should bring about some knowledge in understanding the mechanisms underlying immune to brain interactions and CNS vulnerability stimulated via peripheral inflammation.

Altogether, our results put forwards that peripheral infection could induce a transient neuroinflammatory response in three distinct regions of the brain (HIP-COR-CER) that involved inflammatory (TNF-α, Adiponectin, Autotaxin, and Resistin) and oxidative stress mediators (iNOS) provoked by LPS and TMT challenges. Besides this, our observations on the absence effect of microglial and astrocyte activation suggests that peripheral inflammation induced by infection, will not induce neurodegeneration (unless a massive infection) but could prime the glial cells making them to be more responsive for further stimulation, and can generate transient neuroinflammatory response in the brain.

MATERIALS & METHODS

1. Mice facility or Mice housing:

Fifty six day old OF1 mice and twenty-one day old CD-1 mice were randomly assigned to experimental groups and housed in air conditioned animal facility of the Cyclotron Réunion Océan Indien (CYROI). Mice were maintained in a controlled environment under constant temperature (21° ± 2°C), humidity (50% ± 5%) and photoperiod (12-hours light/12-hours dark in order to habituate to the environment) with free access to food and water. For the sacrifice, mice were euthanized with 4% isoflourane and the complete brain tissue was collected. Half of the hemisphere were fixed in 4% paraformaldehyde-PBS for immunohistochemistry and from the rest of the hemisphere hippocampus, cortex and cerebellum tissues were collected in order to study the gene expression of factors produced in response to peripheral inflammation. The brain samples were flash frozen immediately in dry ice and stored at -80°C.

This study was approved by the regional research ethical committee for animal experimentation of the Cyclotron Réunion Océan Indien (CYROI) and as well by the French government. Mice were kept, handled, and sacrificed in accordance with the European Union regulations and strict efforts were taken concerning the protection of experimenting animals.

2. LPS and TMT Treatment:

Randomly assigned experimental groups of mice were administered a single intraperitoneal (i.p.) injection of either 100ug/kg bwt of LPS (*Escherichia coli* 0111:B4, Sigma, France) or saline (injection vol., 4 ml/kg). In a similar context in order to study the neurodegenerative response, randomly assigned experimental groups of mice were administered a single i.p. injection of either 2 mg/kg trimethyltin hydroxide (TMT, originally obtained from Alfa

Products, Danvers, MA, USA) or saline (injection vol., 2 ml/kg). At 0, 1, 2, 4, 6, 12, 24, 72, 120 and 192 hours after LPS treatment, mice were anesthetized with 4% isoflourane and were cervically dislocated. The brain was quickly removed after decapitation and separated into hemispheres, cerebellum, hippocampus and cortex and stored at -80°C until further assay.

3. RNA extraction:

Flash frozen brain samples stored at -80°C were carefully thawed in ice and all the further experimentation were carried out in ice. Hippocampus, cortex and cerebellar brain tissue were homogenized by the use of Qiagen tissue lyser according to the manufacturer's instructions. After homogenization, total RNA extraction was performed using TRIzol Reagent (Invitrogen Life Technologies, USA) according to the manufacturer's protocol. The RNA samples were resuspended in 35 µL of DEPC (Diethylpyrocarbonate) treated nuclease-free water. The concentration and quantification of total RNA was measured with Eppendorf Biophotometer, with the OD260/OD280 ratio of all RNA samples 1.6-2.0.

4. Quantification of gene expression by using Real Time PCR machine:

Two micro gram of RNA was reversed transcribed to cDNA using random hexamer primers (Sigma, St. Louis, MO) and Moloney Murine Leukemia Virus (MMLV, Invitrogen). Each sample was tested in triplicate. Complementary DNA was amplified by PCR (Applied Biosystems, France) using the SYBR green master-mix (Eurogentec, Belgium) and specific murine primers (Table I, Eurogentec). Each PCR cycle was conducted for 15 s at 95 °C and 1 min at 60 °C. RNA from adipose tissue (AT) of ob/ob mice treated with LPS were used to detect the gene expression of interest (GOI) of TNF-α, iNOS, ATX, Adipo, Res and GAPDH. When once detected, these adipose tissues were used for further assaying to generate standard curves (RNA standard) and as well to investigate the efficiency of the PCR with increasing dilutions of cDNA expressing the studied gene. Relative RNA amounts were calculated with relative standard curves for each mRNA of interest and GlycerAldehyde-3-Phosphate DeHydrogenase (GAPDH). Normalization against the house keeping gene GAPDH was conducted to account for experimental variability in terms of quality, concentration of total RNA, and RT efficiency. Results were analyzed using ABI Prism 7000 Sequence Detection software version 1.2.3.

TABLE RES-1 Primers tested for this study – Quantitative Real Time PCR		
Gene	Sense Sequence	Anti-Sense Sequence
GAPDH	5′- TTCACCACCATGGAGAAGGC-3′	5′- GGCATGGACTGTGGTCATGA-3′
TNF-α	5'-TGGCCTCCCTCTCATCAGTT-3'	5'-GCTTGTCACTCGAATTTTGAGAAG-3'
iNOS	5′-GCAGCCTGTGAGACCTTTG-3′	5′-GCATTGGAAGTGAAGCGTTTC-3′
ATX	5'-TCCTGGAGAGAAGGGAGAGAAAG-3'	5'-CAGCTCCTGTCATTCCAACATC-3'
ADIPO	5'-GACCCTAAAGCCATTATTGCTAA-3'	5'-GGGAAGGTGCTGTTTCATGT- 3'
Resistin	5′-GTA-CCC-ACG-GGA-TGA-AGA-ACC-3	5′-GCA-GAC-CCA-CAG-GAG-CAG-3

5. Immunohistochemistry (IHC):

Immunohistochemistry and microglia labelling was performed on 10µm cryostat section. In brief, frozen brains were embedded in Tissue-Tek O.C.T. compound (product: 361603E, VWR Prolabo) before getting cut and mounted. Slides were allowed to dry for 20 minutes at room temperature and post-fixed in 4%PBS-PFA. They were next washed in PBS (2 x 10 minutes) and antigen retrieval was performed in sodium citrate (pH 6) at 80°C for 5 min. For microglia labeling, slides were rinsed in distilled water then with PBS-triton 0.1% containing 1mM $CaCl_2$ and $MgCl_2$ (PBST-Mg/Ca). A blocking step was performed immersing sections in PBST-Mg/Ca with 1% BSA. Microglia labeling was realized by incubating slides for 2h with GSL I - isolectin B4 (FL-1201, Vector Labs). Sections were rinsed in PBST-Mg/Ca and cell nuclei were stained with DAPI. For astrocytes labeling, they were rinsed in PBS-triton 0.1% containing 1% BSA and incubated for 2h with anti-GFAP coupled with 488 dye (53-9892-80, e-Biosciences). Slides were finally rinsed in PBS-triton 0.1% and counterstained with DAPI before being mounting with the antifading medium Vectashield (Vector Laboratories Inc. Burlingame, CA) before conservation in a fridge at 4°C in the obscurity.

6. Nikon fluorescent microscopy:

Observations were carried out on an Eclipse 80i Nikon fluorescence microscope equipped with a Hamamatsu ORCA-ER digital camera. Micrographs were taken in the TIFF format using NIS Elements Software, allowing image superposition. Images were then prepared with ImageJ software for brightness or contrast adjustment before preparation of the figures. The

nomenclature is according to the Mouse Brain in Stereotaxic Coordinates, Third Edition by Keith B.J. Franklin and George Paxinos (Sep17, 2007).

7. Statistical data analysis:

Gene expression data were analysed by one-way analysis of variance (ANOVA) followed if significant by Tukey post-test for comparison of all pairs of data with multiple time points versus controls using GraphPad PRISM 5.01 software. Values were expressed as mean ± SEM with an N size of 3-8 performed in triplicate. A P-value of <0.05 was considered to indicate statistical significant difference.

RESULTS

Dynamic expression of inflammatory markers - A time frame in vivo LPS kinetic study:

In order to investigate the dynamic expression of inflammatory marker, we intraperitoneally injected OF1 mice with LPS (100µg/kg body weight). Next, we sacrificed mice at different time points (0, 2, 4, 6, 12, 24, 72, 120 hours) and we analyzed inflammatory factor (TNF-α) fat tissue related factors (Adiponectin Autotaxin, Resistin) and oxidative stress factor (iNOS) gene expression using quantitative real time PCR in three brain regions: the cortex, the hippocampus and the cerebellum.

The expression levels of the pro-inflammatory cytokine TNF-α were found to be significantly upregulated at 2 hours point of time period in the three regions of the brain: hippocampus, cortex and cerebellum, and still significantly increased at 4 hours in the hippocampus. Its expression levels plunged down to its basal control levels at almost 12 hours point of time (Fig.1 A, Fig.2 A, and Fig.3 A).

A similar patterned expression of TNF-α's levels were evident in case of iNOS for hippocampus and cerebellum. iNOS showed its transient expression peak at 4-6 hours points post injection in hippocampus, while the upregulation occurred at 2-4 hours in the cerebellum (Fig.1 B and Fig.3 B) but not in cortex (Fig.2 B) and their corresponding expression levels returned to its basal levels from 6 to 12 hours in the three brain regions studied.

In response to LPS, the expression levels of anti-inflammatory cytokine adiponectin were found to be triggered at 6 hours, (Fig.1 C) 120 hour (Fig.2 C) and 24 hours (Fig.3 C) in hippocampus, cortex and cerebellum respectively.

Transcriptional mRNA levels of Resistin showed its peak at 6 hours point of time period in hippocampus (Fig.1 D), whereas resistin failed to reach statistical significance in cortex and cerebellar tissues (Fig.2 D and Fig.3 D).

Autotaxin mRNA levels were not statistically modified in the three regions of the brain (Fig.1 E, Fig.2 E and Fig.3 E).

Dynamic expression of inflammatory markers - A time frame in vivo TMT kinetic study:

Similar experiments were conducted with TMT (2mg/kg bwt) administrated intraperitoneally into mice. *In vivo* inflammatory effects of TMT on OF1 mice were determined by using real time PCR in hippocampus with variable points of time periods (0, 2, 4, 6, 12, 24, 72, 120, 192 hours).

In presence of 2mg/kg bwt of i.p TMT injection, the pro-inflammatory cytokine TNF-α showed its expression peak at 24 hours point of time period (Fig. 4 A) and the pro-oxidative factor iNOS at 4 hours (Fig. 4 B), whereas the anti-inflammatory cytokine Autotaxin and Adiponectin reached its statistical significant inflammatory response at 5 and 8 days respectively (Fig. 4 C and Fig. 4 D).

Mild dosage of 100µg/kg LPS induced neuroinflammation but not glial cell activation:

56 day old mice were subjected to low grade chronic inflammation by the administration of intraperitoneal LPS injections at concentration of 100µg/kg. Following LPS injections brains from mice for each time point of LPS treatment were investigated for the presence of microglial activation and reactive astrogliosis (glial cell activation) at specific points of time period including 24, 72, 120, 192 and 360 hours and were relatively compared to the PBS saline vehicle controls. Since TMT is well known for its potential impact in inducing microglial activation and reactive astrogliosis, we used TMT as our positive controls to compare the labelling between LPS and TMT treated mice brains. Isolectin B4 conjugates were employed to label microglial activation and glial fibrillary acidic protein GFAP antibodies were used to detect reactive astrogliosis in corresponding brain hemispheres with prime focus on dentate gyrus of the hippocampal brain regions.

Following i.p LPS injections, no microglial nor astrocyte activation was observed with lectin and glial fibrillary acidic proteins labelling respectively (Figure 5). The expected reactive microglia cells were observed 3 days post TMT injection as well as reactive astrogliosis (Figure 5).

DISCUSSION

Communication between the cytokine network and brain is essential to maintain homeostasis of the CNS (Peferoen et al., 2014). Over the years, numerous studies have provided an insight into how the brain sense the presence of peripheral inflammation, but still the precise mechanisms responsible for the onset of this inflammatory response remains highly debatable. To further study the mechanisms underlying these observations, we subjected the mice to low grade chronic neuroinflammation and neurodegeneration with LPS and TMT treatments respectively and the potential mediators of neuroinflammatory, neurodegenerative response and the glial cell activation (microglial activation and reactive astrogliosis) in the mice brains were assayed by using quantitative real time PCR and immunohistological approaches.

Inflammation in the CNS is differentially and tightly regulated in distinct regions of the brain such as hippocampus, cortex and cerebellum which are largely regulated by means of cytokines networking generated by the resident cells of the CNS (predominantly by microglia and astrocytes) in response to various environmental insults (Pierson et al., 2012; Perry and Teeling, 2013). Peripheral infection triggers transcriptional activation of genes. This reaction involves inflammatory and oxidative stress mediators and is highly dose and time dependent. This wide spread reactivity mediated by glial cells induces the expression of inflammatory genes, in which some genes are induced rapidly (early mediators of inflammation and oxidative stress - TNF-α, iNOS, Adiponectin, IL-1β, IκBα, CD14) whereas others will be detected from hours to days (late mediators of inflammation and oxidative stress - IL-6, COX, members of the complement family) (Kronfol and Remick, 2000).

Taken together with our experimentation, more precisely in order to have a comprehensive understanding of the overall inflammatory response generated, we studied different localities of the brain (HIP, COR, CER) at defined points of time period (PBS Saline, 2h, 4h, 6h, 12h, 24h, 72h, 120h, 192h and 360h) with defined dosage levels (LPS-100µg/kg, TMT-2 mg/kg) that should fetch us a logical conclusion of the overall coordinated brain inflammatory response happening in the CNS.

LPS is a known inflammation inducing agent which triggers innate immune response characterized by the production of cytokines and immune system activation. Humans are more sensitive to LPS than mice, which can tolerate a dose up to a thousand times higher. For instance a dose of 1 µg/kg induces shock in humans (Warren et al., 2010). In conjunction with

the previous report, administration of Salmonella equi LPS (0.8 ng/kg) to healthy young human subjects resulted in negative effects on verbal and non-verbal declarative memory functions (long-term memory of human beings) and depression (Reichenberg et al., 2001; Cohen et al., 2003). TMT is potential neurotoxicant well known for its neurodegenerative effects on the brain especially on hippocampus (Harry et al., 2004) and acts via TNFa pathway which plays a critical role for this neurodamage.

In the current study we used LPS as a potential inflammation mimicking agent to study neuroinflammatory response in hippocampus, cortex and cerebellum, whereas TMT for studying neurodegenerative response of the hippocampus in the murine models. When once the LPS and TMT have been intraperitoneally injected, it diffused into the mice system,

Peripheral infection induced transient neuroinflammatory response in the brain:

Adiponectin is a translation product of AdipoQ gene. The major actions of adiponectin includes the promotion of insulin sensitivity, increased glucose uptake, clearance of free fatty acids (FFA). It was also reported that, adiponectin antagonizes and counteracts the effects of TNF-α by negatively regulating its expression in various tissues such as liver and macrophages. (Ouchi and Walsh, 2008; Moschen et al., 2012) via the inhibition of endothelial nuclear factor kappa beta (NF-kβ) signalling through cAMP dependent pathway (Zhang et al., 2013). Adiponectin is the most abundant cytokine in the circulation but the levels of adiponectin are inversely proportional in obesity and diabetic subjects. Autotaxin also known as Lysophospholipase D (LysoPLD) is a type II ectonucleotide pyrophosphate phosphodiesterase secreted enzyme that catalyzes the transformation of albumin bound or membrane-derived lysophosphatidylcholine (LPC) to produce equimolar amounts of LPA and choline (Tokumura et al., 2002; Umezu-Goto et al., 2002) with the help of Lysophospholipase D activity. The specific binding of LPA to its LPA receptors triggers various physiological activities including blood vessel development, parturition, adipocytes differentiation and cellular proliferation. Elevated levels of Autotaxin expression was enhanced in frontal cortex of Alzheimer-type dementia patients as reported by (Umemura et al., 2006), multiple sclerosis [Hammack et al., 2004] and cell type ATX specific expression in the brain has been upregulated during development and after neurotrauma (Savaskan et al., 2007). Despite the fact that, the role of these specific targets (ATX and ADIPO) in various physiological functions have been well defined, but the potential role of these inflammatory cytokines in CNS vulnerability still remains controversial.

This study showed the transcriptional mRNA expression of pro and anti-inflammatory cytokines in the neuroinflammatory LPS and neurodegenerative TMT models. A similar kind of study had also demonstrated the heightened production of IL-6, IL-1β and TNF-α in brain followed by intraperitoneal LPS injection (Teeling et al., 2010).

Here in this present study for the first time we showed that, peripheral stimulation of OF1 mice induced a significant upregulation of ADIPO mRNA levels at 6, 120 hours (5 days), 24 hour in hippocampus, cortex and cerebellum respectively in LPS model, and at 192 hours (8 days) in hippocampus of TMT model. In addition to this we also demonstrated that, a significant increase of ATX mRNA expression could be detected at 120 hours (5 days) in the hippocampus of TMT treated mice, but autotaxin failed to reach its statistical significance in hippocampus, cortex and cerebellum in LPS treated OF1 mice. Recently we demonstrated that Autotaxin mRNA levels are expressed in microglial cells and ATX's overexpression downregulated LPS-induced microglial activation and pro-inflammatory cytokine production (TNF-α, IL-6) and elevated the upregulation of the anti-inflammatory cytokine (IL-10) suggesting that ATX could play a role in controlling neuroinflammation (Awada et al., 2014).

Peripheral cytokines produced in response to inflammation are regulated in cascades, where the possible generation of one cytokine tends to augment the production levels of another cytokines and so on. For example, interleukin-1 stimulates the release of IL-2, IL-6, and TNF-α. Circulating or endothelial cytokines can transduce a signal to neurons in the brain through informational substances such as Nitric oxide (NO) catalyzed by the enzyme inducible nitric oxide synthase (iNOS) further leading to signal amplification (Kronfol and Remick, 2000). This statement is in strong corroboration with our *in vivo* results in which we showed that post peripheral LPS injections the genes encoding iNOS are expressed in the brain where there is a significant upregulation of iNOS mRNA levels at 4h point of time period in hippocampus, cortex and cerebellum, associated with higher levels of resistin mRNA expression at 6h point of time period only in hippocampus but not in cortex and cerebellum.

Low-grade chronic inflammation mimicked neuroinflammation but not glial cell activation: The brain is composed of about 1 to 5 trillion of supporting cells known as glia. Glial cells outnumbers the neurons in the brain. Microglia and astrocytes are the major sources of inflammatory factors that plays a decisive role in mediating inflammatory response in the CNS. Glial cell activation is a hallmark feature of neurodegenerative disorders in which microglial activation triggered in response to infectious agents have been implicated and widely studied

in various neuroinflammation mediated neurodegenerative diseases such as Alzheimer disease (Mosher and Wyss-Coray, 2014; Johansson et al., 2015), Parkinson disease (Le et al., 2001; Gao et al., 2002; Gao et al., 2003) amyotrophic lateral sclerosis (Koutsilieri et al., 2002) and multiple sclerosis (Smith, 2001; Nelson et al., 2002; Klesney-Tait et al., 2006). Besides microglia, astrocytes are another cell type that responds to all sorts of insults in the CNS by a process known as reactive astrogliosis or astrocyte activation (Sofroniew and Vinters, 2010). The striking features of glial cell activation includes: the exhibition of cellular hypertrophy, increased phagocytic activity, have characteristic bushy appearance, exhibits remarkable morphological and phenotypic changes, upregulates the expression of inflammatory and oxidative stress factors that exhibits both protective and destructive actions towards the neighboring target cells (neuronal and glial cells) (Sofroniew, 2014). In order to validate our experimental hypothesis, whether this transient neuroinflammation generated in response to LPS probably due to microglia and astrocytes, we performed immunohistochemistry on LPS treated brains with varying time points of treatment (24, 72, 120, 192, 360 hours) and compared to corresponding PBS saline vehicle controls with our primary focus centered on microglial and reactive astrogliosis. Apart from LPS, since trimethyltin (TMT) is an organometal widely used over the years as a model of inducing hippocampal damage, we used TMT treated mice brains as our positive controls in order to compare the labelling of LPS induced microglial and astrocyte activation.

Reactive astrogliosis can be characterized by the rapid synthesis of GFAP and is demonstrated by increase in protein content or by immunostaining with GFAP antibody (Eng et al., 2000). Expression of this filamentous glial fibrillary acidic protein have been found to be upregulated in various neurodegenerative disorders, traumatic brain injury and stroke (Rosengren et al., 1994; Rosengren et al., 1995; Herrmann et al., 2000; Nylen et al., 2002; Vos et al., 2004).

The results of this study demonstrated that 100µg/kg LPS induced a transient inflammation in the brain without triggering microglial and astrocyte activation

Altogether, our results suggests that (TNF-α), adipocytokines (ADIPO and RES), pro-oxidative enzyme (iNOS) and ATX are expressed *in vivo* in the brain in response to LPS and TMT treatments. These data showed that peripheral infection could induce a transient neuroinflammatory response in three distinct regions of the brain (HIP-COR-CER) that involved inflammatory cytokines and oxidative stress. Besides this, absence effect of astrocyte and microglial activation suggests that peripheral inflammation induced by infection, will not

induce neurodegeneration (unless a massive infection) but could prime the glial cells and make them more responsive to the next stimulation.

Conclusively our results suggest that from the site of injection signals propagate from the periphery and reaches the CNS and that there is a potential link between peripheral infection, immune system activation and neuroinflammatory response in the CNS. Therefore, understanding the inter-related mechanisms of inflammation and oxidative stress generated as a result of peripheral infection coupled with glial cells and its potential response towards the CNS vulnerability are of great interest to find out the basic roots of neurodegenerative disorders that should open the doors to novel therapeutic strategies to fight against neurodegeneration.

Overall Theme Of The Project

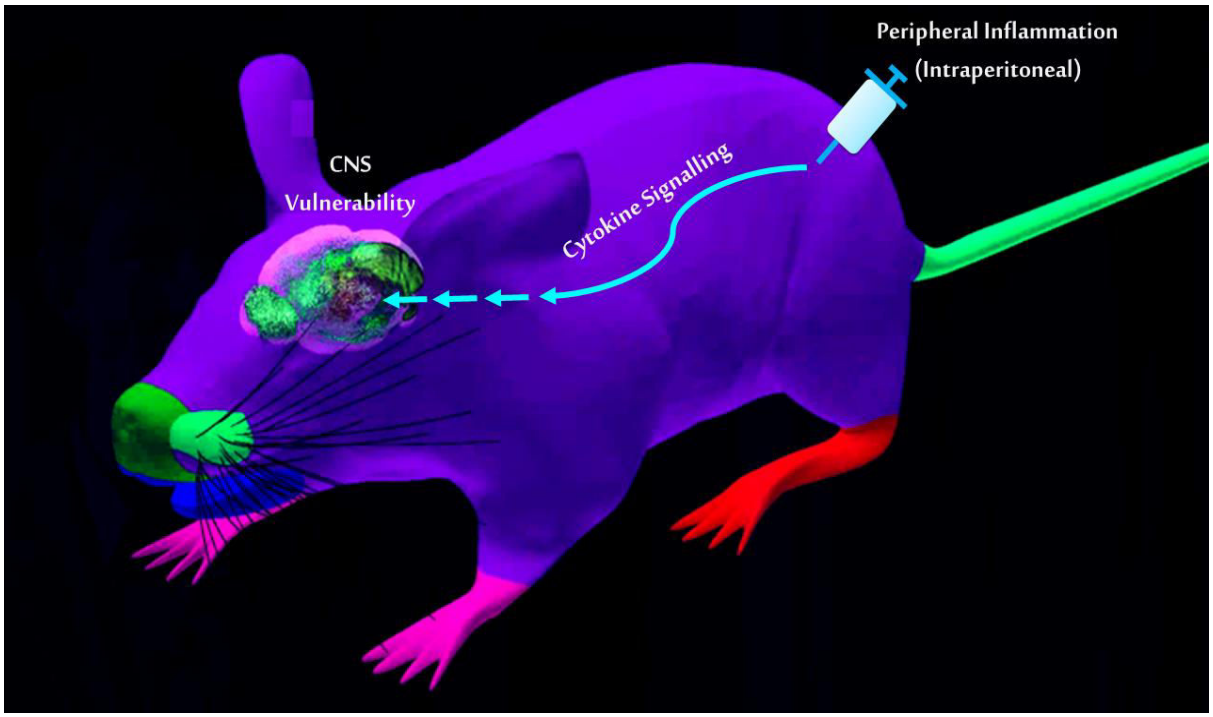


Figure: Brain senses peripheral inflammation via cytokine signalling: This hypothesis proposes the secret talk between peripheral immune system activation and the central nervous system mediated via cytokine signalling. When once the stimulus in the form of peripheral inflammation hits the system, the peripheral immune system will be activated that leads to the recruitment and activation of local inflammatory cells such as macrophages and mast cells and upregulates the elevated production of inflammatory mediators (generation of Pro-inflammatory cytokines, free radicals and chemokines). The inflammatory mediators thus generated in response to peripheral infection at the site of origin of inflammation travels inside

all along the host system acts via autocrine, paracrine and endocrine fashion and makes use of blood as a transporting vehicle to reach other systems (CNS).

Endothelial cells present in the BBB are very stringent and restricts the entry to only few molecules to pass through the brain, but under compromised conditions these inflammatory mediators might cross the blood brain barrier and reaches inside the brain. It is also worth remembering that cytokines and its soluble receptors are not only present in immune cells and adipocytes, but also well expressed in the CNS. Inflammation in the CNS are largely mediated by glial cells especially by microglia and astrocytes. When once this inflammatory stimulus/mediators reaches the CNS, this reaction is further amplified or carried over by the glial cells and the same cytokines, nerve growth factors and trophic factors will be generated within selected regions of the brain.

As a consequence of this reaction, when the stimulus in the form of stress signal (inflammatory mediators) acts upon the brain cells, ramified resting microglia and brain resident astrocytes transforms into reactive migratory microglia (microglial activation) and activated astrocytes (reactive astrogliosis) which accelerates the recruitment of macrophages and activates signalling factors (NFkB, AP-1 or Nrf2, CREB). Depending on the intensity of the stimulus, the response might be detrimental or protective in the CNS. If the produced inflammatory mediators/cytokines are pro-inflammatory they confer neurodegenerative actions on neighboring target cells such as inducing neuroinflammation, neurodegeneration, dementia, affecting memory ultimately leading to central nervous system vulnerability. To a paradox if the cytokines produced are anti-inflammatory they confer neuroprotective actions such as glutamate uptake, neurotropic release and trophic factors release.

CREDITS

This work was supported by the grants from Conseil Régional de La Réunion and Europe (CPER/FEDER). AP is a recipient of fellowship from Conseil Régional de La Réunion. ND and CLH were supported by Europe (CPER/FEDER). We would also like to thank University of La Réunion and CYROI (Cyclotron Réunion Ocean Indian) for providing a platform in executing our research activities.

CITATIONS

Abbas, Abul. 2006. Basic Immunology. Elsevier.

Amor S, Puentes F, Baker D, van der Valk P. 2010. Inflammation in neurodegenerative diseases. Immunology 129(2):154-169.

Awada R, Saulnier-Blache JS, Gres S, Bourdon E, Rondeau P, Parimisetty A, Orihuela R, Harry GJ, d'Hellencourt CL. 2014. Autotaxin downregulates LPS-induced microglia activation and pro-inflammatory cytokines production. Journal of cellular biochemistry 115(12):2123-2132.

Bluher M. 2012. Clinical relevance of adipokines. Diabetes & metabolism journal 36(5):317-327.

Bluher M. 2014. Adipokines - removing road blocks to obesity and diabetes therapy. Molecular metabolism 3(3):230-240.

Blüher M, Mantzoros CS. 2015. From leptin to other adipokines in health and disease: Facts and expectations at the beginning of the 21st century. Metabolism 64(1):131-145.

Bruccoleri, Brown, Harry. 1998. Cellular localization and temporal elevation of tumor necrosis factoralpha, interleukin-1alpha, and transforming growth factor-beta mRNA in hippocampal injury response induced by trimethyltin. Journal of neurochemistry.

Catalan V, Gomez-Ambrosi J, Rodriguez A, Salvador J, Fruhbeck G. 2009. Adipokines in the treatment of diabetes mellitus and obesity. Expert opinion on pharmacotherapy 10(2):239-254.

Choi JW, Herr DR, Noguchi K, Yung YC, Lee CW, Mutoh T, Lin ME, Teo ST, Park KE, Mosley AN, Chun J. 2010. LPA receptors: subtypes and biological actions. Annual review of pharmacology and toxicology 50:157-186.

Cohen O, Reichenberg A, Perry C, Ginzberg D, Pollmacher T, Soreq H, Yirmiya R. 2003. Endotoxin-induced changes in human working and declarative memory associate with cleavage of plasma "readthrough" acetylcholinesterase. Journal of molecular neuroscience : MN 21(3):199-212.

Collins, Cotran, Kumar. 1998. Robbins Pathologic Basis of Disease. Philadelphia: WB Saunders Company.

Contos JJ, Ishii I, Chun J. 2000. Lysophosphatidic acid receptors. Molecular pharmacology 58(6):1188-1196.

Dantzer R, Konsman JP, Bluthe RM, Kelley KW. 2000. Neural and humoral pathways of communication from the immune system to the brain: parallel or convergent? Autonomic neuroscience : basic & clinical 85(1-3):60-65.

Deverman BE, Patterson PH. 2009. Cytokines and CNS development. Neuron 64(1):61-78.

Eng LF, Ghirnikar RS, Lee YL. 2000. Glial fibrillary acidic protein: GFAP-thirty-one years (1969-2000). Neurochemical research 25(9-10):1439-1451.

Eskes C, Juillerat-Jeanneret L, Leuba G, Honegger P, Monnet-Tschudi F. 2003. Involvement of microglia-neuron interactions in the tumor necrosis factor-alpha release, microglial activation, and neurodegeneration induced by trimethyltin. Journal of neuroscience research 71(4):583-590.

Freidin M, Bennett MV, Kessler JA. 1992. Cultured sympathetic neurons synthesize and release the cytokine interleukin 1 beta. Proc Natl Acad Sci U S A 89(21):10440-10443.

Fukushima N, Ishii I, Contos JJ, Weiner JA, Chun J. 2001. Lysophospholipid receptors. Annual review of pharmacology and toxicology 41:507-534.

Gao HM, Jiang J, Wilson B, Zhang W, Hong JS, Liu B. 2002. Microglial activation-mediated delayed and progressive degeneration of rat nigral dopaminergic neurons: relevance to Parkinson's disease. Journal of neurochemistry 81(6):1285-1297.

Gao HM, Liu B, Zhang W, Hong JS. 2003. Critical role of microglial NADPH oxidase-derived free radicals in the in vitro MPTP model of Parkinson's disease. FASEB journal : official publication of the Federation of American Societies for Experimental Biology 17(13):1954-1956.

Green SJ, Scheller LF, Marletta MA, Seguin MC, Klotz FW, Slayter M, Nelson BJ, Nacy CA. 1994. Nitric oxide: cytokine-regulation of nitric oxide in host resistance to intracellular pathogens. Immunology letters 43(1-2):87-94.

GS H, DL M, LN C, BM S. 1994. Tumor necrosis factor α inhibits signaling from the insulin receptor. Proc Natl Acad Sci USA 91:4854.

Harry GJ, McPherson CA, Wine RN, Atkinson K, Lefebvre d'Hellencourt C. 2004. Trimethyltin-induced neurogenesis in the murine hippocampus. Neurotoxicity research 5(8):623-627.

Herrmann M, Vos P, Wunderlich MT, de Bruijn CH, Lamers KJ. 2000. Release of glial tissue-specific proteins after acute stroke: A comparative analysis of serum concentrations of protein S-100B and glial fibrillary acidic protein. Stroke 31(11):2670-2677.

Ishii I, Friedman B, Ye X, Kawamura S, McGiffert C, Contos JJ, Kingsbury MA, Zhang G, Brown JH, Chun J. 2001. Selective loss of sphingosine 1-phosphate signaling with no obvious phenotypic abnormality in mice lacking its G protein-coupled receptor, LP(B3)/EDG-3. The Journal of biological chemistry 276(36):33697-33704.

Johansson JU, Woodling NS, Wang Q, Panchal M, Liang X, Trueba-Saiz A, Brown HD, Mhatre SD, Loui T, Andreasson KI. 2015. Prostaglandin signaling suppresses beneficial microglial function in Alzheimer's disease models. The Journal of clinical investigation 125(1):350-364.

Klesney-Tait J, Turnbull IR, Colonna M. 2006. The TREM receptor family and signal integration. Nature immunology 7(12):1266-1273.

Koutsilieri E, Scheller C, Tribl F, Riederer P. 2002. Degeneration of neuronal cells due to oxidative stress--microglial contribution. Parkinsonism & related disorders 8(6):401-406.

Kozak W, Soszynski D, Rudolph K, Conn CA, Kluger MJ. 1997. Dietary n-3 fatty acids differentially affect sickness behavior in mice during local and systemic inflammation. The American journal of physiology 272(4 Pt 2):R1298-1307.

Kronfol Z, Remick DG. 2000. Cytokines and the brain: implications for clinical psychiatry. The American journal of psychiatry 157(5):683-694.

Le W, Rowe D, Xie W, Ortiz I, He Y, Appel SH. 2001. Microglial activation and dopaminergic cell injury: an in vitro model relevant to Parkinson's disease. The Journal of neuroscience : the official journal of the Society for Neuroscience 21(21):8447-8455.

Lucas S-M, Rothwell NJ, Gibson RM. 2006. The role of inflammation in CNS injury and disease. British Journal of Pharmacology 147(Suppl 1):S232-S240.

Lull ME, Block ML. 2010. Microglial Activation & Chronic Neurodegeneration. Neurotherapeutics : the journal of the American Society for Experimental NeuroTherapeutics 7(4):354-365.

Medzhitov R. 2008. Origin and physiological roles of inflammation. Nature 454(7203):428-435.

Moschen AR, Wieser V, Tilg H. 2012. Adiponectin: key player in the adipose tissue-liver crosstalk. Current medicinal chemistry 19(32):5467-5473.

Mosher KI, Wyss-Coray T. 2014. Microglial dysfunction in brain aging and Alzheimer's disease. Biochemical Pharmacology 88(4):594-604.

Nelson PT, Soma LA, Lavi E. 2002. Microglia in diseases of the central nervous system. Annals of medicine 34(7-8):491-500.

Nishimasu H, Okudaira S, Hama K, Mihara E, Dohmae N, Inoue A, Ishitani R, Takagi J, Aoki J, Nureki O. 2011. Crystal structure of autotaxin and insight into GPCR activation by lipid mediators. Nat Struct Mol Biol 18(2):205-212.

Nylen K, Karlsson JE, Blomstrand C, Tarkowski A, Trysberg E, Rosengren LE. 2002. Cerebrospinal fluid neurofilament and glial fibrillary acidic protein in patients with cerebral vasculitis. J Neurosci Res 67(6):844-851.

Ouchi N, Walsh K. 2008. A novel role for adiponectin in the regulation of inflammation. Arteriosclerosis, thrombosis, and vascular biology 28(7):1219-1221.

Peferoen L, Kipp M, van der Valk P, van Noort JM, Amor S. 2014. Oligodendrocyte-microglia cross-talk in the central nervous system. Immunology 141(3):302-313.

Perry VH, Teeling J. 2013. Microglia and macrophages of the central nervous system: the contribution of microglia priming and systemic inflammation to chronic neurodegeneration. Seminars in Immunopathology 35(5):601-612.

Pierson E, Simmons SB, Castelli L, Goverman JM. 2012. Mechanisms regulating regional localization of inflammation during CNS autoimmunity. Immunological reviews 248(1):205-215.

Qi X, Lin W, Wang D, Pan Y, Wang W, Sun M. 2009. A role for the extracellular signal-regulated kinase signal pathway in depressive-like behavior. Behavioural brain research 199(2):203-209.

Reichenberg A, Yirmiya R, Schuld A, et al. 2001. CYtokine-associated emotional and cognitive disturbances in humans. Archives of general psychiatry 58(5):445-452.

Reuhl KR, Smallridge EA, Chang LW, Mackenzie BA. 1983. Developmental effects of trimethyltin intoxication in the neonatal mouse. I. Light microscopic studies. Neurotoxicology 4(1):19-28.

Rosengren LE, Lycke J, Andersen O. 1995. Glial fibrillary acidic protein in CSF of multiple sclerosis patients: relation to neurological deficit. J Neurol Sci 133(1-2):61-65.

Rosengren LE, Wikkelso C, Hagberg L. 1994. A sensitive ELISA for glial fibrillary acidic protein: application in CSF of adults. J Neurosci Methods 51(2):197-204.

Sahin-Efe A, Katsikeris F, Mantzoros CS. 2012. Advances in adipokines. Metabolism 61(12):1659-1665.

Saper CB, Breder CD. 1992. Endogenous pyrogens in the CNS: role in the febrile response. Progress in brain research 93:419-428; discussion 428-419.

Savaskan NE, Rocha L, Kotter MR, Baer A, Lubec G, van Meeteren LA, Kishi Y, Aoki J, Moolenaar WH, Nitsch R, Brauer AU. 2007. Autotaxin (NPP-2) in the brain: cell type-specific expression and regulation during development and after neurotrauma. Cellular and molecular life sciences : CMLS 64(2):230-243.

Smith ME. 2001. Phagocytic properties of microglia in vitro: implications for a role in multiple sclerosis and EAE. Microscopy research and technique 54(2):81-94.

Sofroniew MV. 2014. Astrogliosis. Cold Spring Harbor perspectives in biology 7(2).

Sofroniew MV, Vinters HV. 2010. Astrocytes: biology and pathology. Acta neuropathologica 119(1):7-35.

Stitt JT. 1986. Prostaglandin E as the neural mediator of the febrile response. The Yale journal of biology and medicine 59(2):137-149.

Teeling JL, Cunningham C, Newman TA, Perry VH. 2010. The effect of non-steroidal anti-inflammatory agents on behavioural changes and cytokine production following systemic inflammation: Implications for a role of COX-1. Brain, behavior, and immunity 24(3):409-419.

Teeling JL, Felton LM, Deacon RM, Cunningham C, Rawlins JN, Perry VH. 2007. Sub-pyrogenic systemic inflammation impacts on brain and behavior, independent of cytokines. Brain, behavior, and immunity 21(6):836-850.

Thundyil J, Pavlovski D, Sobey CG, Arumugam TV. 2012. Adiponectin receptor signalling in the brain. British Journal of Pharmacology 165(2):313-327.

Tokumura A, Majima E, Kariya Y, Tominaga K, Kogure K, Yasuda K, Fukuzawa K. 2002. Identification of human plasma lysophospholipase D, a lysophosphatidic acid-producing enzyme, as autotaxin, a multifunctional phosphodiesterase. The Journal of biological chemistry 277(42):39436-39442.

Umemura K, Yamashita N, Yu X, Arima K, Asada T, Makifuchi T, Murayama S, Saito Y, Kanamaru K, Goto Y, Kohsaka S, Kanazawa I, Kimura H. 2006. Autotaxin expression is enhanced in frontal cortex of Alzheimer-type dementia patients. Neuroscience letters 400(1-2):97-100.

Umezu-Goto M, Kishi Y, Taira A, Hama K, Dohmae N, Takio K, Yamori T, Mills GB, Inoue K, Aoki J, Arai H. 2002. Autotaxin has lysophospholipase D activity leading to tumor cell growth and motility by lysophosphatidic acid production. J Cell Biol 158(2):227-233.

Van Gaal LF, Mertens IL, De Block CE. 2006. Mechanisms linking obesity with cardiovascular disease. Nature 444(7121):875-880.

Vos PE, Lamers KJ, Hendriks JC, van Haaren M, Beems T, Zimmerman C, van Geel W, de Reus H, Biert J, Verbeek MM. 2004. Glial and neuronal proteins in serum predict outcome after severe traumatic brain injury. Neurology 62(8):1303-1310.

Warren HS, Fitting C, Hoff E, Adib-Conquy M, Beasley-Topliffe L, Tesini B, Liang X, Valentine C, Hellman J, Hayden D, Cavaillon JM. 2010. Resilience to bacterial infection: difference between species could be due to proteins in serum. J Infect Dis 201(2):223-232.

Wilson CJ, Finch CE, Cohen HJ. 2002. Cytokines and cognition--the case for a head-to-toe inflammatory paradigm. Journal of the American Geriatrics Society 50(12):2041-2056.

Yamauchi T, Kamon J, Waki H, Terauchi Y, Kubota N, Hara K, Mori Y, Ide T, Murakami K, Tsuboyama-Kasaoka N, Ezaki O, Akanuma Y, Gavrilova O, Vinson C, Reitman ML, Kagechika H, Shudo K, Yoda M, Nakano Y, Tobe K, Nagai R, Kimura S, Tomita M, Froguel P, Kadowaki T. 2001. The fat-derived hormone adiponectin reverses insulin resistance associated with both lipoatrophy and obesity. Nature medicine 7(8):941-946.

Zhang R, Wu J, Liu D, Shan H, Zhang J. 2013. Anti-inflammatory effect of full-length adiponectin and pro-inflammatory effect of globular adiponectin in esophageal adenocarcinoma cells. Oncology research 21(1):15-21.

FIGURE LEGENDS

Figure 1, 2, 3:

Dynamic expression of inflammatory markers - A time frame *in vivo* LPS kinetic study:
OF1 mice aged 8 weeks were subjected to inflammation in the absence or presence of intra-peritoneal LPS injection (100µg/Kg bwt). mRNA encoding TNF-α (A), iNOS (B), Adiponectin (C), Resistin (D), Autotaxin (E) levels were quantified in hippocampus, cortex and cerebellum by using Applied Biosystems quantitative Real Time-PCR. Results are the mean ± SEM from experiments performed having an N size 3-8. Statistics * $p<0.05$; ** $p<0.01$; *** $p<0.001$ LPS treated values (2,4,6,12,24,72,120 hours) were significantly different from control values (PBS Saline) as determined by one way ANOVA followed by post Tukey's test (N=3-8 mice per group).

Figure 4:

Dynamic expression of inflammatory markers - A time frame *in vivo* TMT kinetic study:
CD1 mice aged 8 weeks were subjected to inflammation in the absence or presence of intra-peritoneal TMT injection (2mg/Kg bwt). mRNA encoding TNF-α (A), iNOS (B), Adiponectin (C), Autotaxin (D) levels were quantified in hippocampus by using Applied Biosystems quantitative Real Time-PCR. Results are the mean ± SEM from experiments performed having an N size 3-8. Statistics * $p<0.05$; ** $p<0.01$; *** $p<0.001$ LPS treated values (2,4,6,12,24,72,120, 192 hours) were significantly different from control values (PBS Saline) as determined by one way ANOVA followed by post Tukey's test (N=3-8 mice per group).

Figure 5:

Microglial and astrocyte labelling with Isolectin IB4 and GFAP in the brain of adult mouse
Representative examples of immunohistochemistry labelling for isolectin B4 (A, D, G, J, M, P, S), DAPI (B, E, H, K, N, Q, T), a nuclear marker. Merge corresponded to C, F, I, L, O, R, U. Representative examples of immunohistochemistry labelling for GFAP, DAPI corresponds to (a, b, c, d, e, f, g).

GSL I-B4, Griffonia (Bandeiraea) Simplicifolia Lectin Isolectin B4; GFAP, Glial Fibrillary Acidic Protein; DAPI, 4,6-diamino-2-phenylindole.

Figure.1 - A time frame in vivo LPS kinetic study in HIPPOCAMPUS

Figure.1 A

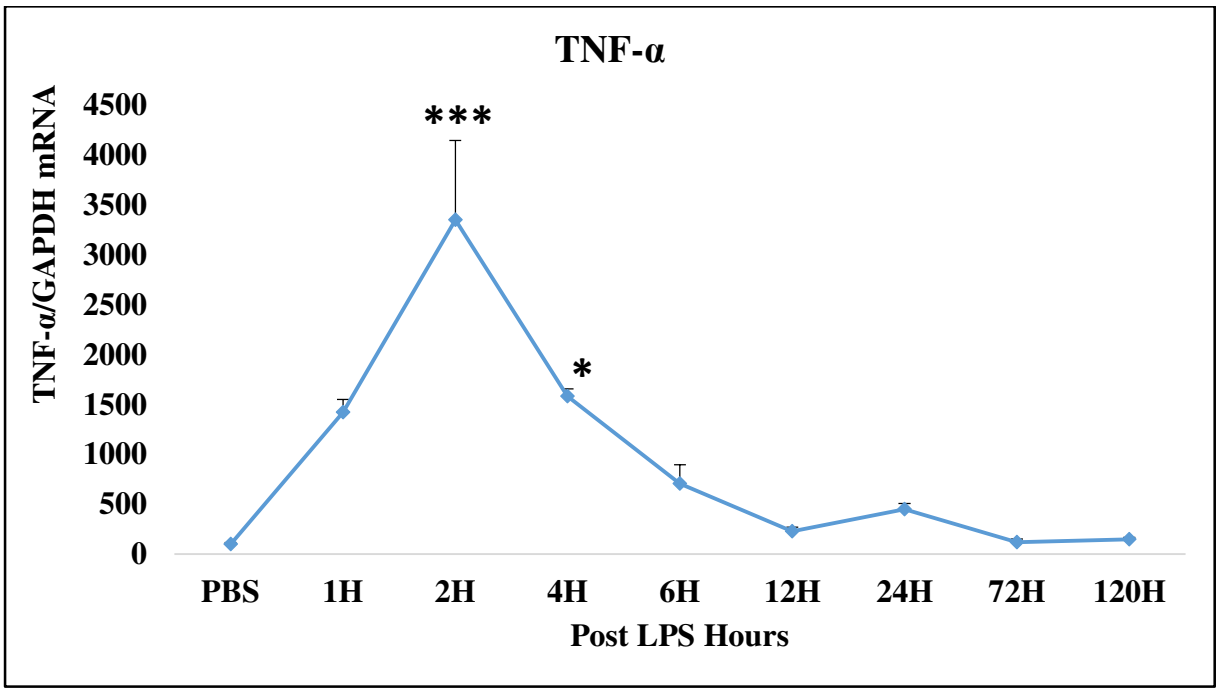


Figure.1 B

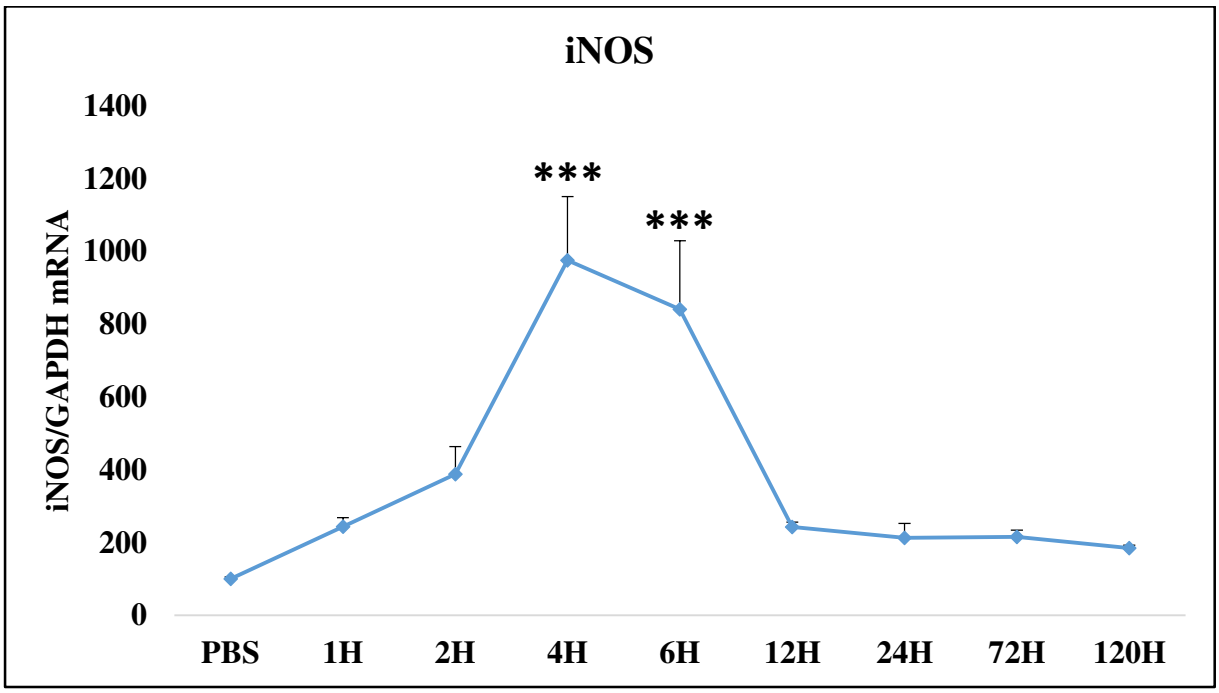


Figure.1 C

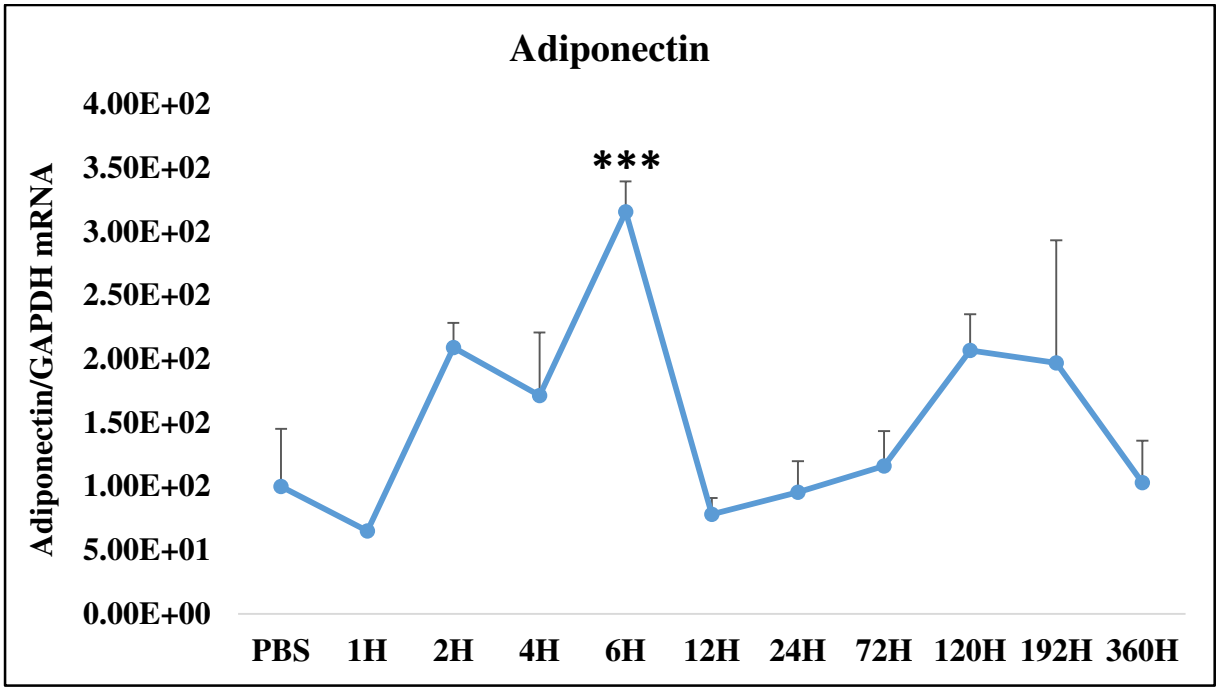


Figure.1 D

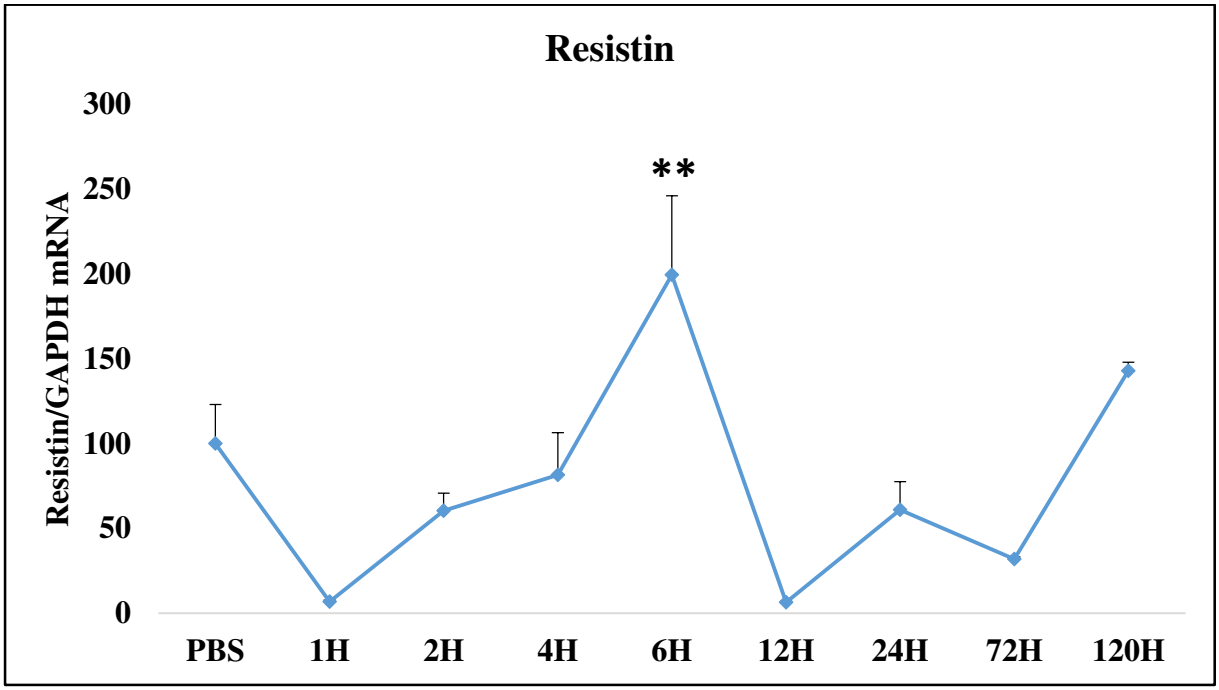


Figure.1 E

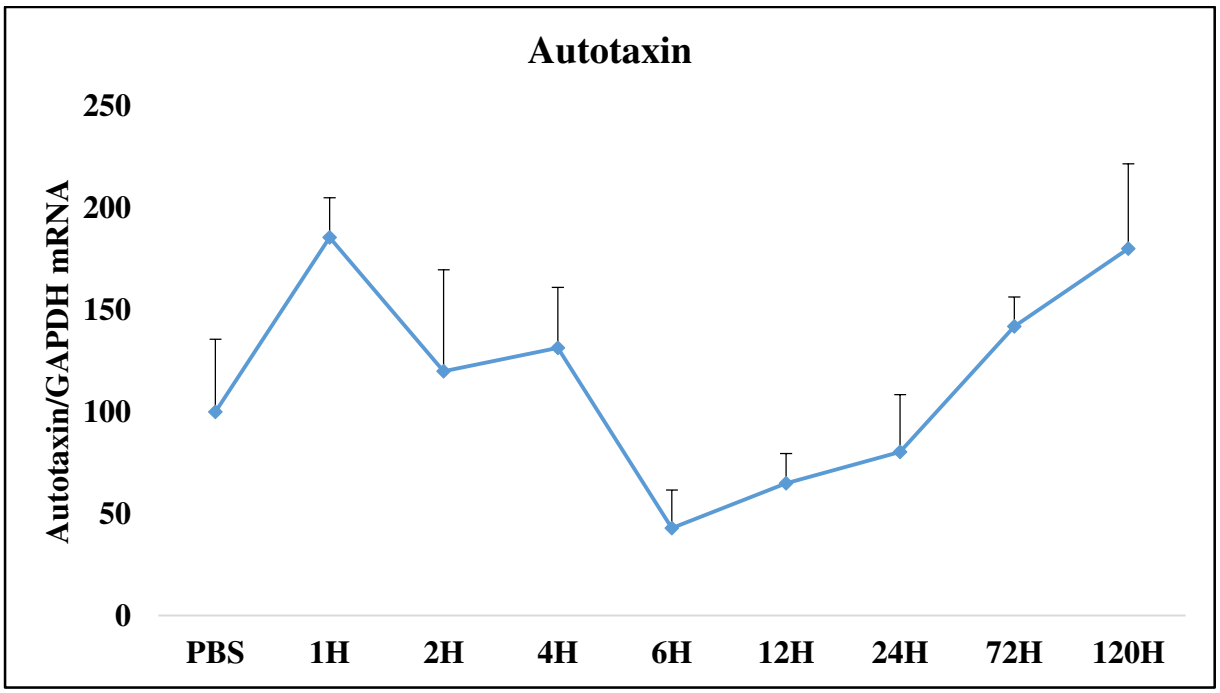

Autotaxin
Autotaxin/GAPDH mRNA
250
200
150
100
50
0
PBS
1H
2H
4H
6H
12H
24H
72H
120H

Figure.2 - A time frame in vivo LPS kinetic study in CORTEX

Figure.2 A

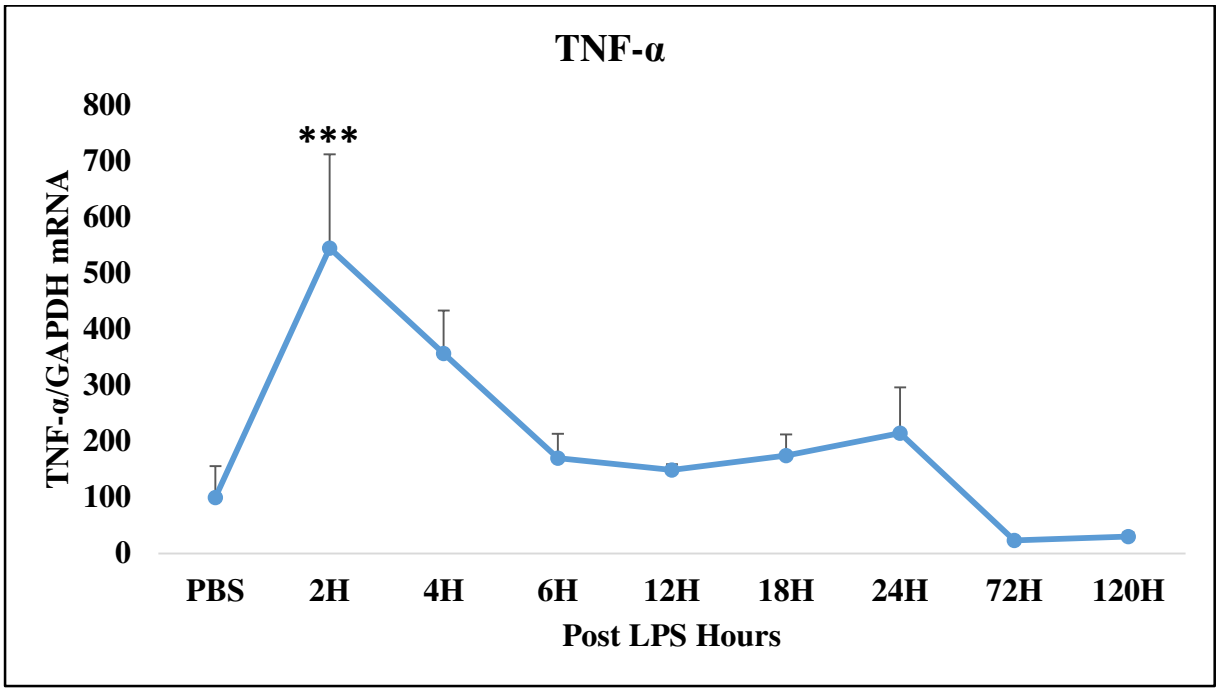


Figure.2 B

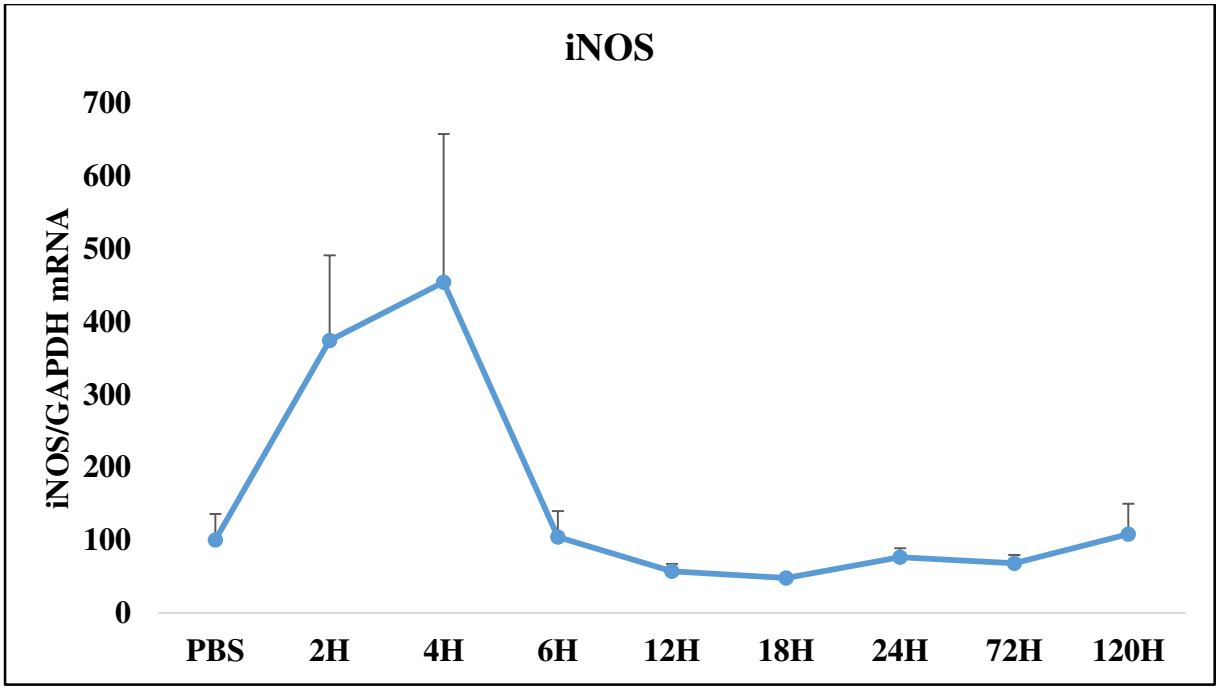


Figure.2 C

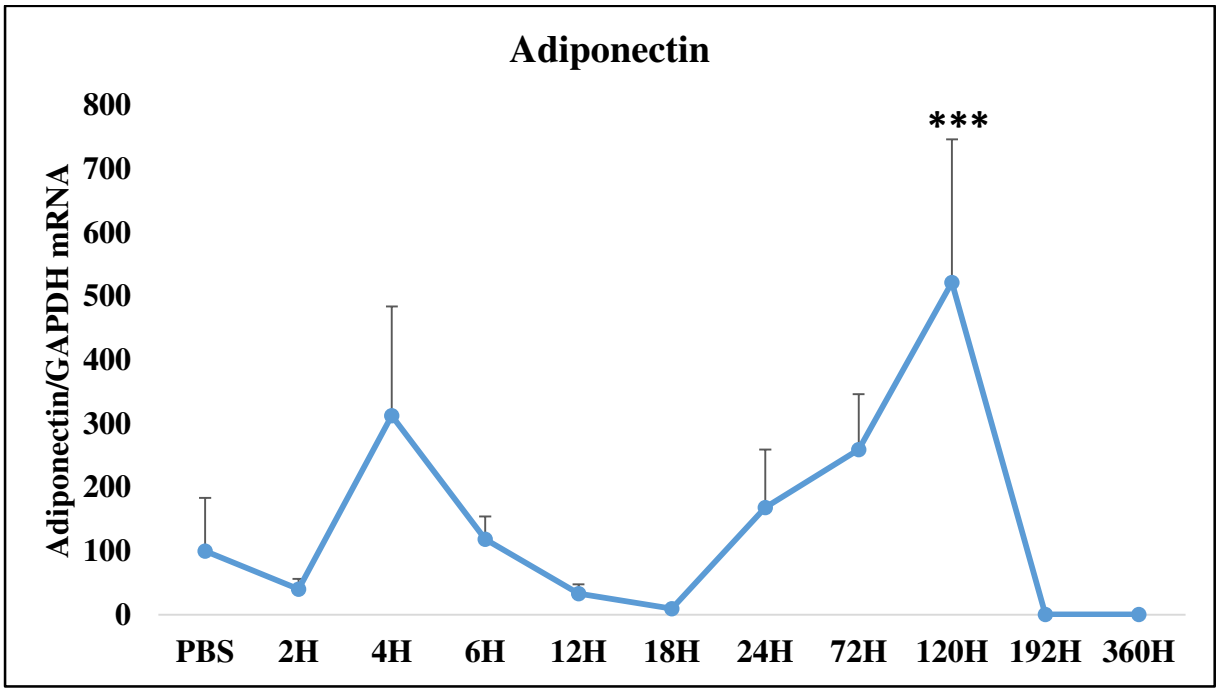


Figure.2 D

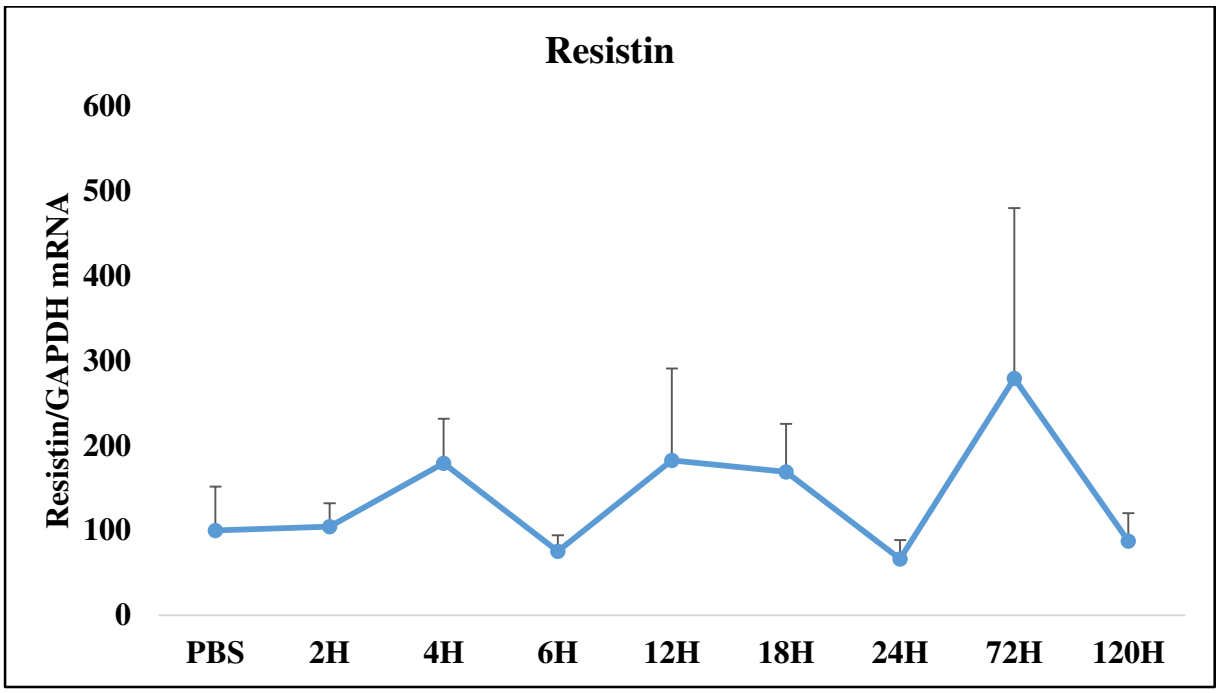


Figure.2 E

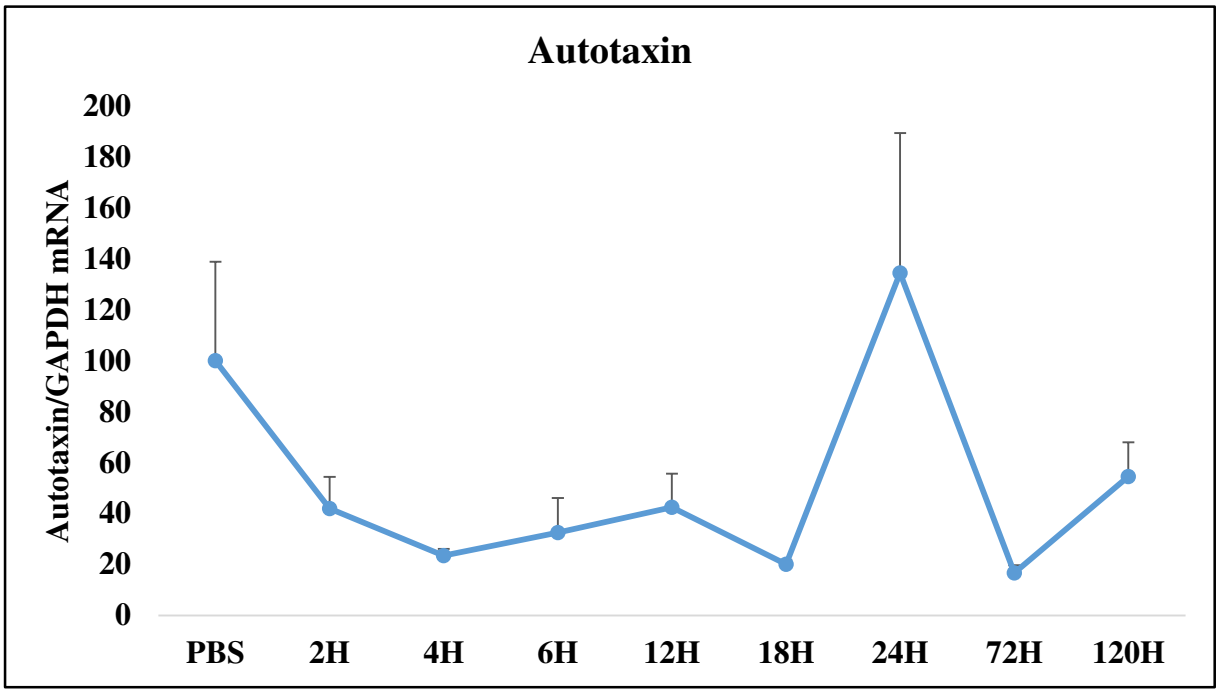

Autotaxin
Autotaxin/GAPDH mRNA
200
180
160
140
120
100
80
60
40
20
0
PBS
2H
4H
6H
12H
18H
24H
72H
120H

Figure.3 - A time frame in vivo LPS kinetic study in CEREBELLUM

Figure.3 A

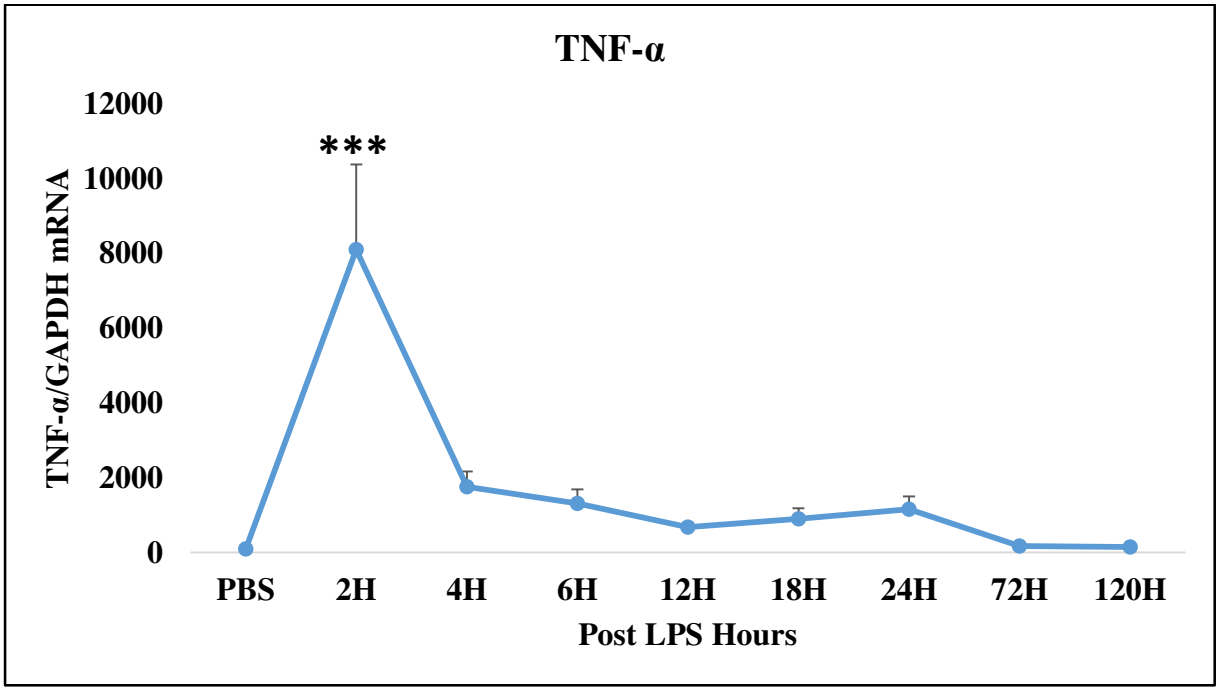


Figure.3 B

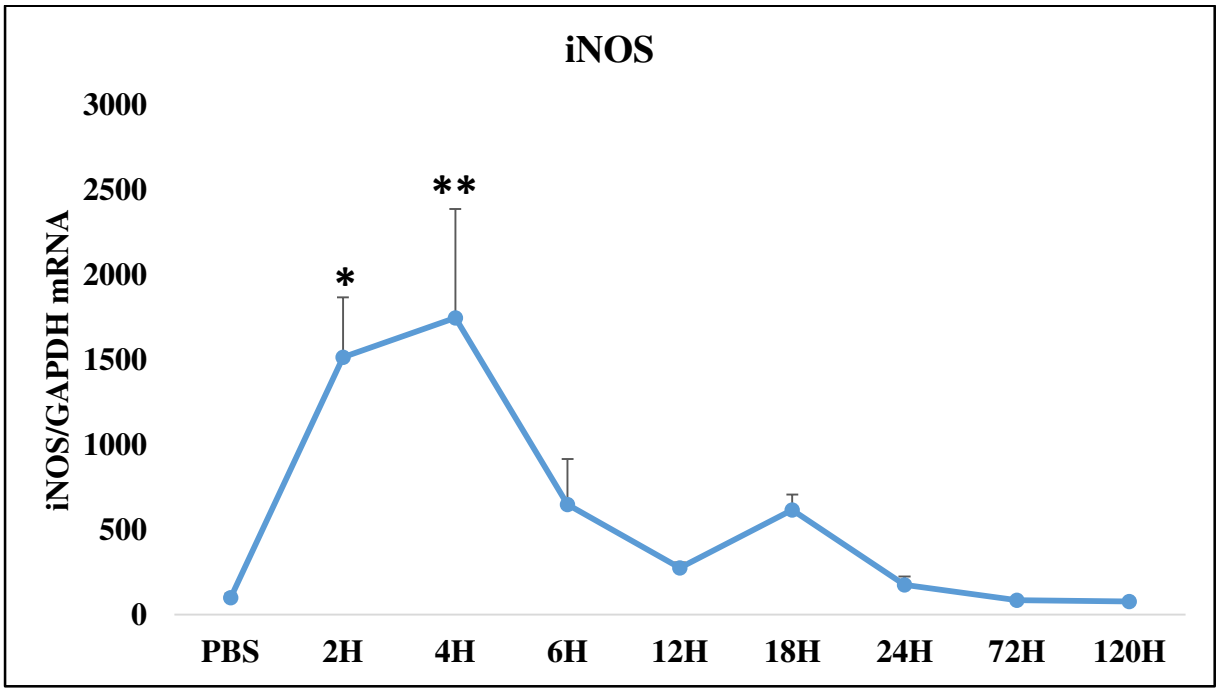


Figure.3 C

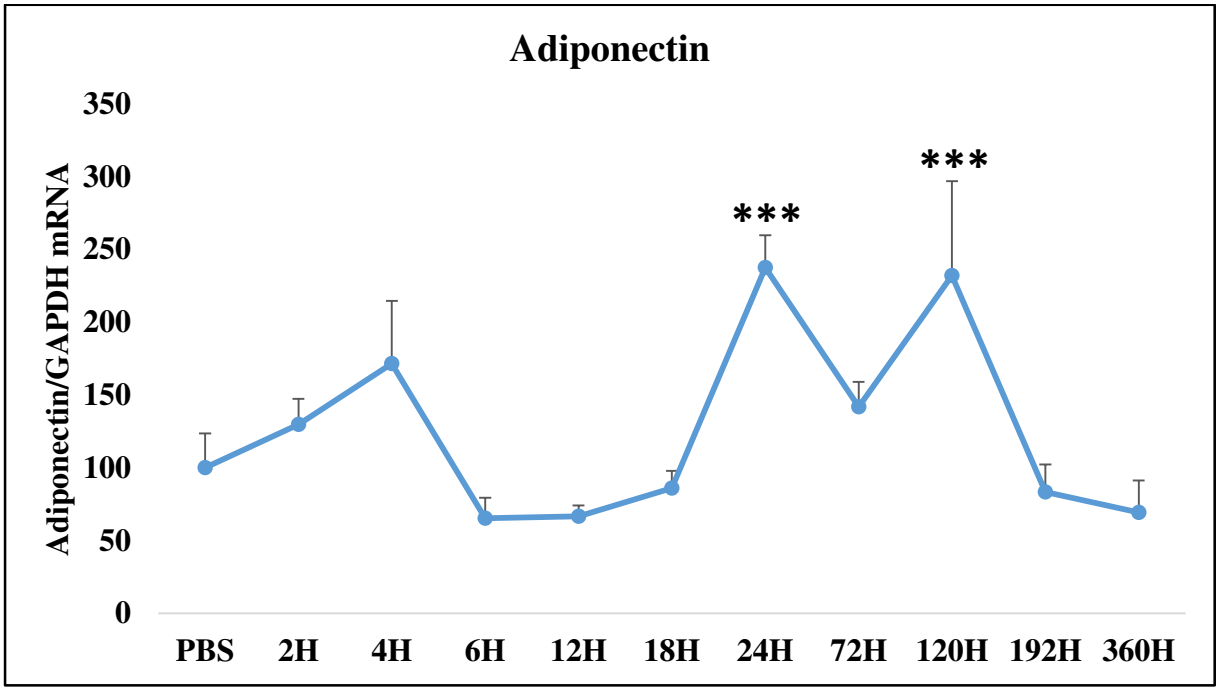


Figure.3 D

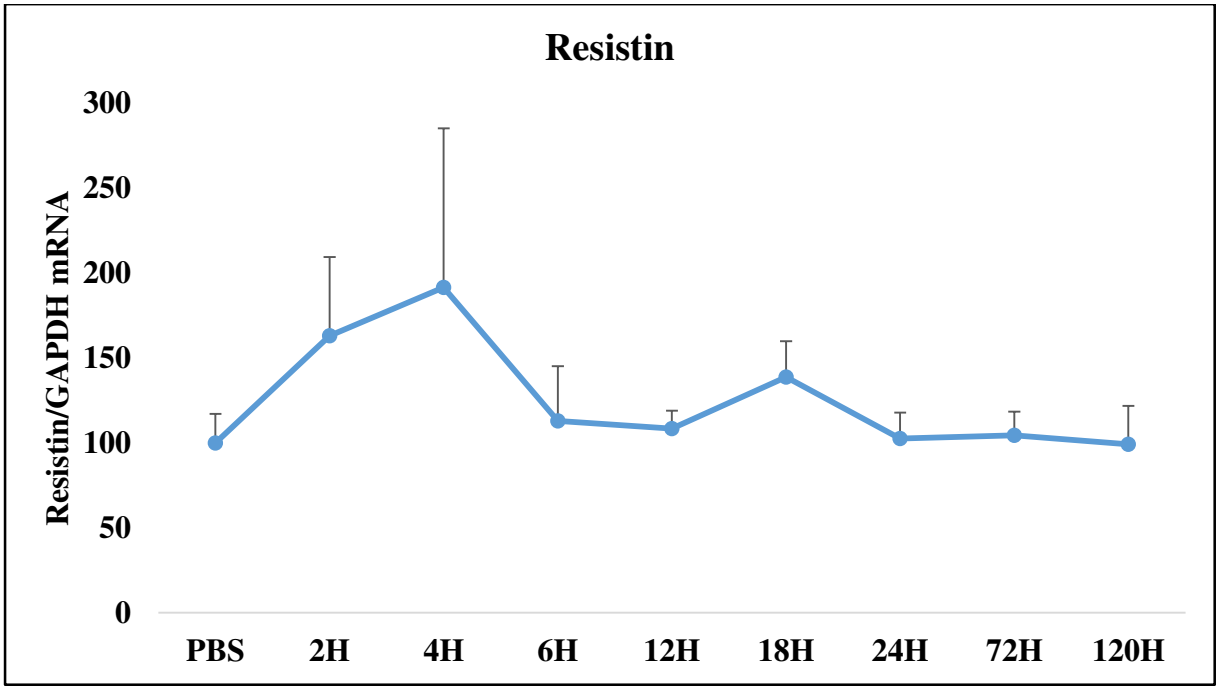


Figure.3 E

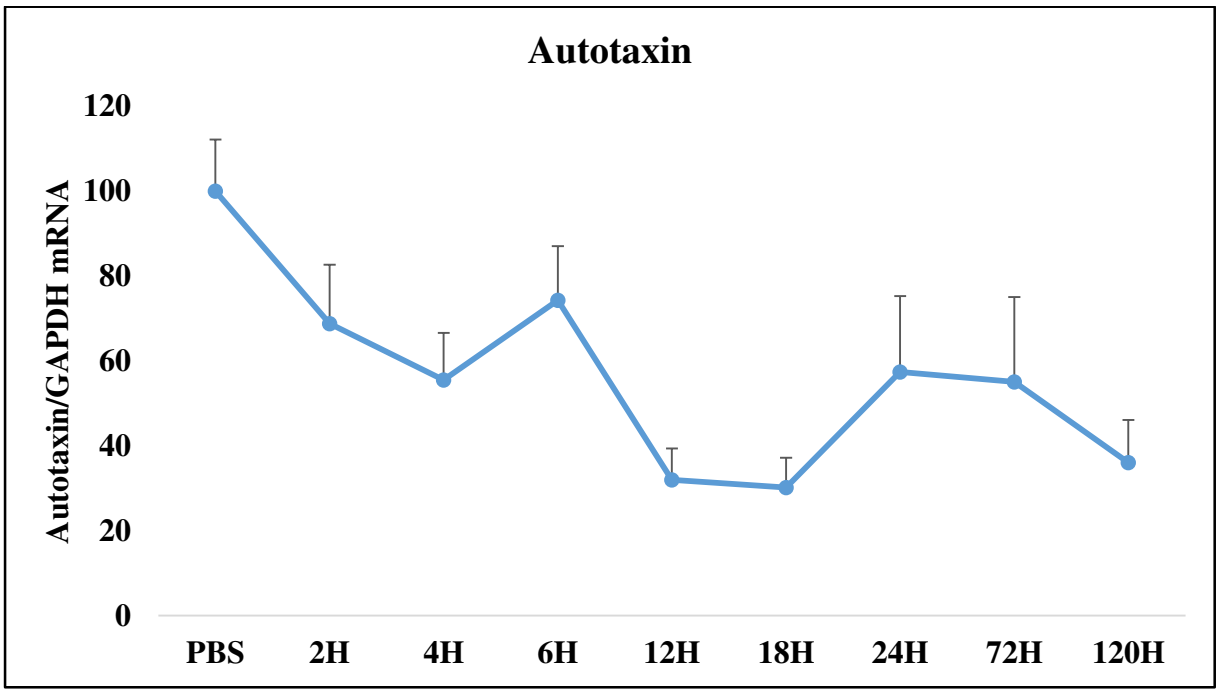

Autotaxin
Autotaxin/GAPDH mRNA
120
100
80
60
40
20
0
PBS
2H
4H
6H
12H
18H
24H
72H
120H

Figure.4 - A time frame in vivo TMT kinetic study in HIPPOCAMPUS

Figure.4 A

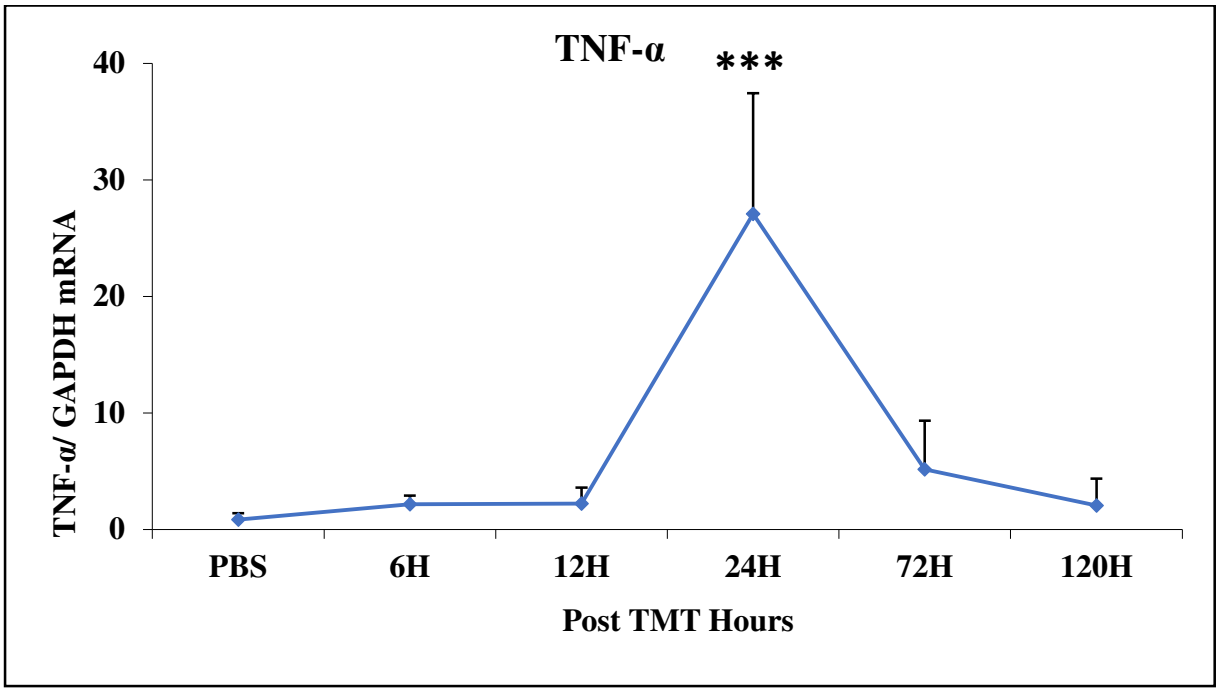


Figure.4 B

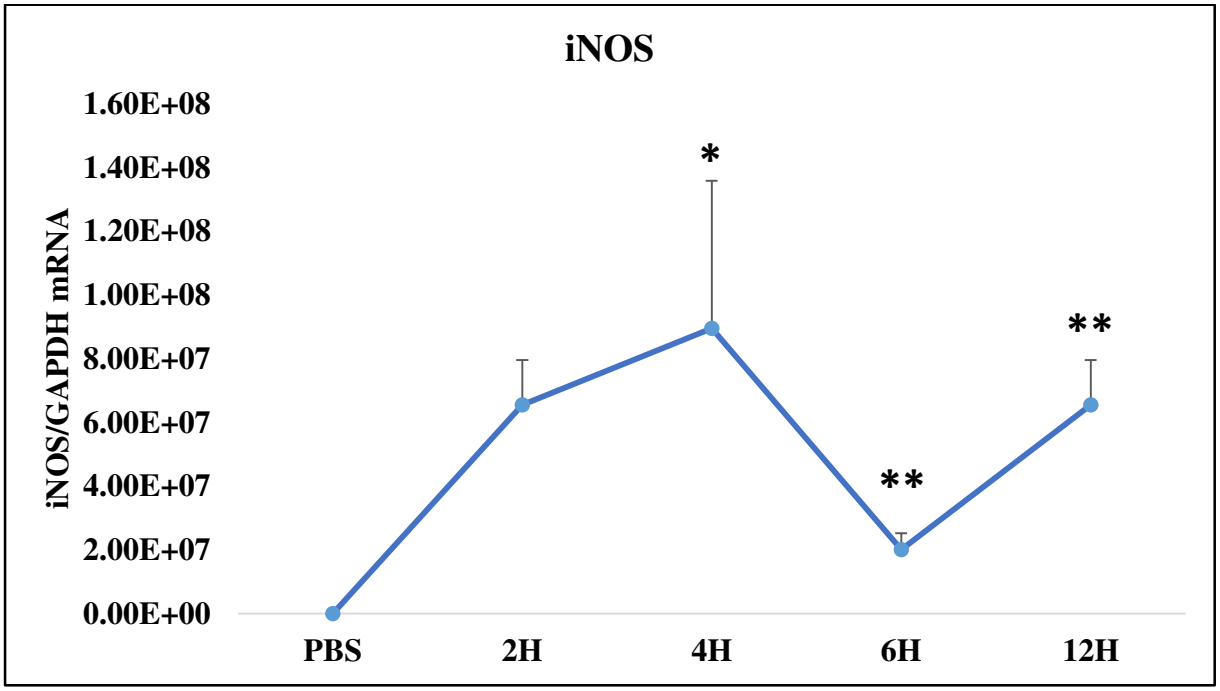


Figure.4 C

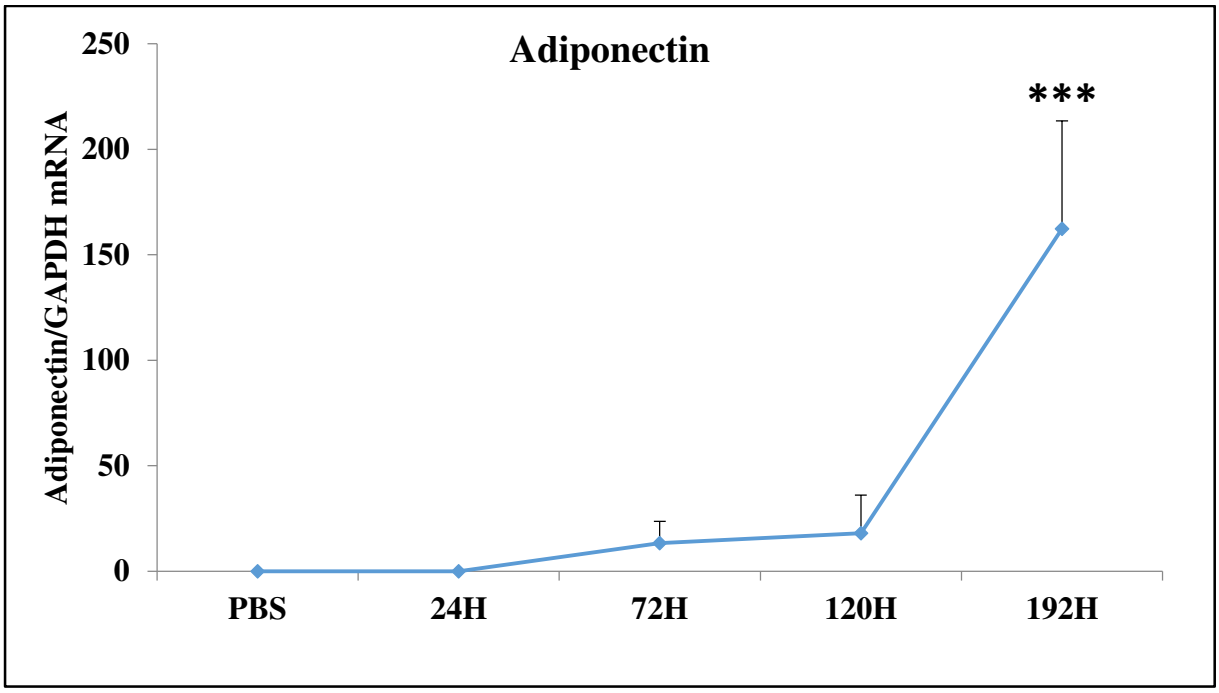


Figure.4 D

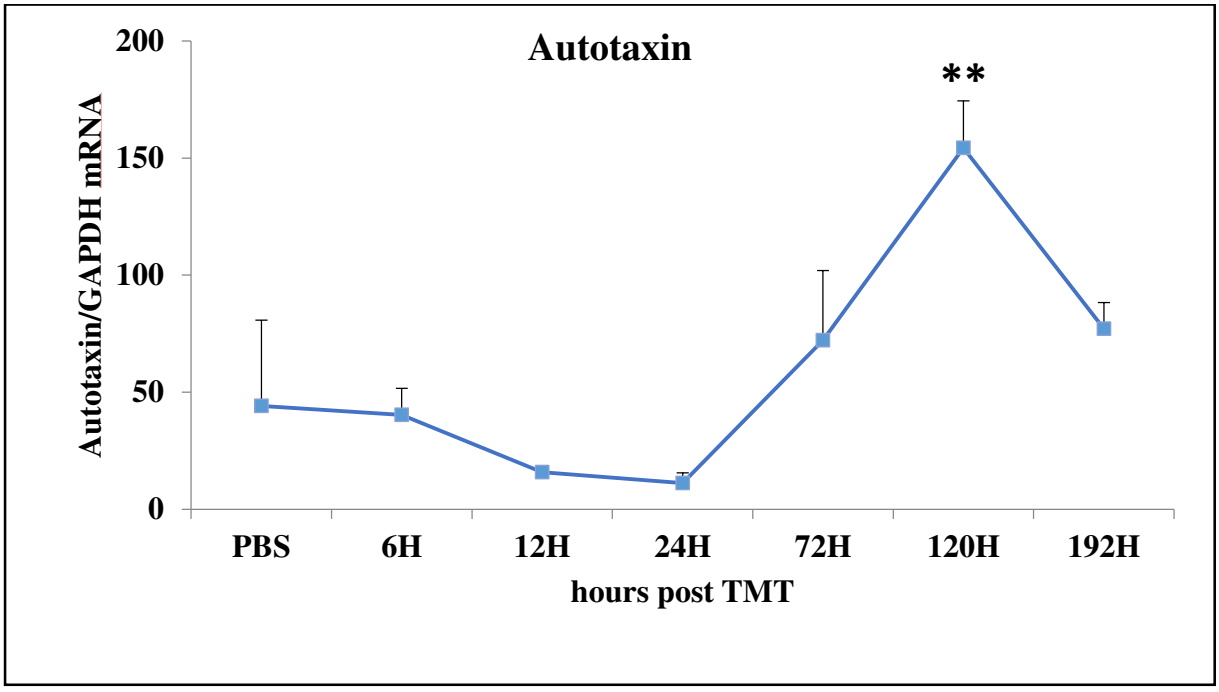
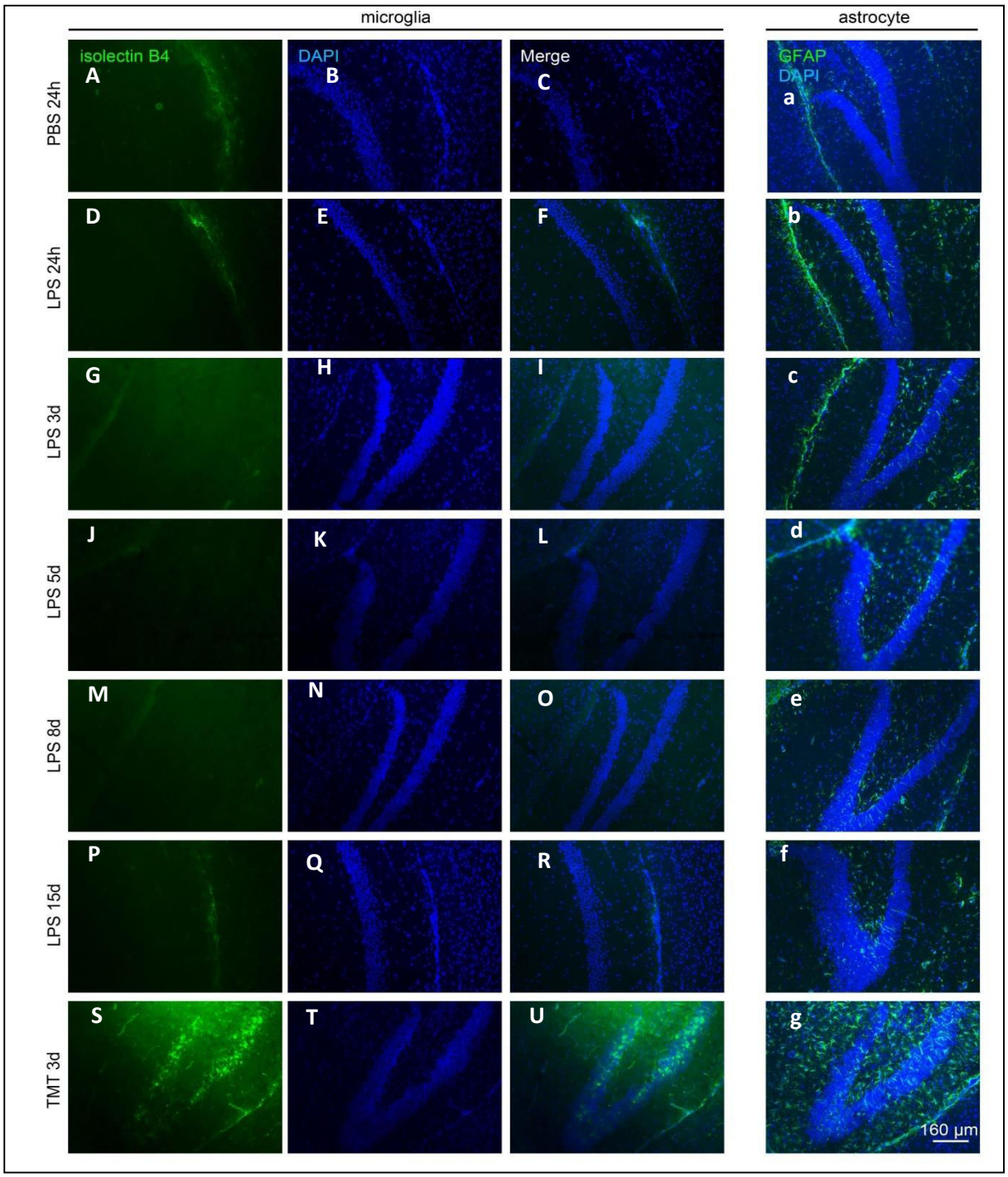


Figure.5 - Microglial and astrocyte labelling with Isolectin IB4 and GFAP in the brain of adult mouse



PROJECT-2

Autotaxin Downregulates LPS-Induced Microglia Activation and Pro-Inflammatory Cytokines Production

Project-2: Article Introductory Preface

Autotaxin Downregulates Lps-Induced Microglia Activation And Pro-Inflammatory Cytokines Production

Aim: Does overexpression of ATX downregulate LPS induced microglial activation?

Experimental Design:

- Murine Bv2 microglial cell line and cortical primary culture were cell cultured.
- BV2 Microglial cells were stably transfected with pCDNA3 empty vector control and as well by the plasmid containing the cDNA of murine ATX.
- Stably transfected cells were challenged with LPS at a concentration of 10ng-1µg/mL and 1µM LPA for a time period of 4-24 hours.
- LPS induced cytotoxicity levels were studied by means of MTT assay.
- TNF-α, IL-10 and ATX mRNA time frame kinetic study were monitored by using qRT-PCR.
- Time frame kinetic study of TNF-α, IL-10 and ATX mRNA quantification by means of qRT-PCR.
- Gene expression mRNA levels of TNF-α, IL-6, IL-10, ATX and LPAR1 were quantified by using quantitative real time PCR (qRT-PCR) approach.
- Protein levels of TNF-α, IL-6, IL-10 levels were studied by using commercial ELISA kits.
- Secreted LPA levels were determined by radio enzymatic assay
- LPS stimulated microglial activation were investigated by the expression of membrane receptors CD11b, CD14, CD80 and CD86 using flow cytometry.
- NF-kB and AP-1 activation were spectrophotometrically measured by using PNPP (para-Nitrophenylphosphate) as a substrate on RAW-Blue macrophage reporter cell line.

Principal Findings:

- ✓ LPS elevated TNF-α and decreased IL-10 mRNA levels in BV2 cells.
- ✓ LPS elevated ATX mRNA levels and induced LPA production.
- ✓ Pro-inflammatory mRNA expression levels of TNF-α, IL-6 were significantly downregulated and the anti-inflammatory mRNA levels of IL-10 were significantly upregulated in ATX cells.
- ✓ Pro-inflammatory protein production levels of TNF-α, IL-6 were significantly downregulated and the anti-inflammatory protein levels of IL-10 were significantly upregulated in ATX cells.
- ✓ Microglial activation cell specific markers including CD11B, CD14, CD80, and CD86 were significantly lowered in the ATX cells under LPS pressure.
- ✓ Conditioned media exposure on RAW-Blue™ cells from the overexpressing ATX clone partially inhibited LPS-induced NF-kB activation.

ARTICLE
Journal of Cellular Biochemistry 115:2123–2132 (2014)

Journal of Cellular Biochemistry

Autotaxin Downregulates LPS-Induced Microglia Activation and Pro-Inflammatory Cytokines Production

Rana Awada,[1] Jean Sébastien Saulnier-Blache,[2] Sandra Grès,[2] Emmanuel Bourdon,[1] Philippe Rondeau,[1] Avinash Parimisetty,[1] Ruben Orihuela,[3] G. Jean Harry,[3] and Christian Lefebvre d'Hellencourt[1]*

[1]*Groupe d'Etude sur l'Inflammation Chronique et l'Obésité (GEICO) EA 4516, Université de La Réunion, 15 avenue R. Cassin, CS 92003, 97715, Saint Denis Cedex and Plateforme CYROI, 2 Rue Maxime Rivière, BP 80 005, Sainte Clotilde Cedex, Reunion Island, France*

[2]*INSERM U1048, Institut des maladies métaboliques et cardiovasculaires (I2MC). 1 avenue Jean Poulhès, BP84225, Toulouse, Cedex 4, France*

[3]*Neurotoxicology Group, National Institute of Environmental Health Sciences (NIEHS), National Institutes of Health, Department of Health and Human Services, Research Triangle Park, North Carolina, USA*

ABSTRACT

Inflammation is essential in defense against infection or injury. It is tightly regulated, as over-response can be detrimental, especially in immune-privileged organs such as the central nervous system (CNS). Microglia constitutes the major source of inflammatory factors, but are also involved in the regulation of the inflammation and in the reparation. Autotaxin (ATX), a phospholipase D, converts lysophosphatidylcholine (LPC) into lysophosphatidic acid (LPA) and is upregulated in several CNS injuries. LPA, a pleiotropic immunomodulatory factor, can induce multiple cellular processes including morphological changes, proliferation, death, and survival. We investigated ATX effects on microglia inflammatory response to lipopolysaccharide (LPS), mimicking gram-negative infection. Murine BV-2 microglia and stable transfected, overexpressing ATX-BV-2 (A+) microglia were treated with LPS. Tumor necrosis factor α (TNFα), interleukin (IL)-6, and IL-10 mRNA and proteins levels were examined by qRT-PCR and ELISA, respectively. Secreted LPA was quantified by a radioenzymatic assay and microglial activation markers (CD11b, CD14, B7.1, and B7.2) were determined by flow cytometry. ATX expression and LPA production were significantly enhanced in LPS treated BV-2 cells. LPS induction of mRNA and protein level for TNFα and IL-6 were inhibited in A+ cells, while IL-10 was increased. CD11b, CD14, and B7.1, and B7.2 expressions were reduced in A+ cells. Our results strongly suggest deactivation of microglia and an IL-10 inhibitory of ATX with LPS induced microglia activation. J. Cell. Biochem. 115: 2123–2132, 2014.

KEY WORDS: AUTOTAXIN; MICROGLIA; INFLAMMATION; LYSOPHOSPHATIDIC ACID

Numerous and highly prevalent pathologies affecting the central nervous system (CNS) are associated with increased inflammation including multiple sclerosis, Parkinson disease, Alzheimer disease, and ischemic stroke [Czlonkowska and Kurkowska-Jastrzebska, 2011]. A controlled inflammatory response to infection or an injury can be beneficial, while an exacerbated or prolonged response can become detrimental. As an important source of inflammatory factors, microglia are integral to many of these disease related pathologies [Munoz-Fernandez and Fresno, 1998]. The activation program of microglia can be sequential and includes morphological, phenotypic, and functional changes [Ponomarev et al., 2006]. The multiple aspects of a microglia response and the

Abbreviations used: ANOVA, analysis of variance; ATX, autotaxin; CM, conditioned medium; CNS, central nervous system; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; IL, interleukin; LPA, lysophosphatidic acid; LPA1, LPA receptor 1; LPC, lysophosphatidylcholine; LPS, lipopolysaccharide; MHC, major histocompatibility complex; SEAP, secreted embryonic alkaline phosphatase; TLR, toll like receptor; TNF, tumor necrosis factor.

Grant sponsor: Conseil Régional de La Réunion, Europe (CPER/FEDER); Grant sponsor: Ministère de L'enseignement Supérieur et de la Recherche; Grant sponsor: Fondation de la Recherche Médicale (France); Grant number: LRM20100618582.

*Corespondence to: Christian Lefebvre d'Hellencourt, GEICO, EA 4516, Université de la Réunion, 15, avenue René Cassin, CS 92003, Cedex 09, 97715 Saint Denis de La Réunion, France. E-mail: Christian.Lefebvre-d-Hellencourt@univ-reunion.fr

Manuscript Received: 5 March 2014; Manuscript Accepted: 18 July 2014

Accepted manuscript online in Wiley Online Library (wileyonlinelibrary.com): 23 July 2014

DOI 10.1002/jcb.24889 • Published 2014. This article is a US Government work and is in the public domain in the USA

need to appropriately regulate the pro-inflammatory and anti-inflammatory stages of a response have raised interest in identifying endogenous factors contributing to this regulation.

Microglia express integrin alpha M (CD11b) and innate immunity receptors such as receptor and co-receptor of lipopolysaccharide (LPS) (Toll-like receptor 4 [TLR4] and CD14). Infection can trigger these receptors and induce subsequent signal transduction, transcription factors, and cytokine production. For instance, LPS can induce NF-κB through TLR4/CD14 interaction and increase proinflammatory cytokines such as, TNF alpha (TNFα), Interleukin-6 (IL-6) as well as anti-inflammatory cytokines such as IL-10 [Bruce et al., 1996; Aloisi et al., 1999; Cacci et al., 2005]. Factors produced by activated microglia including cytokines, chemokines, and free radicals can be neurotoxic [Rock et al., 2004]. In association with the production of pro-inflammatory factors and polarization to an M1 phenotype, microglia increase expression of major histocompatibility complex (MHC) class I and II and co-stimulatory molecules (CD80 and CD86) [Mack et al., 2003; Ponomarev et al., 2005]. Recent data suggest that polarization of microglia to an anti-inflammatory/repair M2 phenotype with expression of IL-10 and TGFβ contributes to their role in reparation and regulation [Boche et al., 2013; Taylor and Sansing, 2013].

Understanding the progression of this injury program and associated regulatory factors have taken a prominent emphasis in neurodegenerative and neuro-reparative research. Within such a framework, a growing interest has emerged for the potential involvement of lysophosphatidic acid (LPA) [Zhao and Natarajan, 1831]. LPA is a phospholipid exerting numerous biological activities, including cell proliferation, differentiation, and migration. These actions occur through activation of specific G-protein coupled receptors [Ishii et al., 2004]. LPA is hydrolyzed from lysophosphatidylcholine (LPC) as a result of the secretion of the ectonucleotide pyrophosphatase phosphodiesterase-2 (ENPP2, autotaxin, ATX) enzyme exhibiting lysophospholipase D (lysoPLD) activity [Umezu-Goto et al., 2002]. ATX is a 125 kDa enzyme released by cultured human melanoma cells [Stracke et al., 1992]. Recent work demonstrated a role for ATX in numerous physiological processes including cell motility, angiogenesis, and adipogenesis [Ferry et al., 2003; van Meeteren et al., 2006; Nakanaga et al., 2010] that are mediated mainly through LPA production [van Meeteren and Moolenaar, 2007; Okudaira et al., 2010]. ATX is known to be upregulated under various inflammatory conditions such the ones that occur in cancer, arthritis, or multiple sclerosis [Hammack et al., 2004; Nochi et al., 2008; Liu et al., 2009a,b]. ATX is expressed within the subventricular zone and choroid plexus epithelium during development, and in the normal adult brain. However, it is also expressed in leptomeningeal cells and oligodendrocyte precursor cells [Savaskan et al., 2007]. Following traumatic injury, ATX expression is increased in reactive astrocytes [Savaskan et al., 2007]. A concurrent developmental profile for LPA receptor expression has been reported [Ohuchi et al., 2008]. The expression of ATX and LPA receptors temporally contribute to the differentiation of oligodendrocyte precursor cells [Yuelling and Fuss, 2008]. We recently reported that ATX could down-regulate an oxidative stress response in cultured microglia providing a protective effect to prevent cell death [Awada et al., 2012]. Given the critical functions of microglia in responding to and modulating brain injury, the ability of ATX to minimize microglia cell death associated with an oxidative stress response suggests a potential beneficial role for ATX in vivo [Awada et al., 2012]. We now demonstrate that LPS induces ATX production in BV-2 microglial cells and ATX overexpression inhibits LPS-induced pro-inflammatory cytokine elevation through up-regulation of the anti-inflammatory cytokine, IL-10.

METHODS

MICROGLIAL CULTURE

A murine microglial BV-2 cell line (obtained from Pr. Philippe Gasque, GRI, la Reunion, France) and BV-2 cells transfected with *pcDNA3 autotaxin* (clones corresponding to the stable transfection of the empty vector [E]) and of ATX [A+] [Awada et al., 2012]) were maintained in Dulbecco's Modified Eagle's Medium (DMEM; Biotech, South America) supplemented with 10% fetal bovine serum (FBS; < 0.02 ng/mL endotoxin), 100 U/mL penicillin, and 100 μg/mL streptomycin at 37 °C in a humidified incubator under 95% air. Viability of ATX (+) cells was similar to that of non-transformed cells as assessed by trypan blue exclusion and cell counting. All culture reagents were obtained from Biotech. Cells were passage by trypsinization and all experiments were conducted on cells with less than 30 passages.

Primary microglia were prepared as previously described [Harry et al., 2002]. Briefly primary mixed glia cultures were prepared from the cortical layer of 2-day-old CD-1 mice. Meninges were carefully removed and tissue digested 20 min in 2.5% trypsin and dissociated by trituration. Cells were collected through a series of centrifugation and filtration, resuspended in 10 mL DMEM/F12 media containing 10% heat inactivated low-endotoxin FBS, penicillin, and streptomycin and plated in T75 tissue culture flasks at a density of 1×10^6 cells/mL. Cells were maintained in a humidified tri-gas incubator under 5% CO_2. Media was changed every 3 days thereafter. After 12 days in culture, cultures were shaken (180–200 rpm/4 h). The media was collected and centrifuged at 1200 rpm for 10 min and pelleted microglia cells were resuspended in complemented media and plated into poly L lysine coated 6-well Costar tissue culture plates at 10^5 cells per well. Cells were maintained under incubator conditions for 24 h prior to experimental manipulation.

LPS TIME COURSE FOR TNF, IL-10, AND ATX

BV-2 cells were plated at a density of 10^5 cells per well in sterile 6-well tissue culture plates and maintained for 24 h. Upon media change, individual wells of confluent cells were randomly assigned, in triplicate, as controls with normal culture medium or exposed for 4–24 h with LPS (10 ng/mL or 1 μg/mL; *Escherichia coli* K-235 > 55.10^4 U/mg, Sigma, France) to determine the time course of response for mRNA levels of TNFα, IL-10, and ATX.

CELL DOSING WITH LPS AND LYSOPHOSPHATIDIC ACID (LPA)

Based upon identification of a peak time for LPS-induced TNFα mRNA elevation (Fig. 1) the 4 h timepoint was selected for further studies. Native BV-2 cells or primary microglia were plated at a density of 10^5 cells per well in 6-well plates (105 mm^2). At 24 h, individual wells, in triplicate, were randomly assigned to treatment

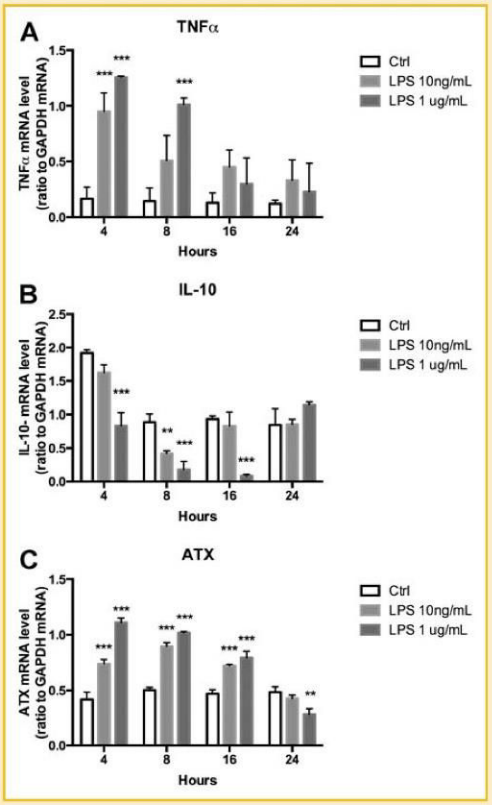


Fig. 1. TNFα, IL-10, ATX expression in LPS stimulated microglia. BV-2 cells were incubated for 4, 8, 16, and 24 h in the absence or presence of LPS (10 ng/mL or 1 μg/mL). mRNA level was determined by qRT-PCR for TNFα (A), IL-10 (B), and ATX (C). Results are the mean ± SD from at least three independent experiments performed in triplicate. Statistics are *** $P < 0.001$, ** $P < 0.01$, significantly different from control values, as determined by Dunnett's t-test following a significant overall two way ANOVA (n = 3–6).

condition of either a media change to normal culture medium (control) or medium containing LPS (1 μg/mL). During the 4 h exposure period, cells were co-exposed to either control vehicle or 1 μM LPA (containing an oleic acid at the sn-1 position of the glycerol; Sigma-Aldridge, France). The presence of 1 μM LPA did not alter cell viability as assessed by trypan blue exclusion and cell counting.

QUANTITATIVE RT-PCR

Following the 4 h incubation with or without LPS in the presence or absence of LPA, total RNA was extracted from BV-2 cells, EV, and A+ cells using Trizol reagent (Invitrogen, France). Six micro gram of RNA was reversed transcribed to cDNA using random primers (Sigma, St. Louis, MO) and Moloney Murine Leukemia Virus (MMLV, Invitrogen). cDNA was amplified by PCR (Biosystems, France) using the SYBR green master-mix (Eurogentec, Belgium) and specific murine primers (Table I, Eurogentec). Each PCR cycle was conducted for 15 s at 95 °C and 1 min at 60 °C. Relative RNA amounts were calculated with relative standard curves for each mRNA of interest and glyceraldehyde-3-phosphate dehydrogenase (GAPDH). Data was normalized relative to GAPDH for each individual sample and analyzed using ABI Prism 7000 SDS software.

QUANTIFICATION OF LPA BY RADIOENZYMATIC ASSAY

Following the 4 h incubation with 1 μg/mL LPS, media was collected and the level of LPA was determined from normal BV-2 cells. Lipids were extracted from the media with an equal volume of 1-butanol and evaporated (SC210A SpeedVac Plus; Thermoscientific, France). The total amount of LPA in the media was determined using a radioenzymatic assay as previously described [Saulnier-Blache et al., 2000]. Briefly, extracted lipids were converted to ^{14}[C]-phosphatidic acid with recombinant LPA acyl-transferase in the presence of ^{14}[C]-oleoyl-CoA. Reaction products were extracted with methanol and separated by one-dimensional silica gel 60 thin-layer chromatography (TLC) glass plate (Merck, Rahway, NJ). The separated bands were detected by direct digital autoradiography and X-ray film after contact autoradiography.

TNFα, IL-6, AND IL-10 PROTEIN QUANTITATION

After 4 h of exposure to normal media or LPS (1 μg/mL), media was collected from each of the cell lines, aliquoted (100 μL) and stored at − 20 °C. Protein levels of TNFα, IL-6 (eBioscience; Cliniscience, France), and IL-10 (Southern Biotech; Cliniscience, France) were determined by ELISA according to the manufacturer's protocol. Briefly, adherent cells were scrapped from each well in 1 mL of ice-cold phosphate buffer saline (PBS) then lysed with 50 μL of lysis buffer (25 mM Tris, 10 mM KCl, 1 mM EDTA and 1% Triton). Total protein levels were determined by bicinchoninc acid agent as previously described [Smith et al., 1985].

QUANTIFICATION OF CD11B, CD14, CD80, AND CD86 EXPRESSION USING FLOW CYTOMETRY

Following 4 h incubation with normal media or LPS (1 μg/mL), cells were washed with ice cold PBS, scraped, and incubated with blocking buffer (PBS/1% bovine serum albumin [BSA]) for 10 min to block

TABLE I. Primers for Real-time Q-rt-pcr

Gene	Forward primer	Reverse primer
GAPDH	5′-TTCACCACCATGGAGAAGGC-3′	5′-GGCATGGACTGTGGTCATGA-3′
ATX	5′-GACCCTAAAGCCATTATTGCTAA-3′	5′-GGGAAGGTGCTGTTTCATGT-3′
TNFα	5′-TGGCCTCCCTCTCATCAGTT-3′	5′-GCTTGTCACTCGAATTTTGAGAAG-3′
IL-6	5′-CAGAATTGCCATCGTACAACTCTTTTCTCA-3′	5′-AAGTGCATCATCGTTGTTCATACA-3′
IL-10	5′-ACCTCCTCCACTGCCTTGCT-3′	5′-GGTTGCCAAGCCTTATCGGA-3′
LPA1	5′-CATGGTGGCAATCTACGTCAA-3′	5′-AGGCCAATCCAGCGAAGAA-3′

non-specific binding. Free-floating cells were incubated with mouse monoclonal antibodies to either CD11b, CD14, CD80, or CD86 (1:200 dilution in PBS/BSA 1%; Bioscience), for 1 h at 4 °C. Cells were then washed in PBS, incubated with phycoerythrin PE-conjugated secondary IgG (1:1000 dilution, Molecular Probes®, France) for 30 min at 4 °C and analyzed by flow cytometry using FACScan flow cytometer (Becton Dickinson, France). The cytometer was operated at 488 nm excitation laser. Red fluorescence was measured with a high pass filter at 630 nm (FLH-2). The forward scatter (FSC) and side scatter (SSC) of particles passing the laser were determined.

NF-κB AND AP-1 ACTIVATION

RAW-Blue™ cells (Invivogen, France) derived from RAW 264.7 macrophages and stably transfected with a construct in which NF-κB and AP1 activation lead to the secretion of secreted embryonic alkaline phosphatase (SEAP) were used to assess NF-κB and AP-1 activation [Hoo et al., 2010]. Cells were maintained in DMEM supplemented with 10% FBS and 200 mg/mL G418 (normal culture medium) in a humidified incubator under 5% CO_2/95% air. Cells (< 15 passages) were resuspended in fresh normal culture medium and an 180 μL aliquot of cell suspension (100,000 cells) was added per well of a 96-well tissue culture plate. Condition media (CM) was obtained as supernatants of BV-2 or A+ cells collected after 4 h. RAW-Blue cells were incubated (37 °C; 5% CO_2) for 4 h with normal culture medium, LPS (1 μg/mL), LPA (1 μM), or CM. After incubation, 1.10^{-3} M p-nitrophenyl-phosphate (PNPP, Sigma, France; dissolved in 0.1 M glycine buffer containing 1 mM $MgCl_2$, 1 mM $ZnCl_2$, pH 10.4) was added to each well and the cells incubated at 37 °C for 30 min. The conversion of PNPP + H_2O to para-nitrophenol (PNP) + phosphate due to the presence of SEAP resulted in a change to a yellow color allowing for detection at 460 nm measured using a microplate reader (Fluostar; BMG, France). Data is presented as total absorbance relative to control.

STATISTICAL ANALYSIS

Data were analyzed by either a Student's *t*-test for two group comparisons or by a one way or two way ANOVA followed by a Dunnett's or Tukey's test for comparisons of multiple doses or times (GraphPad Prism version 6.0c for Mac OsX, GraphPad Software, La Jolla, CA). Statistical significance was set at $P < 0.05$. Data are expressed as mean ± SD from a minimum of three independent experiments (n = 3–6) performed in triplicate.

RESULTS

LPS ELEVATED TNFα AND DECREASED IL-10 mRNA LEVELS

Exposure of BV-2 cells to LPS produced a dose related elevation in TNFα mRNA levels (Fig. 1A). At both dose levels, TNFα mRNA levels were significantly elevated at 4 h. At 8 h, significant elevations were seen only in the 1 μg/mL dose group with the lower 10 ng/mL dose group showing elevation but not reaching statistical significance. This was a transient response and by 16 and 24 h mRNA levels were not statistically different from control. At 1 μg/mL LPS, IL-10 mRNA levels were significantly decreased at 4, 8, and 16 h, returning to within control levels at 24 h (Fig. 1B). For the lower LPS dose (10 ng/mL), a significant decrease in IL-10 was observed only at 8 h, returning to control levels by 16 h (Fig. 1B).

LPS ELEVATED ATX MRNA LEVELS AND INDUCED LPA PRODUCTION

Control cells displayed a detectable level of ATX mRNA that was significantly increased by LPS (1 μg/mL) at 4–16 h, returning to within control levels by 24 h (Fig. 1C). Following 4 h of exposure to LPS (1 μg/mL) the elevation in ATX mRNA levels was accompanied by a significant elevation of LPA released into the culture media compared to controls (Fig. 2) suggesting an increase in ATX activity.

MODULATION OF THE LPS RESPONSE BY ATX

To examine the impact of ATX on LPS-induced TNFα, BV-2 cells and primary microglia were exposed to 1 μg/mL LPS in the absence or presence of 1 μM LPA for 4 h. In control cells, LPS induced changes in cell morphology consistent with activation and elevated the production of TNFα protein at a level comparable to that observed in the dose-ranging study. In the presence of LPA, the normal LPS-induced change in cell morphology was not observed and elevations in TNFα mRNA and protein levels were significantly lower as compared to the LPS-induced elevations observed in the absence of LPA (Figs. 3A and B). BV-2 cells transfected with the original plasmid PCDNA3 (EV) or with a PCDNA-ATX plasmid (A+) showed no evidence of decreased cell viability and failed to display the normal LPS (1 μg/mL) induced morphological changes. Rather, the cells maintained morphological features similar to control cells (data not shown).

In BV2 cells, mRNA levels of LPA receptor 1 (LPA1) were expressed at a low level under control culture conditions and were not altered with LPS (1 μg/mL) exposure (Fig. 4A). In EV cells, the basal level of LPA1 mRNA was slightly, but not significantly, higher than controls. Upon stimulation with 1 μg/mL LPS, levels were significantly elevated. In the A+ cells, the overexpression of ATX resulted in a significant increase in mRNA levels for the LPA1 under control conditions and in presence of LPS (Fig. 4A). Upon stimulation with 1 μg/mL LPS, an increase in ATX mRNA levels was observed in all cell lines however, the level of induction over their

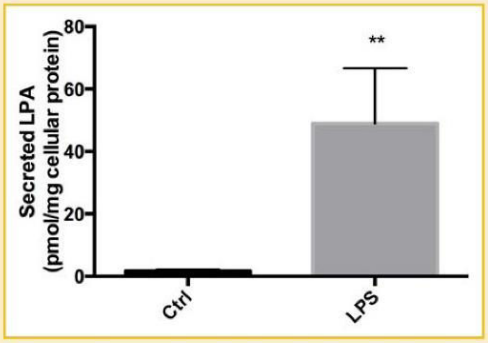


Fig. 2. LPA accumulation in LPS stimulated BV-2.LPA accumulation was quantified by radioenzymatic assay after 4 h of culture media from BV-2 cells untreated or in the presence of 1 μg/mL of LPS. Student *t*-test $^{**}P < 0.01$.

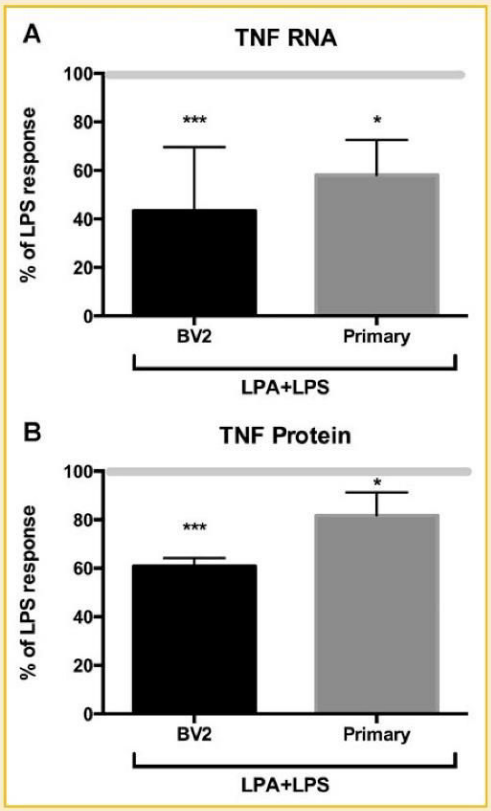


Fig. 3. Effect of LPA on LPS-induced TNFα protein. BV-2 cells or primary microglia were treated for 4 h with 1 μg/mL of LPS in the absence or presence of 1 μM LPA. TNFα mRNA levels were determined by qRT-PCR and TNFα protein levels by ELISA. Results are expressed as % of LPS response (LPS 100%). LPA alone did not change basal levels of TNFα mRNA or protein. Data represents the mean ± SD (n = 3–6). *** $P < 0.001$, * $P < 0.05$ significantly different from LPS treated cells, as determined by Dunnett's *t*-test following a significant overall ANOVA.

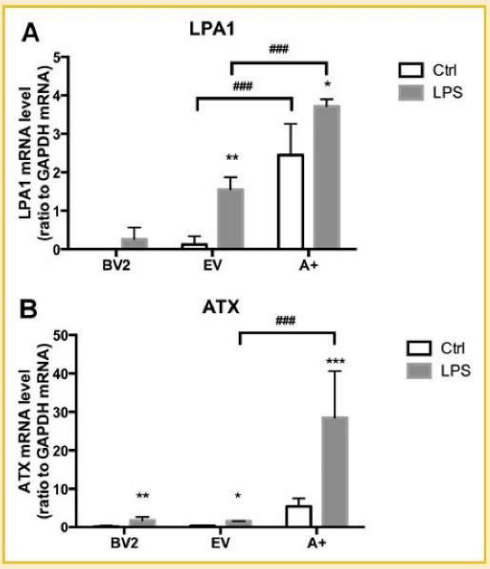


Fig. 4. Effect of LPS and ATX on LPA receptor (LPA1) expression and ATX expression. BV-2 cell, empty vector (EV), or autotaxin (A+) transfected cells were incubated for 4 h in the absence or presence of LPS (1 μg/mL). mRNA encoding LPA1 (A) and ATX (B) were quantified by qRT-PCR as described in the Materials and Methods. Results are the mean ± SD from experiments performed in triplicate. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, LPS treated values significantly different from control values (BV2, EV, A+); ### $P < 0.001$, Overexpressing ATX (A+) values significantly different from EV values, as determined by Tukey's post hoc analysis following a significant overall two way ANOVA (n = 3).

cell specific controls was relatively small in the BV-2 and EV cells (Fig. 4B). As predicted, in the A+ cells, basal ATX mRNA levels were higher than those seen in the other two cell lines (data not shown) and, with LPS stimulation, there was a significant and pronounced elevation in ATX mRNA levels (Fig. 4B).

TNFα, IL-6, AND IL-10 MRNA LEVELS IN BV-2, EV, AND A+ CELLS IN THE PRESENCE OF LPS

Basal mRNA levels for TNFα were similar in the BV-2 and EV cells and slightly lower in the A+ cells. LPS induced similar elevations in TNFα mRNA levels in the BV-2 and EV cells while, in the A+ cells a decrease was observed (Fig. 5A). IL-6 mRNA levels showed a similar pattern with basal and LPS-induced levels similar for the BV-2 and EV cells. Basal levels were similar in the A+ cells and no changes were observed with LPS (Fig. 5B). For the anti-inflammatory cytokine, IL-10, basal levels were very low in the BV-2 cells and failed to show an induction by LPS (Fig. 5C). EV cells showed a basal level of mRNA expression for IL-10 that was decreased following LPS exposure. With an overexpression of ATX in the A+ cells, the basal mRNA level for IL-10 was significantly higher than that observed in the other two cell lines and significantly decreased by LPS (Fig. 5C).

TNFα, IL-6, AND IL-10 PROTEIN IN BV-2, EV AND A+ CELLS IN THE PRESENCE OF LPS

Basal protein levels for TNFα, IL-6, and IL-10 were similar in the BV-2 and EV cells (Figs. 6A–C). In the A+ cells, a slightly lower basal level of TNF protein was detected; however, this failed to reach statistical significance (Fig. 6A). For IL-6, the lower protein level in the A+ cells was significantly lower than the other two cell lines (Fig. 6B) and for the anti-inflammatory cytokine, IL-10 the basal levels were significantly higher (Fig. 6C). Following LPS, TNFα protein levels were increased in the BV-2 and EV cells but were slightly decreased in the A+ cells (Fig. 6A). IL-6 was elevated in the BV-2 cells by LPS but was not elevated in the EV cells. For the A+ cells the relative increase in IL-6 following LPS was similar to that

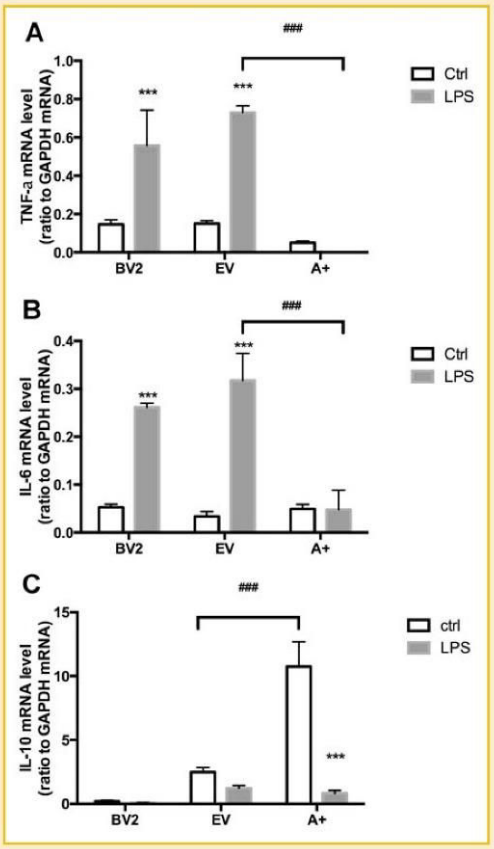


Fig. 5. Cytokines mRNA expression in presence of LPS. BV-2 cell, empty vector (EV), or autotaxin (A+) transfected cells were incubated for 4 h in the absence or presence of LPS (1 μg/mL). mRNA encoding TNFα (A), IL-6 (B), and IL-10 (C), were quantified by RT-PCR as described in the Materials and Methods. Results are the mean ± SD of three independent experiments. *** $P < 0.001$, LPS treated values significantly different from control values (BV2, EV, A+), ### $P < 0.001$, Overexpressing ATX (A+) values significantly different from EV values, as determined by Tukey's post hoc analysis following a significant overall two way ANOVA (n = 3).

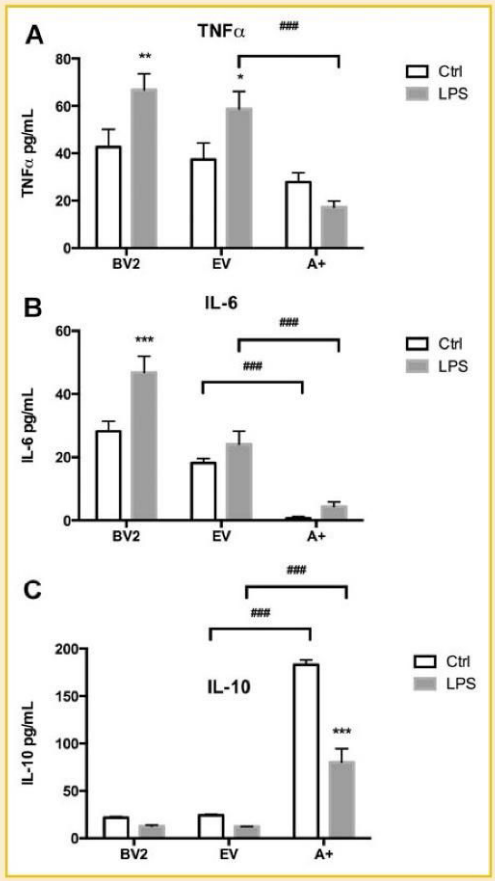


Fig. 6. Cytokines proteins production in presence of LPS. BV-2, empty vector (EV), or autotaxin (A+) transfected cells were incubated for 4 h in the absence or presence of LPS (1 μg/mL). TNFα (A), IL-6 (B), and IL-10 (C) protein levels were determined by ELISA. Results are the mean ± SD from experiments performed in triplicate. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, LPS treated values significantly different from control values (BV2, EV, A+), ### $P < 0.001$, Overexpressing ATX (A+) values significantly different from EV values as determined by by Tukey's post hoc analysis following a significant overall two way ANOVA (n = 3).

seen in the BV-2 cells; however, the absolute protein level was substantially less (Fig. 6C). In all cell lines, the protein levels for IL-10 were significantly lower following LPS as compared to vehicle controls. For the anti-inflammatory cytokine IL-10, all three of the cell lines demonstrated a lower protein level following LPS. In the A+ cells, basal levels of IL-10 were significantly higher than those observed in either the BV-2 or EV cells and while the proportional decrease of approximately 50% following LPS was similar, there remained a significantly higher level of IL-10 protein (Fig. 6C).

CD11B, CD14, CD80, AND CD86 MRNA LEVELS IN THE PRESENCE OF LPS

Stimulation of microglia was estimated by measuring mRNA levels of different CDs. Basal levels for CD11b, CD14, CD80, and CD86 were significantly lower in the A+ cells as compared to BV-2 cells (Figs. 7A–D). Upon stimulation with LPS, CD11b was found to be significantly elevated in the BV-2 cells with no concurrent significant elevation in CD14, CD80, or CD86 (Figs. 7A and D). A+ cells displayed no evidence of stimulation by LPS on the expression levels for CD11b, CD14, CD80, or CD86 (Figs. 7A–F).

NF-κB AND AP-1 ACTIVATION IN THE PRESENCE OF LPA OR CONDITIONED MEDIA FROM A+ CELLS

In RAW-Blue™ cells, LPS significantly induced NF-κB activation (Fig. 8). A similar level of activation over controls was observed in cells co-exposed to LPS (1 μg/mL) and CM from normal BV-2 cells.

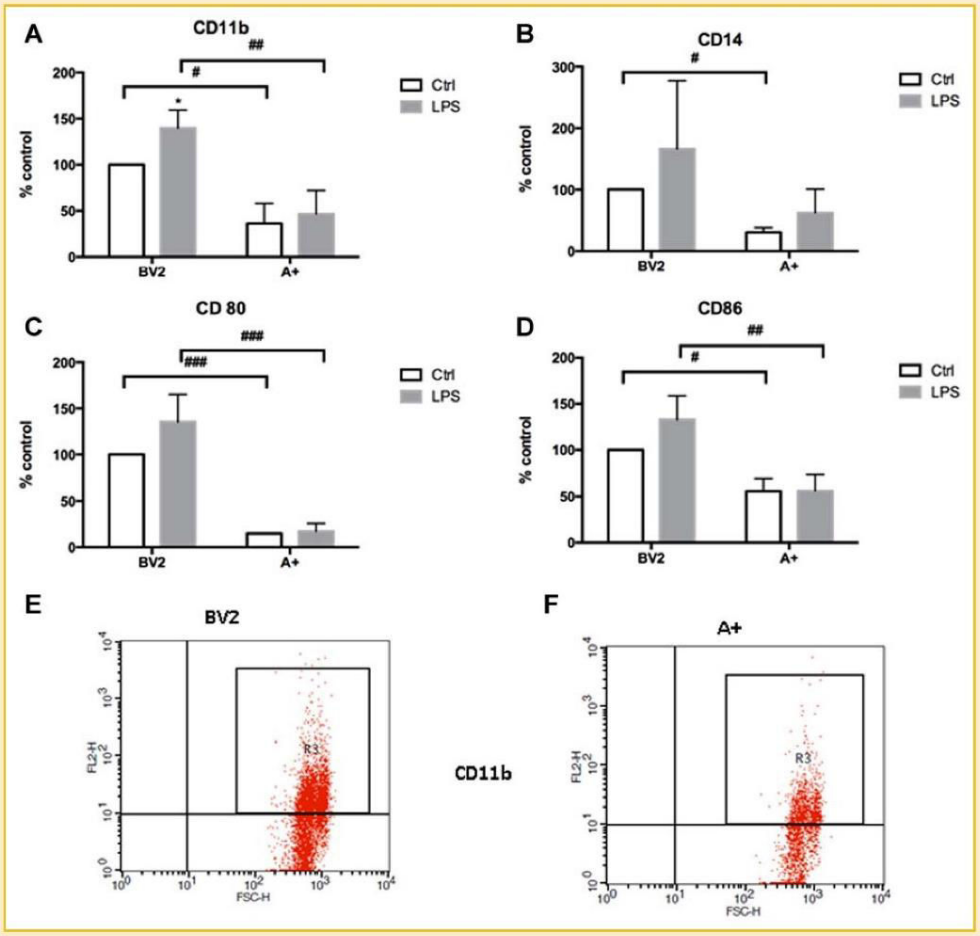


Fig. 7. Autotaxin and microglial membrane proteins expression. Non-transfected cells (NT) or autotaxin (A+) transfected cells were incubated for 24 h in the presence or absence of LPS. (A) CD11b, (B) CD14, (C) CD80, and (D) expression were evaluated by FACS. Results are the mean ± SD from three independent experiments conducted in triplicate, *$P < 0.05$, LPS treated significantly different from respective control values. # $P < 0.05$, ## $P < 0.01$, ### $P < 0.001$, Overexpressing A+ significantly different from BV-2 values, as determined by Tukey's post-hoc analysis following a significant overall two way ANOVA ($n = 3$). Representative FACS scatter plot for CD11b, (E) BV-2 non-transfected cells, and (F). A+ cells in presence of LPS (1 μg/mL).

Co-exposure of LPA (1 μM) with LPS resulted in an approximate 30% inhibition of NF-κB activation. A similar reduction was seen with the co-administration of CM from A+ cells. This combined exposure resulted in an approximate 50% inhibition of LPS-induced NF-κB activation.

DISCUSSION

Activation of microglia and increase in inflammatory factors are physiological responses to various injuries or diseases, including infection, which are necessary processes to restore homeostasis. In addition to removal of cellular debris or aberrant proteins, microglia cells are also involved in the reparation process. However, extended microglial activation and excessive pro-inflammatory response may be detrimental to cells within the environment and for that reason, their activation/deactivation is closely regulated under normal conditions. In the process of regulation, it has been proposed that the pro-inflammatory M1 phenotype of microglia can be down-regulated by a concurrent or subsequent microglia polarization to the anti-inflammatory/repair M2 phenotype. The induction of an M2 phenotype has been proposed for promotion of tissue regeneration or regulation of the inflammatory response and repair [Chawla et al., 2011; Aguzzi et al., 2013]. In the current study, we demonstrate a potential role for ATX in regulating a pro-inflammatory response and activation of BV-2 microglia cells. The concomitant elevation of

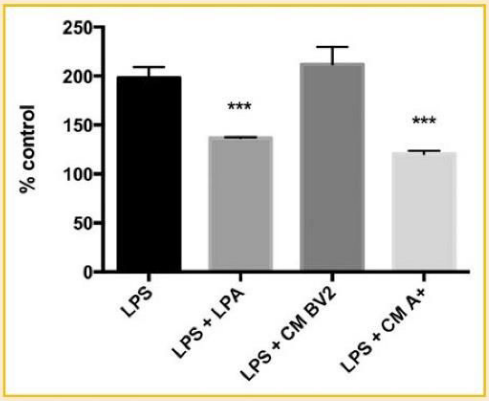


Fig. 8. NF-κB activation. Non-transfected cells (NT) or autotaxin (A+) transfected cells were incubated for 24 h with or without LPS (1 μg/mL). The supernatant (conditioned medium [CM]) from the different cell cultures were then added to RAW-Blue cell. NF-κB activity is determined as described in material and methods. Results are the mean ± SD from three independent experiments performed in triplicate. ***$P < 0.001$, significantly different from LPS values, as determined by Dunnett's t-test post hoc analysis following a significant overall ANOVA ($n = 3$).

the anti-inflammatory protein, IL-10, suggests a role in regulating this inhibition. The induction of ATX production and activity by LPS and the inhibition of an LPS response in ATX overexpressing cells suggest an active role for ATX in the down regulation of an acute inflammatory response.

ATX, through its phospholipase D activity, constitutes the main source of LPA, by catalyzing the conversion of LPC into LPA [Tokumura et al., 2002; Umezu-Goto et al., 2002]. LPA binding to its receptors leads to several different physiological activities including angiogenesis, adipocytes differentiation, and myelination [Ferry et al., 2003; Fox et al., 2003; van Meeteren et al., 2006]. However, the role of ATX in inflammation remains controversial. Elevations in ATX and/or LPA receptors have been reported in inflammatory conditions, including human rheumatoid arthritis (RA) [Nochi et al., 2008], multiple sclerosis [Hammack et al., 2004], and mammary gland inflammatory diseases [Liu et al., 2009a,b]. Yet, LPA inhibition of LPS-induced inflammation in macrophages and in mice suggests anti-inflammatory properties [Fan et al., 2008]. Here we show, for the first time, that stimulation of microglia by LPS induces an increase in ATX mRNA levels and LPA production. A similar increase of ATX in response to LPS has been described in the human monocytic cell line, THP-1 involving PKR, JNK, and p38 MAPK-mediated mechanisms [Li and Zhang, 2009; Li et al., 2012]. In the current study, the partial inhibition of NF-κB by conditioned medium from the clone overexpressing ATX suggesting that ATX/LPA affects multiple pathways including NF-κB.

While not examined in the current study, the various transcription pathways induced in monocytes for the production of ATX in the presence of LPS are likely to be similar in microglia [Li and Zhang, 2009; Li et al., 2012]. Microglia, as well as, monocytes/macrophages express TLR4 and CD14, the receptor and co-receptor for LPS recognition. These receptors are involved in the innate immunity functions of microglia and binding of the receptor by LPS induces a pro-inflammatory cytokine response. Based on our findings, the decrease in CD14 expression seen with ATX suggests a role for ATX in down-regulating the LPS response through inhibition of co-receptor expression.

To date, a total of five LPA receptors belonging to the family of G-protein-coupled receptors have been described. Each of the LPA receptors is encoded by a different gene (lpA1/Edg2, lpA2/Edg4, lpA3/Edg7, lpA4/$P2Y_9$/GPR23, lpA5/GPR92) and difference in the LPA receptor type has been reported depending on the species [Moller et al., 2001; Meyer and Jakobs, 2007]. In cultured rat microglia cells, the main microglia LPA receptor is LPA3 while in mouse LPA1 serves as the primary receptor [Moller et al., 2001]. Our results are consistent with the expression of LPA1 in mouse BV-2 microglia as previously demonstrated [Moller et al., 2001], and suggest that ATX increase leads to an increase of LPA that could bind to the microglia LPA1 and activates the corresponding signaling pathway. The basal expression level of LPA1 is significantly higher in microglia overexpressing ATX as well as the level of induction by LPS.

Microglia can be stimulated to an M1 phenotype in different environments including inflammation and oxidative stress [Vijitruth et al., 2006]. Recently, we have shown that ATX mRNA levels are elevated in microglia upon oxidative stress, suggesting an up-regulation under such environmental conditions [Awada et al., 2012]. Over expression of ATX in microglia improves their resistance to hydrogen peroxide, decreases intracellular reactive oxygen species and increases enzyme involved in the detoxification such as catalase demonstrating that ATX have a protective role in oxidative stress [Awada et al., 2012]. A role for ATX in the regulation of the acute inflammatory response has been previously proposed [Li and Zhang, 2009; Li et al., 2012]. In vivo studies reported a significant decrease in LPS-induced plasma TNFα levels in mice pre-treated with LPA while IL-6 was not changed [Fan et al., 2008]. A similar finding has been reported for cultured peritoneal macrophages with a decreased production of TNFα protein and no change in IL-6 following LPS in the presence LPA [Fan et al., 2008]. Our results demonstrate the ability of ATX to minimize LPS induced activation of microglia and the release of pro-inflammatory cytokines including TNFα and IL-6. In ATX overexpressing cells (A+), the basal level of TNFα mRNA was lower and LPS did not induce an elevation of TNFα. The differences between our findings and those of Fan et al. [2008] in the effect on IL-6 could be due to a continuous production of LPA via overexpression of ATX in our study as compared to the previous study's use of an acute LPA treatment. The observed elevation in IL-10 in cells over-expressing ATX suggests that the inhibition of TNFα following LPS is related to the higher basal level of the anti-inflammatory cytokine IL-10 and a potential polarization of cells to the M2 state.

Microglia in various phenotypic stages express integrin alpha M (CD11b) with elevations observed in activated microglia [Ladeby et al., 2005]. A study from Ma and co-workers examining spinal cord microglia in vivo suggested that LPA induced microglia activation and increased CD11b [Ma et al., 2010]. Under these in vivo conditions it is likely that the response to LPA observed in microglia reflected the diverse cell population and potential mediation of the

response. Using defined microglia culture systems, we found that ATX overexpression decreased BV-2 activation and that LPA inhibited TNFα expression and production in LPS stimulated primary microglia. CD14 and TLRs are also involved in the activation of the microglia as antigen-presenting cell (APC) [Olson and Miller, 2004] and we found a similar expression pattern for CD14 as was observed for CD11b. Interestingly, we found that the expression of the co-stimulatory molecules B7.1 and B7.2 was lowered in the A+ clone, indicating that not only was the innate immunity of microglia down-regulated but also their adaptive immune functions.

The association between ATX and cancer, as well as, inflammation-associated pathologies have lead to the development of ATX inhibitors in the perspective of therapeutic strategies [Federico et al., 2008; Gierse et al., 2010]. However, in contrast, our results on microglia and those by Fan et al. [2008] on macrophages suggest a beneficial role of ATX in CNS injury by protecting the microglia cells and down-regulating their activation. These potential beneficial actions may open new therapeutic perspectives for ATX or LPA.

ACKNOWLEDGMENTS

This work was supported by the Intramural Research Program of NIEHS/NIH and by grants from 'Conseil Régional de La Réunion', Europe (CPER/FEDER) and 'Ministère de L'enseignement Supérieur et de la Recherche'. RA was a recipient of fellowships for both teaching and research activities from the Ministère de l'Education Nationale, de l'Enseignement Supérieur et de la Recherche. AP is a recipient of fellowship from Conseil Régional de La Réunion. SG and JSSB were supported by INSERM (France), and by the Fondation de la Recherche Médicale (France) (grant #LRM20100618582). We would like to thanks Janine Santos and Shih-Heng (David) Chen for their reading and input in the manuscript.

REFERENCES

Aguzzi A, Barres BA, Bennett ML. 2013. Microglia: scapegoat, saboteur, or something else. Science 339:156–161.

Aloisi F, De Simone R, Columba-Cabezas S, Levi G. 1999. Opposite effects of interferon-gamma and prostaglandin E2 on tumor necrosis factor and interleukin-10 production in microglia: a regulatory loop controlling microglia pro- and anti-inflammatory activities. J Neurosci Res 56:571–580.

Awada R, Rondeau P, Gres S, Saulnier-Blache JS, Lefebvre d'Hellencourt C, Bourdon E. 2012. Autotaxin protects microglial cells against oxidative stress. Free Radic Biol Med 52:516–526.

Boche D, Perry VH, Nicoll JA. 2013. Review: activation patterns of microglia and their identification in the human brain. Neuropathol Appl Neurobiol 39:3–18.

Bruce AJ, Boling W, Kindy MS, Peschon J, Kraemer PJ, Carpenter MK, Holtsberg FW, Mattson MP. 1996. Altered neuronal and microglial responses to excitotoxic and ischemic brain injury in mice lacking TNF receptors. Nat Med 2:788–794.

Cacci E, Claasen JH, Kokaia Z. 2005. Microglia-derived tumor necrosis factor-alpha exaggerates death of newborn hippocampal progenitor cells in vitro. J Neurosci Res 80:789–797.

Chawla A, Nguyen KD, Goh YP. 2011. Macrophage-mediated inflammation in metabolic disease. Nat Rev Immunol 11:738–749.

Czlonkowska A, Kurkowska-Jastrzebska I. 2011. Inflammation and gliosis in neurological diseases–clinical implications. J Neuroimmunol 231:78–85.

Fan H, Zingarelli B, Harris V, Tempel GE, Halushka PV, Cook JA. 2008. Lysophosphatidic acid inhibits bacterial endotoxin-induced pro-inflammatory response: potential anti-inflammatory signaling pathways. Mol Med 14:422–428.

Federico L, Pamuklar Z, Smyth SS, Morris AJ. 2008. Therapeutic potential of autotaxin/lysophospholipase d inhibitors. Curr Drug Targets 9:698–708.

Ferry G, Tellier E, Try A, Gres S, Naime I, Simon MF, Rodriguez M, Boucher J, Tack I, Gesta S, Chomarat P, Dieu M, Raes M, Galizzi JP, Valet P, Boutin JA, Saulnier-Blache JS. 2003. Autotaxin is released from adipocytes, catalyzes lysophosphatidic acid synthesis, and activates preadipocyte proliferation. Up-regulated expression with adipocyte differentiation and obesity. J Biol Chem 278:18162–18169.

Fox MA, Colello RJ, Macklin WB, Fuss B. 2003. Phosphodiesterase-Ialpha/autotaxin: a counteradhesive protein expressed by oligodendrocytes during onset of myelination. Mol Cell Neurosci 23:507–519.

Gierse J, Thorarensen A, Beltey K, Bradshaw-Pierce E, Cortes-Burgos L, Hall T, Johnston A, Murphy M, Nemirovskiy O, Ogawa S, Pegg L, Pelc M, Prinsen M, Schnute M, Wendling J, Wene S, Weinberg R, Wittwer A, Zweifel B, Masferrer J. 2010. A novel autotaxin inhibitor reduces lysophosphatidic acid levels in plasma and the site of inflammation. J Pharmacol Exp Ther 334:310–317.

Hammack BN, Fung KY, Hunsucker SW, Duncan MW, Burgoon MP, Owens GP, Gilden DH. 2004. Proteomic analysis of multiple sclerosis cerebrospinal fluid. Mult Scler 10:245–260.

Harry GJ, Tyler K, d'Hellencourt CL, Tilson HA, Maier WE. 2002. Morphological alterations and elevations in tumor necrosis factor-alpha, interleukin (IL)-1alpha, and IL-6 in mixed glia cultures following exposure to trimethyltin: modulation by proinflammatory cytokine recombinant proteins and neutralizing antibodies. Toxicol Appl Pharmacol 180:205–218.

Hoo RL, Wong JY, Qiao C, Xu A, Xu H, Lam KS. 2010. The effective fraction isolated from Radix Astragali alleviates glucose intolerance, insulin resistance and hypertriglyceridemia in db/db diabetic mice through its anti-inflammatory activity. Nutr Metab (Lond) 7:67.

Ishii I, Fukushima N, Ye X, Chun J. 2004. Lysophospholipid receptors: signaling and biology. Annu Rev Biochem 73:321–354.

Ladeby R, Wirenfeldt M, Garcia-Ovejero D, Fenger C, Dissing-Olesen L, Dalmau I, Finsen B. 2005. Microglial cell population dynamics in the injured adult central nervous system. Brain Res Brain Res Rev 48:196–206.

Li S, Xiong C, Zhang J. 2012. ATX and LPA receptor 3 are coordinately up-regulated in lipopolysaccharide-stimulated THP-1 cells through PKR and SPK1-mediated pathways. FEBS Lett 586:792–797.

Li S, Zhang J. 2009. Lipopolysaccharide induces autotaxin expression in human monocytic THP-1 cells. Biochem Biophys Res Commun 378:264–268.

Liu S, Murph M, Panupinthu N, Mills GB. 2009a. ATX-LPA receptor axis in inflammation and cancer. Cell Cycle 8:3695–3701.

Liu S, Umezu-Goto M, Murph M, Lu Y, Liu W, Zhang F, Yu S, Stephens LC, Cui X, Murrow G, Coombes K, Muller W, Hung MC, Perou CM, Lee AV, Fang X, Mills GB. 2009b. Expression of autotaxin and lysophosphatidic acid receptors increases mammary tumorigenesis, invasion, and metastases. Cancer Cell 15:539–550.

Ma L, Nagai J, Ueda H. 2010. Microglial activation mediates de novo lysophosphatidic acid production in a model of neuropathic pain. J Neurochem 115:643–653.

Mack CL, Vanderlugt-Castaneda CL, Neville KL, Miller SD. 2003. Microglia are activated to become competent antigen presenting and effector cells in the inflammatory environment of the Theiler's virus model of multiple sclerosis. J Neuroimmunol 144:68–79.

Meyer zu Heringdorf D, Jakobs KH. 2007. Lysophospholipid receptors: signalling, pharmacology and regulation by lysophospholipid metabolism. Biochim Biophys Acta 1768:923–940.

Moller T, Contos JJ, Musante DB, Chun J, Ransom BR. 2001. Expression and function of lysophosphatidic acid receptors in cultured rodent microglial cells. J Biol Chem 276:25946–25952.

Munoz-Fernandez MA, Fresno M. 1998. The role of tumour necrosis factor, interleukin 6, interferon-gamma and inducible nitric oxide synthase in the development and pathology of the nervous system. Prog Neurobiol 56:307–340.

Nakanaga K, Hama K, Aoki J. 2010. Autotaxin–an LPA producing enzyme with diverse functions. J Biochem 148:13–24.

Nochi H, Tomura H, Tobo M, Tanaka N, Sato K, Shinozaki T, Kobayashi T, Takagishi K, Ohta H, Okajima F, Tamoto K. 2008. Stimulatory role of lysophosphatidic acid in cyclooxygenase-2 induction by synovial fluid of patients with rheumatoid arthritis in fibroblast-like synovial cells. J Immunol 181:5111–5119.

Ohuchi H, Hamada A, Matsuda H, Takagi A, Tanaka M, Aoki J, Arai H, Noji S. 2008. Expression patterns of the lysophospholipid receptor genes during mouse early development. Dev Dyn 237:3280–3294.

Okudaira S, Yukiura H, Aoki J. 2010. Biological roles of lysophosphatidic acid signaling through its production by autotaxin. Biochimie 92:698–706.

Olson JK, Miller SD. 2004. Microglia initiate central nervous system innate and adaptive immune responses through multiple TLRs. J Immunol 173:3916–3924.

Ponomarev ED, Novikova M, Maresz K, Shriver LP, Dittel BN. 2005. Development of a culture system that supports adult microglial cell proliferation and maintenance in the resting state. J Immunol Methods 300:32–46.

Ponomarev ED, Shriver LP, Dittel BN. 2006. CD40 expression by microglial cells is required for their completion of a two-step activation process during central nervous system autoimmune inflammation. J Immunol 176:1402–1410.

Rock RB, Gekker G, Hu S, Sheng WS, Cheeran M, Lokensgard JR, Peterson PK. 2004. Role of microglia in central nervous system infections. Clin Microbiol Rev 17:942–964.

Saulnier-Blache JS, Girard A, Simon MF, Lafontan M, Valet P. 2000. A simple and highly sensitive radioenzymatic assay for lysophosphatidic acid quantification. J Lipid Res 41:1947–1951.

Savaskan NE, Rocha L, Kotter MR, Baer A, Lubec G, van Meeteren LA, Kishi Y, Aoki J, Moolenaar WH, Nitsch R, Brauer AU. 2007. Autotaxin (NPP-2) in the brain: cell type-specific expression and regulation during development and after neurotrauma. Cell Mol Life Sci 64:230–243.

Smith PK, Krohn RI, Hermanson GT, Mallia AK, Gartner FH, Provenzano MD, Fujimoto EK, Goeke NM, Olson BJ, Klenk DC. 1985. Measurement of protein using bicinchoninic acid. Anal Biochem 150:76–85.

Stracke ML, Krutzsch HC, Unsworth EJ, Arestad A, Cioce V, Schiffmann E, Liotta LA. 1992. Identification, purification, and partial sequence analysis of autotaxin, a novel motility-stimulating protein. J Biol Chem 267:2524–2529.

Taylor RA, Sansing LH. 2013. Microglial responses after ischemic stroke and intracerebral hemorrhage. Clin Dev Immunol 2013:746068.

Tokumura A, Majima E, Kariya Y, Tominaga K, Kogure K, Yasuda K, Fukuzawa K. 2002. Identification of human plasma lysophospholipase D, a lysophosphatidic acid-producing enzyme, as autotaxin, a multifunctional phosphodiesterase. J Biol Chem 277:39436–39442.

Umezu-Goto M, Kishi Y, Taira A, Hama K, Dohmae N, Takio K, Yamori T, Mills GB, Inoue K, Aoki J, Arai H. 2002. Autotaxin has lysophospholipase D activity leading to tumor cell growth and motility by lysophosphatidic acid production. J Cell Biol 158:227–233.

van Meeteren LA, Moolenaar WH. 2007. Regulation and biological activities of the autotaxin-LPA axis. Prog Lipid Res 46:145–160.

van Meeteren LA, Ruurs P, Stortelers C, Bouwman P, van Rooijen MA, Pradere JP, Pettit TR, Wakelam MJ, Saulnier-Blache JS, Mummery CL, Moolenaar WH, Jonkers J. 2006. Autotaxin, a secreted lysophospholipase D, is essential for blood vessel formation during development. Mol Cell Biol 26:5015–5022.

Vijitruth R, Liu M, Choi DY, Nguyen XV, Hunter RL, Bing G. 2006. Cyclooxygenase-2 mediates microglial activation and secondary dopaminergic cell death in the mouse MPTP model of Parkinson's disease. J Neuroinflammation 3:6.

Yuelling LM, Fuss B. 2008. Autotaxin (ATX): a multi-functional and multi-modular protein possessing enzymatic lysoPLD activity and matricellular properties. Biochim Biophys Acta 1781:525–530.

Zhao Y, Natarajan V. 1831. Lysophosphatidic acid (LPA) and its receptors: Role in airway inflammation and remodeling. Biochim Biophys Acta 1831:86–92.

PROJECT-3

Role Of Adiponectin On Astrocytes Under Oxidative Stress Situation

Project-3: Article Introductory Preface

Role Of Adiponectin On Astrocytes Under Oxidative Stress Situation

Aim: Does overexpression of ADIPO protect astrocytes against H_2O_2 induced oxidative stress?

Experimental Design:

- Murine CLTT astrocyte cell line were cell cultured.
- CLTT astrocytes were stably transfected with pCDNA3 empty vector control and as well by the plasmid containing the cDNA of murine ADIPO.
- Varying levels of overexpressing ADIPO clones were assessed by qRT-PCR.
- Stably transfected cells were challenged with H_2O_2 at a concentration of 100μM for a time period of 24 hours.
- H_2O_2 induced cytotoxicity levels were studied by means of MTT assay.
- Gene expression mRNA levels of COX-2, iNOS, CAT, SOD, ADIPO and AdipoR1, AdipoR2 were quantified by using quantitative real time PCR (qRT-PCR) approach.
- Astrocyte cell morphology and their nuclei were assessed by hematoxylin and eosin staining.
- H_2O_2 induced intracellular oxidized proteins were studied by carbonyl ELISA kits.
- H_2O_2 induced intracellular ROS generation were measured by DCF-HDA approach.

Principal Findings:

- ✓ Overexpressing ADIPO clone improved cell resistance against H_2O_2 mediated stress
- ✓ Overexpressing ADIPO clone significantly mitigated ROS generated in response to H_2O_2.
- ✓ Overexpressing ADIPO clone significantly mitigated H_2O_2 mediated ROS generation.
- ✓ Overexpressing ADIPO clone significantly mitigated H_2O_2 mediated carbonyl protein accumulation.
- ✓ Pro-oxidative mRNA expression levels of iNOS, COX-2 were significantly downregulated and the anti-oxidative mRNA levels of SOD and CAT were significantly upregulated in ADIPO cells.
- ✓ H_2O_2 significantly elevated AdipoR1 mRNA levels in overexpressing ADIPO clone.

Biological Sciences - Neuroscience

ROLE OF ADIPONECTIN ON ASTROCYTES IN OXIDATIVE STRESS SITUATION

Rana Awada[1], Rachelle Gerges[1], Avinash Parimisetty [1], Wildriss Viranaicken[1,2], Jean Harry[3], Philippe Rondeau[1], Christian Lefebvre d'Hellencourt[1]*

[1] ***UMR 1188 Diabète athérothrombose Thérapies Réunion Océan Indien (DéTROI), Sainte-Clotilde, F-97490, France***
2 Université de La Réunion, UMR 1188, Sainte-Clotilde, F-97490, France – Groupe d'Etude sur l'Inflammation Chronique et l'Obésité (GEICO), Plateforme CYROI- Fédération Environnement Biodiversité Santé, Université de La Réunion- 15, Avenue René Cassin - BP 7151- Cedex 9 97715 Saint Denis Messag - France

[2] ***EA 4517 – Groupe de recherche en immunopathologies et maladies infectieuses (GRI), Plateforme CYROI- 2, rue Maxime Rivière Technopole 97 491 Ste-Clotilde– France***

[3] ***Neurotoxicology Group, Laboratory of Toxicology and Pharmacology (LTP), National Institute of Environmental Health Sciences (NIEHS), National Institutes of Health, Dept. of Health and Human Services, Research Triangle Parc, North Carolina, USA***

***Corresponding author:** Christian Lefebvre d'Hellencourt, Groupe d'Etude sur l'Inflammation Chronique et l'Obesité (GEICO), EA 4516, Plateforme CYROI, Faculté des Sciences, Faculté de Santé, Université de La Réunion, Ile de La Réunion, France

Tel: (+262) 262 93 82 01 - Fax: (+262) 262 93 82 37
Email: Christian.Lefebvre-d-Hellencourt@univ-reunion.fr

Running title: Adiponectin in oxidative stressed astrocytes.

ABSTRACT

Oxidative stress (OS) reflects that antioxidant defenses are overwhelmed with reactive oxygen species (ROS) generation, inducing cellular damages evidenced in several neurodegenerative disorders. Astrocytes, the most abundant glial cells in the central nervous system (CNS), appear as key players in neuroprotection. Adiponectin is an adipocytokine secreted by fat tissue, but also in other organs. Its functions in metabolism regulation are well described, but its role in the CNS during OS is still unclear. Here, we investigate the potential role of adiponectin in astrocytes submitted to OS.

We subcloned the cDNA of adiponectin in eukaryotic expression vectors and stably transfected in mouse CLTT astrocyte cells. Overexpressing adiponectin cells, as monitored by qRT-PCR, as well as non-transfected cells were treated with hydrogen peroxide (H_2O_2). Relative toxicity was determined using trypan blue and MTT assays. Protein oxidation and ROS levels were quantified by measuring carbonyl content and rates of DCF oxidation, respectively. CLTT cell expression of adiponectin receptors (AdipoR1, AdipoR2), SOD, iNOS, COX-2 and Catalase expression were determined by qRT-PCR.

We demonstrate that adiponectin overexpression in CLTT cells protect from OS-induced cellular damages, improve cell viability, reduce intracellular ROS formation and carbonylated protein accumulation. Pro-oxidative enzymes iNOS, COX-2 were inhibited in H_2O_2 treated cells while the anti-oxidative enzyme catalase was increase at both mRNA and activity levels.

Our results suggest that adiponectin set up in the CNS could represent a mechanism to protect nervous system against oxidative stress induced during an inflammatory response.

Keywords: Oxidative stress, neurodegeneration, astrocytes, adiponectin

Introduction

Oxidative stress (OS) is the imbalance between reactive oxygen species (ROS) generation and the actions of antioxidant defenses [1]. ROS such as hydrogen peroxide (H_2O_2) are chemically reactive molecules containing oxygen, produced during cellular respiration and enzymatic activity and are involved in various signaling pathways [2]. However, ROS used to be considered potentially toxic because of their ability to damage vital cellular components, such as lipids, proteins and DNA, or act directly as a signaling molecule to activate apoptotic pathways and lead to cell death [3]. The human brain, even if it represents 2% of the body weight, uses 20% of breathed oxygen. The brain is also an abundant provider of transition metals and produces higher level of ROS than any other organs during increased oxidative metabolic state.

Many evidences show that OS is one of the most important causes of neuronal injury and cell death in acute and chronic conditions. In consequence, many neurodegenerative diseases like Parkinson's, Huntington's and Alzheimer's diseases and brain injury states such as ischemia, hypoxia and trauma have been shown to be coupled to increased OS level [4–6]. To maintain the brain functions, neurons and glial cells play an important role in modulating the cell death in addition to neuronal injury in neurodegenerative situations (Rojo et al., 2008). Glial cells which are about 10 fold more abundant than neurons in the brain, maintain the capacity to undergo cell division and replace themselves in the adult central nervous system (CNS) where most neurons cannot regenerate. The most important glial cells types in the CNS are: microglial cells which are the CNS macrophages and macroglial cells constituted by oligodendrocytes that constitute the myelin sheath and astrocytes which are star shaped cells that anchor neurons to blood supply with their numerous projections. Astrocytes, the most abundant macroglial and glial cells, were at first identified as cells holding neurons together and expressing the intermediate filament glial fibrillary acidic protein (GFAP). But this notion has evolved by subsequent findings that revealed many essential astrocytes functions in the CNS. In fact astrocytes maintain the brain regular physiology due to their regulation of the blood flow [7]. In addition, they are implicated in neurotransmission *via* glutamate uptake and glutamine release [8], in ionic buffering, in the elimination of extracellular toxic compounds and in the control of neurons functions *via* many secreted cytokines [9]. On the one hand, when a damage occurs in the CNS, astrocyte cells protect neurons by restricting neuroinflammation [10] and by an upregulation of antioxidants enzymes expression like superoxide dismutase (SOD) that decomposes superoxide to H_2O_2, in addition to glutathione peroxidase and catalase that are

both involved in conversion of H_2O_2 into water [11]. On the other hand, in response to brain insults, astrocytes can increase the expression of inducible nitric oxide synthase (iNOS) [12], and cyclooxygenase COX-2 [13] that induce the release of nitric oxide (NO) and prostaglandin (PGE) respectively, triggering detrimental role in neurodegeneration [14].

It has been recently suggested that adiponectin and its receptors could be involved in neuronal functions. Indeed, adiponectin presence has been described in human cerebrospinal fluid (CSF)[15], and the expression of adiponectin receptors Adipo R1 and R2 in neurons and astrocytes [16]. Adiponectin is an adipocytokine which secretion by white adipose tissue is reduced in obese patients, inhibits energy expenditure, promotes food intake centrally, and stimulates free fatty acids (FFA) consumption in peripheral tissues and plays a role in down regulating inflammation [16]. Adiponectin is involved in peripheral tissues OS mechanisms: it has been shown that adiponectin inhibits oxidative stress *via* generation of catalase and SOD in human prostate carcinoma cells [17]. Despite the expression of adiponectin in the CNS [18], its effects in neurodegenerative conditions is still unclear.

Hence this study is achieved to evaluate the potential role of adiponectin in astrocyte cells submitted to oxidative stress. Overexpressing adiponectin astrocytes were generated and submitted to an oxidative stress. Then we assessed the response of astrocytes to OS by evaluation of cells viability, estimation of the OS state and quantification of pro-oxidative and anti-oxidative enzymes expression.

Materials and Methods

Cell Culture

The astrocyte CLTT cell line derived from transgenic mice expressing a polyoma virus oncogene T was obtained from Pr. Philippe Gasque's laboratory (GRI, la Reunion, France), and was cultured in Dulbecco's modified Eagle's medium (DMEM; Biotech, South America) with 10% fetal bovine serum (FBS with < 0.02 ng/mL endotoxin), L-glutamine (2 mM), penicillin (10000 U/mL), and streptomycin (10 mg/mL). Cells were grown in a 5% CO_2 incubator at 37°C. Confluent cultures were passaged 30 times maximum by trypsinization. For all experiments, cells were plated in triplicate for each condition, at a density of 10^4 cells per well in sterile 96-well plates for cytotoxicity analyses and at a density of 10^5 cells per well in sterile 6-well plates for reactive oxygen species (ROS) assays.

Plasmid construction and generation of stable cell line

Murin adiponectin (mAdiponectin) complete cDNA in pT7T3-Pac (clone image 1329799) were purchase from Geneservice (Cambridge, UK). To construct the recombinant pcDNA3.1-Zeo-mAdiponectin vector, Clone image were submitted to a double digestion with EcoRI and NotI (NewEnglands Biolabs, Ozyme, France). The digested fragments corresponding to mAdiponectin were purified and finally subcloned in pcDNA3.1-Zeo(+) (Invitrogen, FRANCE) previously linearized with the same restriction enzyme and dephosphorylated with Calf Alkaline Phosphaste as indicated by manufacturer (NewEnglands Biolabs, Ozyme, France). Ligation was performed using fast ligation system as describe by supplier (Promega, Madison, USA). To reduced self-ligation of vector, EcoRV (Promega, Madison, USA) were added at the end of ligation reaction. Insert in pCDNA3.1-zeo (+) was controlled by DNA sequencing (Genoscreen, France).

CLTT cells were plated in sterile 6 wells plates then transfected with 2 µg of pCDNA3.1zeo (+) vector alone (empty-vector control) or pCDNA3.1-zeo (+) expression vector containing the cDNA of adiponectin using Lipofectamine and Plus Reagent (Invitrogen, France). Stable transfectants were selected with 700 µg/mL Zeocin (Invivogen,USA) for 48 hours. A control clones empty-vector (EV) and adiponectin-overexpressing clones (Adipo) were chosen for characterization. All further experiments were performed on at least three independent experiments with selected clones.

Hydrogen peroxide (H_2O_2) Treatment

CLTT untransfected, empty-vector-transfected cells (EV) and adiponectin-overexpressing cells (Adipo) were cultured in 96-, 6- or 4-well plates. Once 80% confluent, cells were washed and fresh medium containing hydrogen peroxide (H_2O_2) (sigma, France) was added for 24 hours. In control experiments untransfected CLTT were culture in presence of recombinant adiponectin 10µg/mL (mAcrp30, Biological industries; KBH Israel). This concentration was used based on previously published data [19]. For almost all the experiments H_2O_2 treatment concentration was 100µM, except for MTT assay where increasing concentrations of H_2O_2 (50 µM-1000 µM) were added for 24 hours. Upon medium change, individual wells of confluent cells were randomly assigned to serve as controls with normal culture medium. Cell viability was assessed by trypan blue counting.

MTT assay

A 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (Sigma, France) assay was conducted to evaluate the effect of H_2O_2 on cell proliferation. The MTT assay is based on cleavage of a yellow tetrazolium salt (MTT) by metabolically active cells, yielding a purple formazan, which can be photometrically quantified. An increase in the number of living cells results in an increase in total metabolic activity and thus increased color formation. After overnight incubation of cells in the presence of various reagents, MTT dye (5 mg/mL) was added to each well, followed by 4 h of incubation. After the medium was discarded, 100 µL of dimethylsulfoxide (DMSO) (Sigma, France) was added to each well and plates were agitated in the dark for 30 min to solubilize the resulting dark blue formazan crystals. Plates were read using a microplate reader at a wavelength of 595 nm.

Cell viability

Cell viability was monitored by the trypan blue exclusion technique. Briefly, 20 µL of trypan blue (Sigma, France) was added to 20 µL of cell suspension; cell count was assessed by Mallasez counting chamber and results were expressed as the percentage of dye-impermeable cells to the total cell number.

Cytology staining

To assess cells morphology, cell nuclei were stained blue with hematoxylin (Vector Laboratories, Cliniscience, France), and cytoplasm was counterstained red with eosin. CLTT

cells, EV cells, Adipo-overexpressing cells were seeded on glass coverslips (Assistant, Germany) in four-well plates and randomly selected wells were treated with 100 µM H_2O_2 (control cells were untreated). After 24 h treatment, cells were fixed in pure acetone at −20°C for 5-10 min and stained with hematoxylin solution. Cells were washed 10 times in 2% glacial acetic solution and incubated in bluing solution (1.5 mL of ammonium hydroxide (NH_4OH) 30% stock solution in 98.5 mL of 70% ethanol (C_2H_6O)) for 1 min, rinsed with water, and stained by incubation with eosin solution for 7 min. Cells were imaged using a Nikon Eclipse microscope and NIS-Element software (Nikon, USA).

Quantification of astrocyte genes expression by real time q PCR

Twenty-four hours after treatment in six well-plates, total RNA was extracted from cell cultures using Trizol reagent (Invitrogen, France). RNA integrity was checked by running an aliquot containing 1 µg of RNA on agarose gel stained with ethidium bromide (Et Br). RNA (6 µg) was reverse transcript to cDNA using Random Primers hexamers (Sigma, France) and 1 µL of 200 U RT superscript II enzyme (Invitrogen, Canada) incubated for 50 min at 42ºC. The cDNA was amplified by PCR (ABI Prism), using the SYBR green master mix and two specific oligonucleotide murine primers (purchased from Eurogentec, Belgium). The primer sequences for all studied genes expression are cited in table 1. Each PCR cycle was run for 15 s at 95°C and 1 min at 60°C. For each gene expression a standard curve was established and mRNA quantified. Results were analyzed using ABI Prism 7000 SDS software. All primer mRNA levels were normalized to GAPDH.

Quantification of intracellular ROS levels

After Twenty-four hours treatment, cells were washed with PBS and incubated for 30 min at 37°C with 10 µM dichlorohydrofluorescein diacetate (DCF-HDA). Then, cells were washed once with PBS, and the fluorescence intensity of the oxidized form 2′, 7′ dichlorofluorescin (DCF) was measured in presence of PBS, in a 96 well spectrofluorimeter microplate reader (BMG Labtech, Germany) at 492 nm (excitation) and 520 nm (emission). Fluorescence values per well were calculated after control value subtraction, by the formula: $(Fx - F_0)$ / Total cell number per well, where Fx = fluorescence of the sample x and F_0 = fluorescence of the control using identical conditions without cells. Values expressed in percentage of ROS generation were normalized by division of the value in each treated cell type over the untreated of the same type (CTRL).

Quantification of intracellular oxidated proteins: Carbonyl contents by ELISA

Confluent cells in six well plates, treated or not, were scraped with cold PBS then lysed with 50 µl of lysis buffer (Tris 25 mM, KCl 10 mM, EDTA 1mM and Triton 1%). Carbonylated proteins were analysed as previously described [20]. Briefly, Protein concentration measurements were perfomed on cell lysates using the bicinchoninc acid agent. carbonylated proteins were analysed using an Oxyblot kit (oxyblot Detection, Chemicon International Inc). Absorbances were red at 450 nm and subtracted from the absorbance at 570 nm. The data were shown as a function of increasing amount of protein and the degree of oxidation (carbonylation) of modified proteins and was calculated as follows: % carbonyl = [(B−A)/A] ×100, where A is the slope value of carbonylated proteins in cells expressing adiponectin and B is the slope value of control proteins cells (in this experiment protein levels in CLTT and EV at basal conditions or after treatment were considered as control).

Catalase activity assay

The catalase activity assay is based on the properties of catalase enzyme to reduce hydrogen peroxide (H_2O_2) into oxygen (O_2) and water (H_2O) [21]. Transfected and non-transfected CLTT cells were grown to near confluence in 25-T flasks before treatment with and without H_2O_2 (100 µM) in DMEM-BSA 1% for 4 hours. After these stimulations, treated cells were washed with PBS and lysed with 200 µL of phosphate buffer (NaH_2PO_4 [50 mM], protease inhibitor, pH 7.4) using a Hielscher GmbH ultrasonic device model UP200S (4 probe sonications for -10 sec, pause 60 sec, amplitude 70% on ice) and stored at -80°C. Assays were carried on approximatively 80 µg of protein lysate in 50 mM phosphate buffer (pH 7.4). A first measure at 240 nm is performed with only the samples (blank) before adding 80 µL of H_2O_2 (10 mM final) to start the reaction. Catalase activity was assayed spectrophotometrically by measuring the decrease in absorbance of H_2O_2 at 240 nm in Infinite M200 pro spectrophotometric analyzer (TECAN). The decomposition of hydrogen peroxide initially follows that of a first-order with H_2O_2 concentration and the rate constant K for the overall reaction is given by:

$$K = \frac{2.3}{\Delta t} * \log(\frac{DO_{zero}}{DO_t})$$

Each measurement was considered with 4 replicates and data are expressed as catalytic unit (U) per mg of total protein. Protein concentrations of cell lysates were measured according to the bicinchoninic acid (BCA) method.

Statistical analysis

Data were expressed as the means ± Standard error of the mean (SEM) from three independent experiments, which were each performed in triplicate. Statistical analyses were performed using unpaired Student's t tests or one-way ANOVA (followed by the Tukey test). In some cases, two-way ANOVA was used for multiple comparisons. P values <0.05 were considered statistically significant.

Results

Expression of adiponectin, Adipo R1 and R2 receptors in astrocyte cells

We confirmed that the astrocytic CLTT cells expressed adiponectin receptors adipoR1 and adipoR2. These receptors expression has been demonstrated on human astrocytic cells and in rat brain [22][18][15]. As assessed by quantitative real time PCR (qRT-PCR) the level for adiponectin was low but detectable as well as the level of adipo R2. The level of AdipoR1 was higher (data not shown). Adipo R1 and Adipo R2 protein levels in the cells were also investigated by western blot and low levels were detected (data not shown).

Characterization of stably adiponectin-transfected astrocyte cells

To evaluate the effects of adiponectin over expression, CLTT cell line has been transfected separately with pcDNA3.1-Zeo (+) empty vector (EV control cells) or with pcDNA3.1-Zeo (+) containing murine adiponectin cDNA (Adipo cells). Stably transfected cells were used to investigate the role of adiponectin in the astrocyte cells response to oxidative stress. To have the best assessment we selected several clones from each transfected CLTT. Then by qRT-PCR we found that adiponectin was significantly overexpressed in two clones, with clone 3 expressing the most abundantl (Fig 1). We selected this clone for the upcoming experiments. As the expressions of adiponectin were similar CLTT and EV cells, either cellular types have been used as controls for the following experiments.

Effect of adiponectin on H_2O_2 - mediated stress

To evaluate if there is any protective or deleterious effect induced by adiponectin on astrocytes viability in OS states, we investigated the relative cellular metabolic activity and the cell proliferation (MTT assay) and the cell viability (trypan blue counting). CLTT, EV and Adipo were treated with H_2O_2 (50-1000 µM) which represents an acute oxidative stress for 24 h. In presence of H_2O_2, overexpression of adiponectin maintained the metabolic activity induced high levels in presence of 50 to 500 µM of H_2O_2 in comparison with EV or CLTT cells (Fig 2A). Therefore, in the following experiments, we chose a H_2O_2 concentrations of 100 µM for cellular treatments for 24 h. This concentration has been chosen as physiologically, in extreme conditions as oxidative burst during pathogen invasion H_2O_2 concentration can reach 100 µM locally *in vivo* (Chen et al.).

In addition, cell viability was assessed by the trypan blue exclusion technique after treatment with 100 µM H_2O_2. As shown in (Fig 2B), the adiponectin overexpression increased significantly cells viability in comparison with EV cells. Furthermore, this protective effect was confirmed in CLTT non-transfected cells in presence of recombinant adiponectin (Fig 2B). Cell morphology was investigated by Hematoxylin-eosin staining. Overexpression of adiponectin modified the cell morphology, which appeared bigger, rounder and showed a loss of the star-shape, typical of astrocytes (Fig 2C). Upon exposure to 100 µM H_2O_2, many control cells lost their star-shaped morphology. In contrast, some adiponectin overexpressing cells cultured for 24 h in presence of 100 µM H_2O_2 maintained the round shape seen without treatment, whereas others appears stretched out. These results showed that adiponectin improved cell resistance against H_2O_2-mediated stress.

Adiponectin effect on the regulation of oxidative stress

To assess the regulation of OS in presence of adiponectin, two techniques have been used, namely, DCF-HDA and Carbonyl ELISA. We evaluated intracellular ROS levels using DCF-HDA assay in basal conditions considered as control and in treated cells with H_2O_2. Results showed that treatment with H_2O_2 increased significantly ROS generation in CLTT cells as well as in cells transfected with the EV cells. Addition of recombinant adiponectin in non tranfected cells or overexpression of adiponectin (adipo clone) regulates ROS generation as compared to CTRL after H_2O_2 treatment (Fig 3A). As an index of OS, we evaluate also the amount of carbonyl content in modified proteins using Carbonyl ELISA based on phenylhydrazine formation reaction. As shown in figure 3B, H_2O_2 treatment induced an accumulation of oxidized proteins in the controlled cells. A decrease in protein oxidation was

observed when adiponectin is overexpressed is the non-stressed cells and this low amount of oxidated proteins is maintained after H_2O_2 treatment.

Expression of pro and anti-oxidants enzymes in adiponectin overexpressing cells

Adiponectin overexpression in non-treated astrocytes down regulated the pro-oxidative enzymes COX-2 and iNOS. In response to H_2O_2 treatment, COX-2 and iNOS mRNA levels were statistically increased in CLTT and EV cells, while the level of COX-2 remained very low in overexpressing adiponectin cells (Fig 4A and B). A similar pattern of response was observed with iNOS, but did not reached significance during OS (Fig 4B).

There was not significant change in SOD or Catalase mRNA expression due to OS in CLTT cells. But once adiponectin was present an important variation in antioxidative enzymes was seen in comparison with CLTT (CTRL). The adiponectin overexpression induced an increase in SOD mRNA expression in basal and under OS situations (Fig 4C). While an increase of catalase mRNA expression was observed only in OS situations (Fig 4D). The changes in mRNA level seen with SOD were not observed for its activity (Fig 5B). In accordance with mRNA expression results, there were no significative differences in catalase activity attributed to oxidative stress induced by H_2O_2 treatment in transfected astrocyte cells as well as non-transfected CLTT. Besides, as shown in figure 5A, adiponectin overexpression in CLTT cells induced an increase in catalase activity in normal and oxidative conditions. By contrast, no upregulation of catalase activity is noticed for adiponectin transfected cells due to oxidative stress. Thus in OS conditions, adiponectin down regulated pro oxidant and up regulated anti-oxydant enzymes.

Expression of AdipoR1 and AdipoR2 receptors

To determine the effect of OS on the expression of Adipo R1 receptors, we assessed their expression by qRT-PCR after OS formation. The overexpression of adiponectin upregulates Adipo R1 expression in basal state and after H_2O_2 treatment (Fig 6). No significative difference was observed between CTRL cells (CLTT and EV cells). Adipo R1 expression increased in presence of adiponectin in basal state and in OS situation. No significant change in Adipo R2 expression was observed upon H_2O_2 treatment (data not shown).

The presence of adiponectin receptors in CLTT cells was monitored by fluorescence microscopy after immunostaining and no receptors were detected in untransfected cells. However, in cells overexpressing adiponectin a detectable fluorescence appears due to Adipo R1 presence (data not shown).

Discussion

Neurodegenerative diseases are a major health issue and with the increase of aging population the rate of people affected by these diseases are likely to continue to progress. Neurodegenerative diseases are usually associated with OS which plays a key role in CNS cells death [23]. Adiponectin is an abundantly expressed adipokine in adipose tissue and has recently attracted much attention because of its antidiabetic and antiatherogenic effects [5]. In addition to these peripheral actions, adiponectin has also been reported to have central actions [24]. It has been shown to be present in the CSF of rodents and human and to enter the CSF from the circulation [15].

Beside its anorexigenic effects [25], other roles of adiponectin in the CNS has been recently investigated. Studies with mice deficient, overexpressing or injected with adiponectin demonstrated that adiponectin have protective effects in ischemic stroke through endothelial nitric oxide synthase and by inhibiting NADPH oxidase-mediated oxidative damage [26,27]. The protective effects of adiponectin are not limited to ischemic stroke, as osmotin, a plant homolog of adiponectin protect against ethanol-induced apoptotic neurodegeneration in the developing brain [28]. Furthermore, *in vitro*, Adiponectin protects hippocampal neurons from excitotoxicity induced by kanaic acid [29].

In addition to adiponectin presence, the adiponectin receptors adipoR1was found to be expressed in most the region of the brain while adipoR2 expression was more restricted to region such as cortex, hippocampus and hypothalamus [25].
Different cell types in the brain express adiponectin receptors including endothelial cells, neurons and astrocytes [18,30,31].

We confirmed the expression of adiponectin receptors in the cellular model of astrocyte (CLTT astrocytic cell line) we used in this study in agreement with previous study *in vivo* and in other cell lines [18,22]. To evaluate the potential role of adiponectin in astrocytes submitted to OS, we stably transfected the CLTT astrocytic cells with a plasmid overexpressing adiponectin and submit these cells to OS (H_2O_2 challenge). Here, we show that adiponectin had a protective effect against cell-death induced OS. The role of adiponectin in the astrocyte cells is further supported by the decreased free radical formation and reduced accumulation of carbonylated proteins observed in adiponectin overexpressing cells treated with 100µM H_2O_2. The concentration used in our study may be physiologically relevant, and could be encountered *in vivo* under extreme conditions [32].

Our results are in agreement with studies in others cell types showing an inhibition of oxidative stress by adiponectin in colon cancer [17], in hyperlipidemia [33] as well as in cardiac and cerebral ischemia [27,34].

Several studies have demonstrated inhibition of adiponectin secretion during inflammation [35]. OS connects the inflammation of adipose tissue to ROS generation and the inhibition of adiponectin secretion. In addition, H_2O_2 treatment in cultured adipocytes confirms the negative modulation of adiponectin by OS (42). This is in agreement with our results showing that ROS generation in astrocyte cells after H_2O_2 treatment tends to down regulate the adiponectin expression suggesting the same effect in the CNS (data not shown).

Adiponectin appears to modulate OS *via* upregulation of anti-oxidative enzymes as SOD that transform the ion superoxide (O^{2-}) to H_2O_2 coupled with Catalase action that abolish cytotoxicity effect of H_2O_2 *via* converting it to water. Our data are consistent with previous work showing that adiponectin inhibits oxidative stress *via* generation of catalase in human prostate carcinoma cells [17]. SOD seems to have modification of its mRNA level, while its activity didn't change significantly. This difference in regulation between mRNA and activity has been already described. In addition, adiponectin presence induces a down regulation of pro-oxidative stress enzymes triggering a decrease of proteins oxidation.

AdipoR1 and AdipoR2, the two adiponectin receptors, appear to be integral membrane proteins; the N-terminus is internal and the C-terminus is external-opposite to the topology of all other reported G protein-coupled receptors (GPCRs) (40). AdipoR1 and AdipoR2 serve as receptors for full-length adiponectin and mediate increased AMP-activated protein kinase (AMPK), peroxisome proliferator-activated receptor (PPAR)-alpha and p38 MAP kinase activities as well as fatty-acid oxidation and glucose uptake by adiponectin.

Adiponectin adjusts generation of OS-related products in the liver and adipose tissue *via* adipo R1 and R2 receptors by increasing AMPK activation and PPARα signaling pathways, respectively. Activation of AMPK reduced gluconeogenesis, whereas expression of the receptors increased fatty acid oxidation and lead to a regulation of glucose and lipid metabolism, inflammation and oxidative stress *in vivo* (43). The antioxidant effects of adiponectin have been recently linked also to activation of AdipoR1 and the resulting downstream release of intracellular calcium together with increased activation of AMPK (44). AdipoR1 and AdipoR2 were found to be abundantly expressed in the hypothalamus, and their expression levels were comparable to those in the liver (40). In addition, the expression of

AdipoR1 increased significantly action in overexpressing adiponectin cells (Adipo) during OS (Fig.6), whereas that of AdipoR2 remained unchanged (data not shown).

In conclusion, our study shows for the first time that adiponectin modulate the OS in astrocytes and could be objects of interest as new therapeutic targets in neuroprotection strategies due to their protective effect on astrocytes cells.

Acknowledgments

This work was supported by grants from "Conseil Régional de La Réunion", Europe (CPER FEDER) and "Ministère de L'enseignement Supérieur et de la Recherche"

References

[1] Sies H. Oxidative stress: oxidants and antioxidants. Exp Physiol 1997;82:291–5.

[2] Janssen-Heininger YMW, Mossman BT, Heintz NH, Forman HJ, Kalyanaraman B, Finkel T, et al. Redox-based regulation of signal transduction: principles, pitfalls, and promises. Free Radic Biol Med 2008;45:1–17. doi:10.1016/j.freeradbiomed.2008.03.011.

[3] Cross CE, Halliwell B, Borish ET, Pryor WA, Ames BN, Saul RL, et al. Oxygen radicals and human disease. Ann Intern Med 1987;107:526–45.

[4] Browne SE, Ferrante RJ, Beal MF. Oxidative stress in Huntington's disease. Brain Pathol Zurich Switz 1999;9:147–63.

[5] Andersen JK. Oxidative stress in neurodegeneration: cause or consequence? Nat Med 2004;10 Suppl:S18–25. doi:10.1038/nrn1434.

[6] Rao AV, Balachandran B. Role of oxidative stress and antioxidants in neurodegenerative diseases. Nutr Neurosci 2002;5:291–309. doi:10.1080/1028415021000033767.

[7] Abbott NJ, Rönnbäck L, Hansson E. Astrocyte-endothelial interactions at the blood-brain barrier. Nat Rev Neurosci 2006;7:41–53. doi:10.1038/nrn1824.

[8] Swanson RA, Ying W, Kauppinen TM. Astrocyte influences on ischemic neuronal death. Curr Mol Med 2004;4:193–205.

[9] Sofroniew MV, Vinters HV. Astrocytes: biology and pathology. Acta Neuropathol (Berl) 2010;119:7–35. doi:10.1007/s00401-009-0619-8.

[10] Sofroniew MV. Astrocyte barriers to neurotoxic inflammation. Nat Rev Neurosci 2015;16:249–63. doi:10.1038/nrn3898.

[11] Röhrdanz E, Schmuck G, Ohler S, Tran-Thi QH, Kahl R. Changes in antioxidant enzyme expression in response to hydrogen peroxide in rat astroglial cells. Arch Toxicol 2001;75:150–8.

[12] Endoh M, Maiese K, Pulsinelli WA, Wagner JA. Reactive astrocytes express NADPH diaphorase in vivo after transient ischemia. Neurosci Lett 1993;154:125–8.

[13] Hirst WD, Young KA, Newton R, Allport VC, Marriott DR, Wilkin GP. Expression of COX-2 by normal and reactive astrocytes in the adult rat central nervous system. Mol Cell Neurosci 1999;13:57–68. doi:10.1006/mcne.1998.0731.

[14] Pasinetti GM, Aisen PS. Cyclooxygenase-2 expression is increased in frontal cortex of Alzheimer's disease brain. Neuroscience 1998;87:319–24.

[15] Kos K, Harte AL, da Silva NF, Tonchev A, Chaldakov G, James S, et al. Adiponectin and resistin in human cerebrospinal fluid and expression of adiponectin receptors in the human hypothalamus. J Clin Endocrinol Metab 2007;92:1129–36. doi:10.1210/jc.2006-1841.

[16] Kadowaki T, Yamauchi T, Kubota N. The physiological and pathophysiological role of adiponectin and adiponectin receptors in the peripheral tissues and CNS. FEBS Lett 2008;582:74–80. doi:10.1016/j.febslet.2007.11.070.

[17] Lu J-P, Hou ZF, Duivenvoorden WC, Whelan K, Honig A, Pinthus JH. Adiponectin inhibits oxidative stress in human prostate carcinoma cells. Prostate Cancer Prostatic Dis 2012;15:28–35. doi:10.1038/pcan.2011.53.

[18] Guillod-Maximin E, Roy AF, Vacher CM, Aubourg A, Bailleux V, Lorsignol A, et al. Adiponectin receptors are expressed in hypothalamus and colocalized with proopiomelanocortin and neuropeptide Y in rodent arcuate neurons. J Endocrinol 2009;200:93–105. doi:10.1677/JOE-08-0348.

[19] Tsatsanis C, Zacharioudaki V, Androulidaki A, Dermitzaki E, Charalampopoulos I, Minas V, et al. Adiponectin induces TNF-alpha and IL-6 in macrophages and promotes tolerance to itself and other pro-inflammatory stimuli. Biochem Biophys Res Commun 2005;335:1254–63. doi:10.1016/j.bbrc.2005.07.197.

[20] Somanah J, Bourdon E, Rondeau P, Bahorun T, Aruoma OI. Relationship between fermented papaya preparation supplementation, erythrocyte integrity and antioxidant status in pre-diabetics. Food Chem Toxicol Int J Publ Br Ind Biol Res Assoc 2014;65:12–7. doi:10.1016/j.fct.2013.11.050.

[21] Aebi H. Catalase in vitro. Methods Enzymol 1984;105:121–6.

[22] Wan Z, Mah D, Simtchouk S, Klegeris A, Little JP. Globular adiponectin induces a pro-inflammatory response in human astrocytic cells. Biochem Biophys Res Commun 2014;446:37–42. doi:10.1016/j.bbrc.2014.02.077.

[23] Dasuri K, Zhang L, Keller JN. Oxidative stress, neurodegeneration, and the balance of protein degradation and protein synthesis. Free Radic Biol Med 2013;62:170–85. doi:10.1016/j.freeradbiomed.2012.09.016.

[24] Thundyil J, Pavlovski D, Sobey CG, Arumugam TV. Adiponectin receptor signalling in the brain. Br J Pharmacol 2012;165:313–27. doi:10.1111/j.1476-5381.2011.01560.x.

[25] Coope A, Milanski M, Araújo EP, Tambascia M, Saad MJA, Geloneze B, et al. AdipoR1 mediates the anorexigenic and insulin/leptin-like actions of adiponectin in the hypothalamus. FEBS Lett 2008;582:1471–6. doi:10.1016/j.febslet.2008.03.037.

[26] Nishimura M, Izumiya Y, Higuchi A, Shibata R, Qiu J, Kudo C, et al. Adiponectin prevents cerebral ischemic injury through endothelial nitric oxide synthase dependent mechanisms. Circulation 2008;117:216–23. doi:10.1161/CIRCULATIONAHA.107.725044.

[27] Song W, Huo T, Guo F, Wang H, Wei H, Yang Q, et al. Globular adiponectin elicits neuroprotection by inhibiting NADPH oxidase-mediated oxidative damage in ischemic stroke. Neuroscience 2013;248:136–44. doi:10.1016/j.neuroscience.2013.05.063.

[28] Naseer MI, Ullah I, Narasimhan ML, Lee HY, Bressan RA, Yoon GH, et al. Neuroprotective effect of osmotin against ethanol-induced apoptotic neurodegeneration in the developing rat brain. Cell Death Dis 2014;5:e1150. doi:10.1038/cddis.2014.53.

[29] Qiu G, Wan R, Hu J, Mattson MP, Spangler E, Liu S, et al. Adiponectin protects rat hippocampal neurons against excitotoxicity. Age Dordr Neth 2011;33:155–65. doi:10.1007/s11357-010-9173-5.

[30] Fry M, Smith PM, Hoyda TD, Duncan M, Ahima RS, Sharkey KA, et al. Area postrema neurons are modulated by the adipocyte hormone adiponectin. J Neurosci Off J Soc Neurosci 2006;26:9695–702. doi:10.1523/JNEUROSCI.2014-06.2006.

[31] Spranger J, Verma S, Göhring I, Bobbert T, Seifert J, Sindler AL, et al. Adiponectin does not cross the blood-brain barrier but modifies cytokine expression of brain endothelial cells. Diabetes 2006;55:141–7.

[32] Dröge W. Free radicals in the physiological control of cell function. Physiol Rev 2002;82:47–95. doi:10.1152/physrev.00018.2001.

[33] Wang W-Q, Zhang H-F, Gao G-X, Bai Q-X, Li R, Wang X-M. Adiponectin inhibits hyperlipidemia-induced platelet aggregation via attenuating oxidative/nitrative stress. Physiol Res Acad Sci Bohemoslov 2011;60:347–54.

[34] Zhang Y, Wang X-L, Zhao J, Wang Y-J, Lau WB, Yuan Y-X, et al. Adiponectin inhibits oxidative/nitrative stress during myocardial ischemia and reperfusion via PKA signaling. Am J Physiol Endocrinol Metab 2013;305:E1436–43. doi:10.1152/ajpendo.00445.2013.

[35] Fantuzzi G. Adiponectin in inflammatory and immune-mediated diseases. Cytokine 2013;64:1–10. doi:10.1016/j.cyto.2013.06.317.

Figure legends

Figure 1 : Characterization of stably adiponectin-transfected astrocyte cells.

Adiponectin mRNA levels were quantified after 24 hours of culture from CLTT cells transfected with pcDNA3.1-Adiponectin (clones 1,2,3,4 and 5) or empty vector (EV 1, 2 and 3), or from untransfected CLTT cells. Results are the mean ± SD from experiments performed in triplicate. ***p<0.001 significantly different from CLTT as determined by Dunnett's t-test post hoc analysis following a significant overall ANOVA.

Figure 2: Hydrogen peroxide effect on adiponectin overexpression cell proliferation and viability.

Non transfected cells (CLTT), Empty-vector (EV), adiponectin (Adipo) transfected cells and recombinant adiponectin treated cells were incubated for 24 h at 37ºC in the absence or presence of H_2O_2. Various concentrations of H_2O_2 were used for proliferation MTT assay (A). After incubation with 100 µM H_2O_2 for 24 h, the cell viability was evaluated by trypan blue exclusion (B) and the cell morphology was visualized following cell H&E staining as described in the methods section (C). Bars correspond to 20 µm. Results are the mean ± SD from experiments performed in triplicate. ***p<0.001 values were obtained using Tukey's two ways ANOVA to compare Adipo with EV cells (n=3).

Figure 3: Effect of adiponectin overexpression on oxidative stress.

Intracellular ROS generation was evaluated by DCF-HDA assay (A) in CLTT and recombinant adiponectin treated cells incubated for 24 h in the absence (CTRL) or presence of 100 µM H_2O_2. Evaluation of carbonyl contents using Carbonyl ELISA (B) was performed on non-transfected cells (CLTT), Empty-vector (EV), adiponectin (Adipo) transfected cells incubated for 24 h in the absence (CTRL) or presence of 100 µM H_2O_2. EV cells were used to assess the carbonyl formation in absence of adiponectin. Results are the mean ± SD from experiments performed in triplicate. *P<0.05 and **P<0.01 compare to control cells using Tukey's two ways ANOVA for unpaired samples (n=3).

Figure 4: Regulation of anti-oxidative and pro-oxidative enzymes expression *via* adiponectin's overexpression during oxidative stress.

Non transfected cells (CLTT), Empty-vector (EV) and adiponectin (Adipo) transfected cells were incubated for 24 h in the absence (CTRL) or presence of 100 µM H_2O_2. Adiponectin's

effect respectively on COX-2 (A), iNOS (B), SOD (C) and Catalase (D) expression were evaluated by Real Time Q-PCR. Catalase activity (A.U/100 mg protein) of non-transfected cells (CLTT), EV and Adipo transfected cells incubated for 4 h in the absence (CTRL) or presence of 100 µM H_2O_2 (E). Results are the mean ± SD from experiments performed in triplicate or quadruplet. Tukey's two ways ANOVA analysis is *P<0.05, **P<0.01 and ***P<0.001 compared to non-tranfected CLTT.

Figure 5: Regulation of catalase and SOD activity *via* adiponectin's overexpression during oxidative stress.

Non transfected cells (CLTT), Empty-vector (EV) and adiponectin (Adipo) transfected cells were incubated for 24 h in the absence (CTRL) or presence of 100 µM H_2O_2. Catalase (A) and SOD (B) activities (A.U/100 mg protein) of non-transfected cells (CLTT), EV and Adipo transfected cells incubated for 4 h in the absence (CTRL) or presence of 100 µM H_2O_2. Results are the mean ± SD from experiments performed in triplicate or quadruplet. Tukey's two ways ANOVA analysis is *P<0.05, **P<0.01 and ***P<0.001 compared to non-tranfected CLTT.

Figure 6: Overexpression of adiponectin upregulates the expression of Adipo R1 and Adipo R2 receptor.

Non transfected cells (CLTT), empty vector (EV) or adiponectin (ADIPO) transfected cells were incubated for 24 hours in the absence or presence of 100 µM H_2O_2. Adipo R1 and Adipo R2 mRNA expression were assessed by Real time q-PCR. Results are the mean ± SD from experiments performed in triplicate. Tukey's two ways ANOVA analysis for RT q-PCR results is *P<0.05 compared to non-tranfected CLTT.

Fig.1

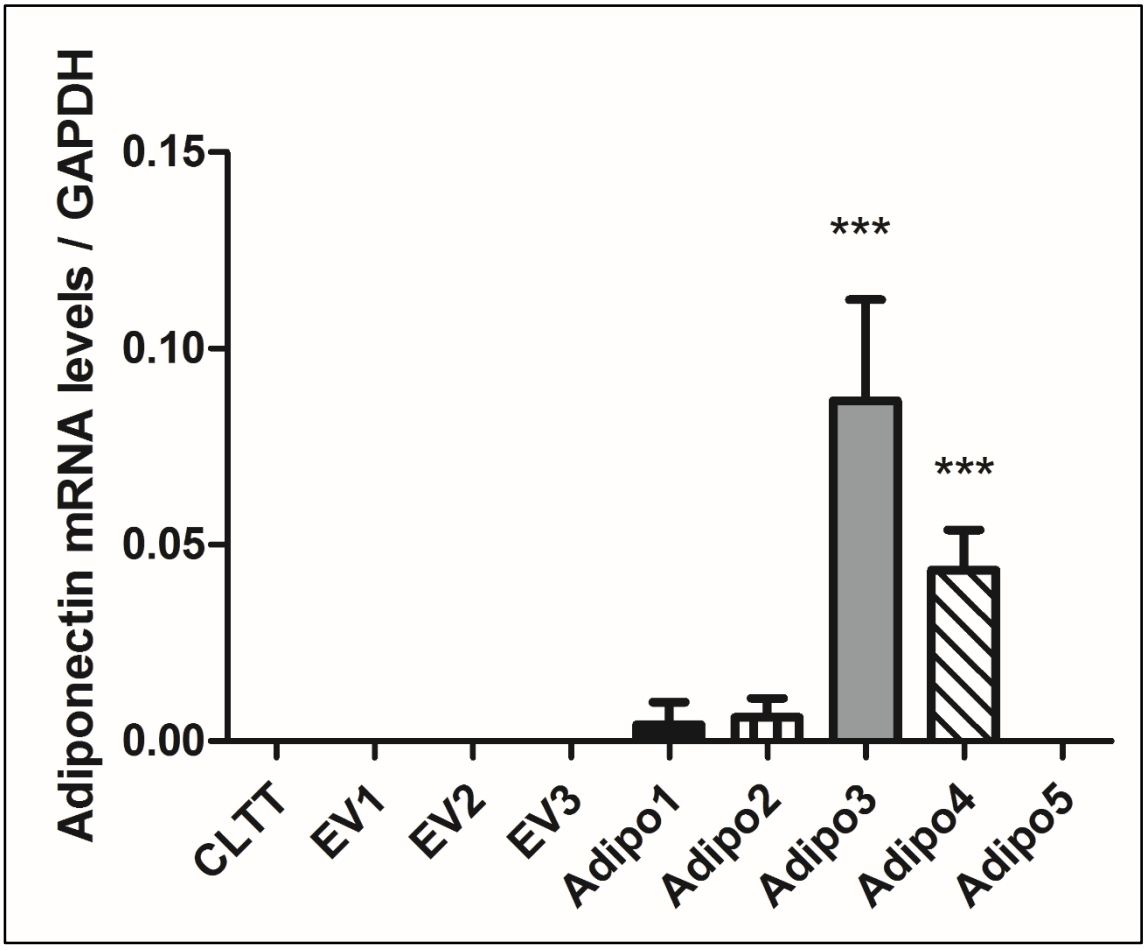
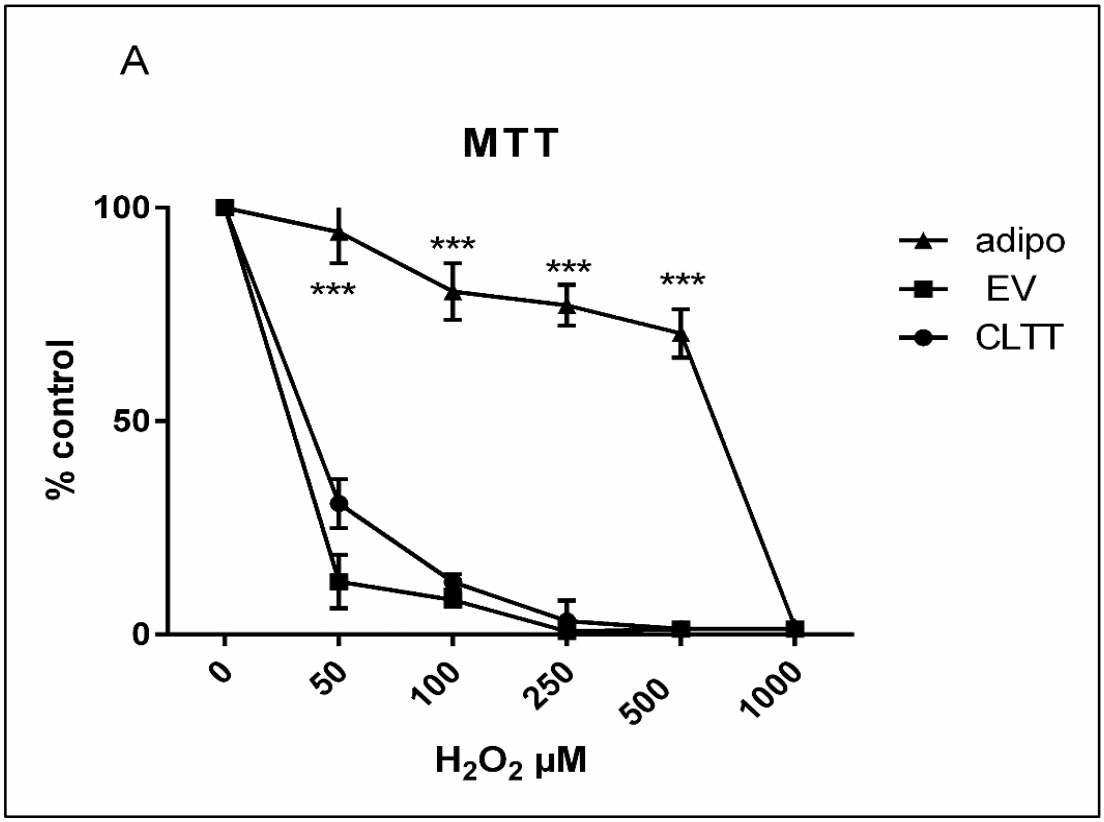

Adiponectin mRNA levels / GAPDH
0.15
0.10
0.05
0.00
***
***
CLTT
EV1
EV2
EV3
Adipo1
Adipo2
Adipo3
Adipo4
Adipo5

Fig.2


A
MTT
100
50
0
% control
***
***
***
***
adipo
EV
CLTT
0
50
100
250
500
1000
H2O2 µM

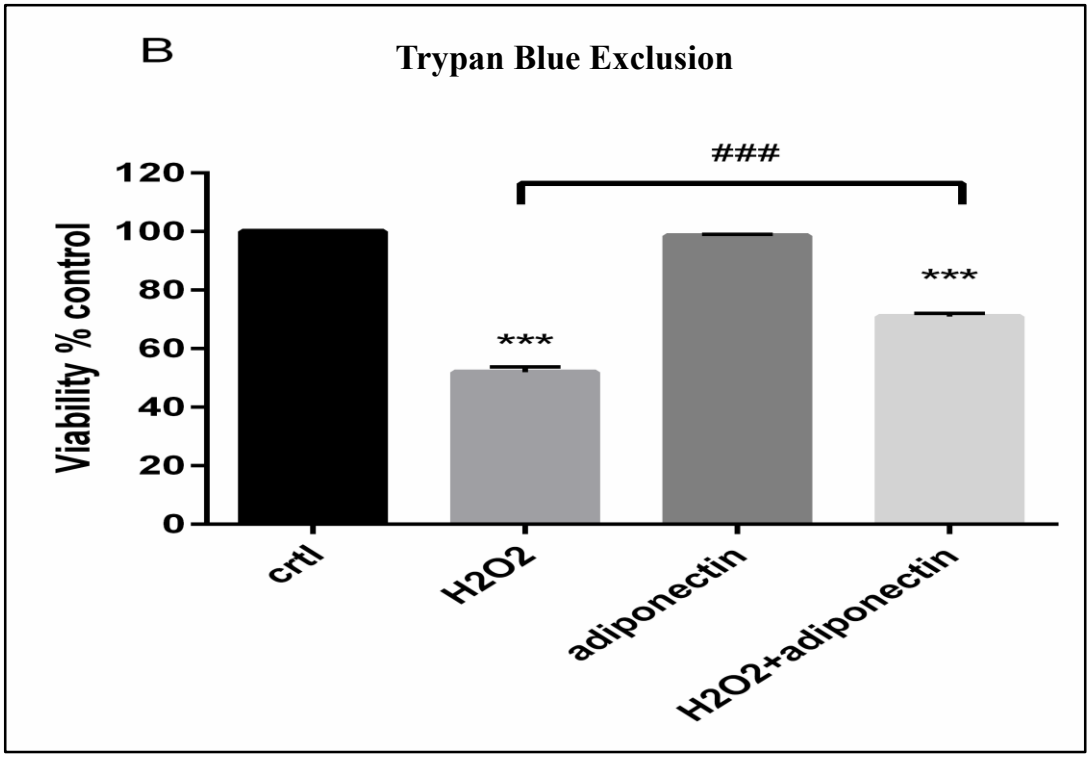

B
Trypan Blue Exclusion
###
120
100
80
60
40
20
0
Viability % control
***
***
crtl
H2O2
adiponectin
H2O2+adiponectin

Fig.2.C Hematoxylin and eosin staining

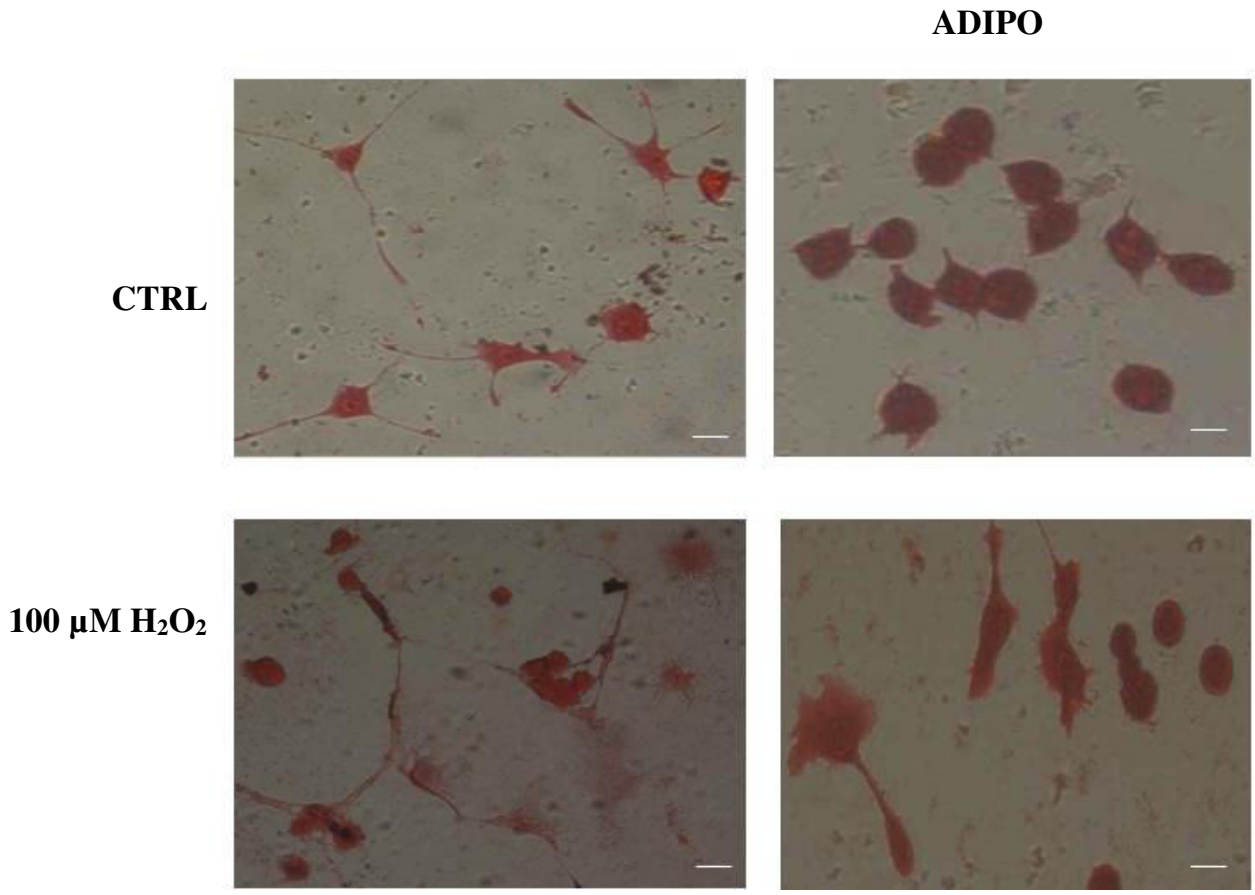
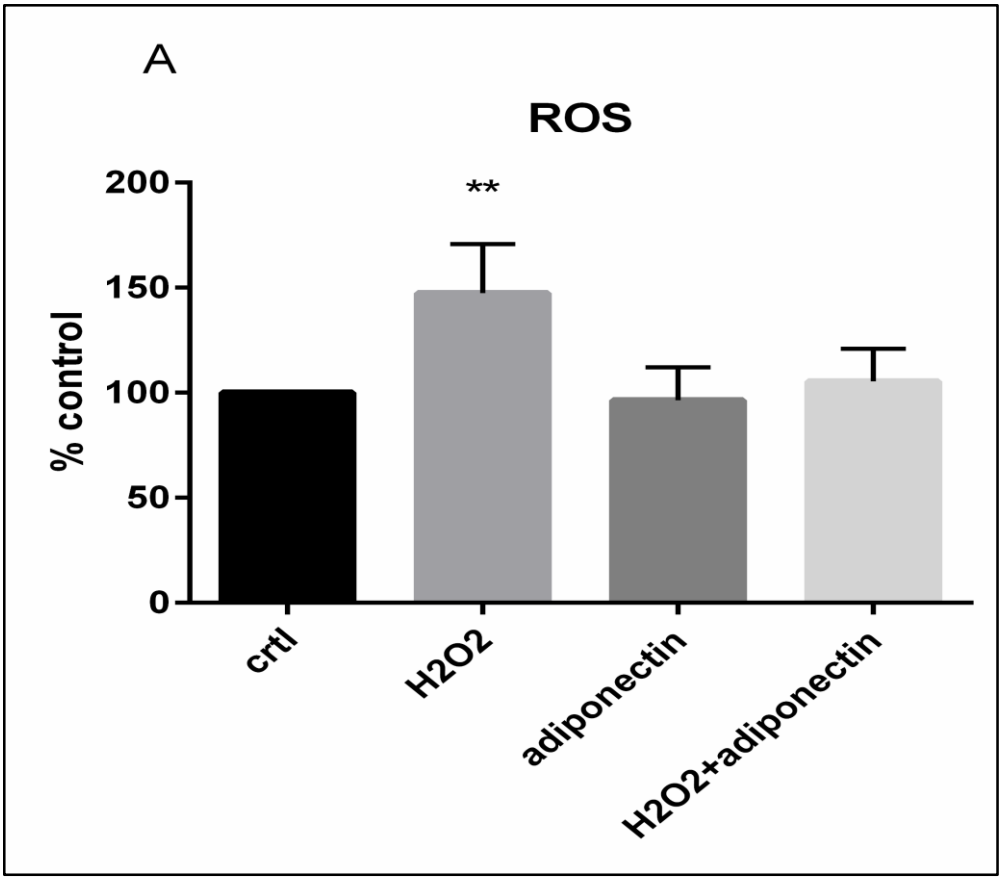


Fig.3


A
ROS
200
150
100
50
0
% control
**
crtl
H2O2
adiponectin
H2O2+adiponectin

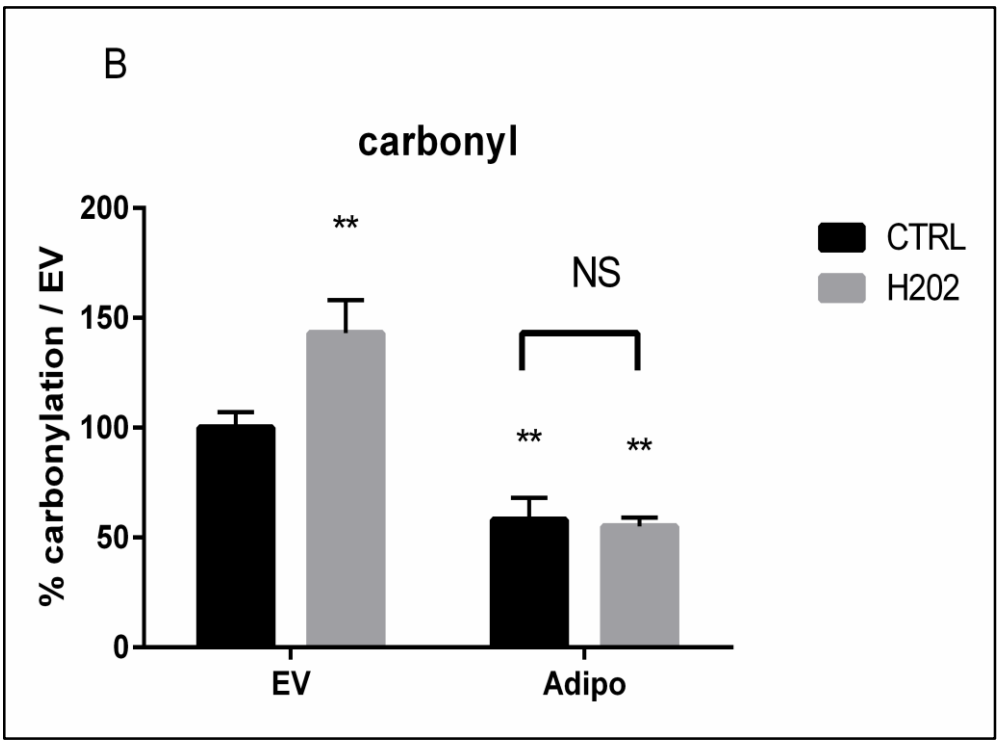

B
carbonyl
200
150
100
50
0
% carbonylation / EV
**
NS
**
**
CTRL
H202
EV
Adipo

Fig. 4

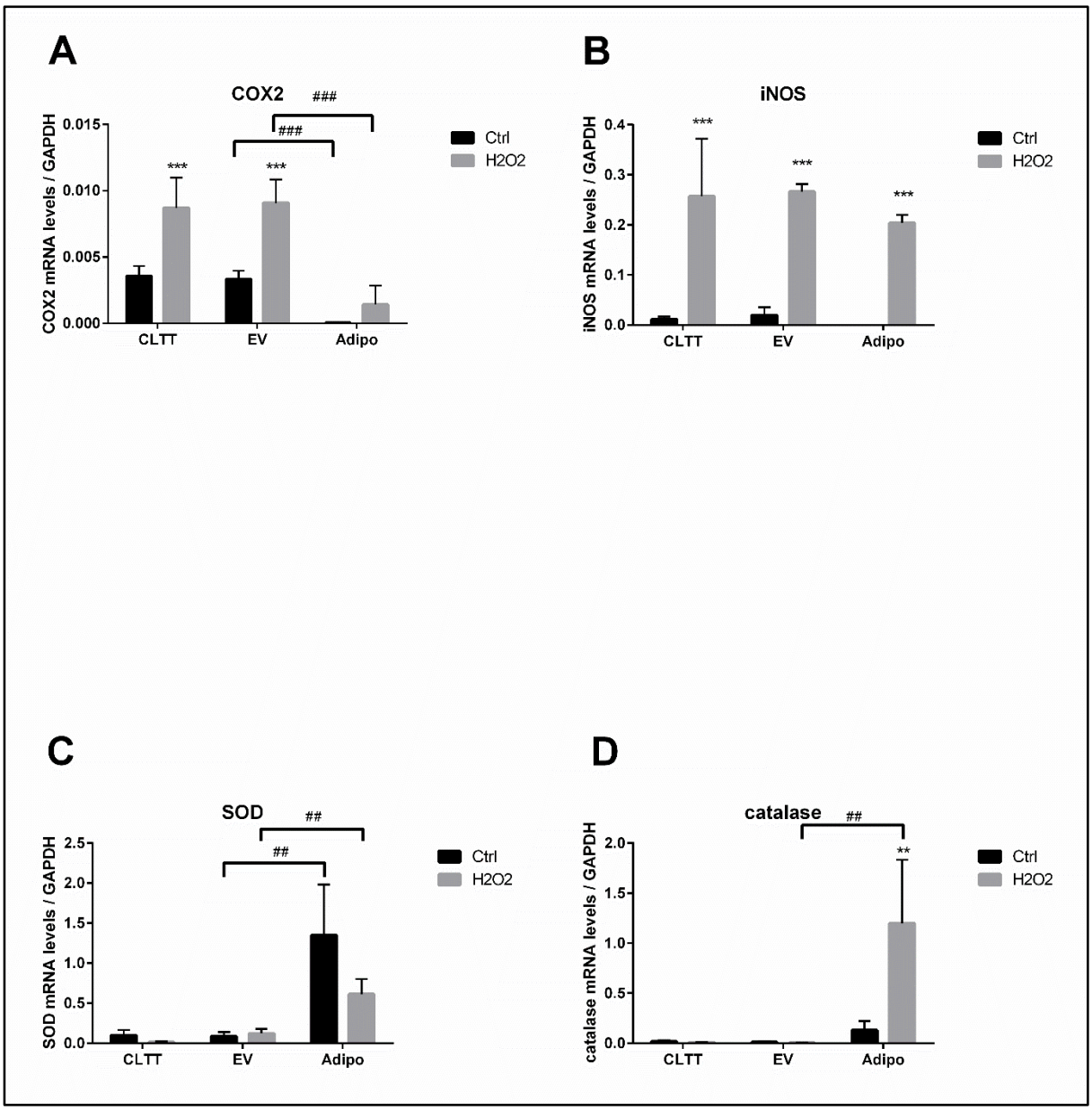

A
COX2
###
###
***
***
COX2 mRNA levels / GAPDH
0.015
0.010
0.005
0.000
CLTT
EV
Adipo
Ctrl
H2O2
B
iNOS
***
***
***
iNOS mRNA levels / GAPDH
0.4
0.3
0.2
0.1
0.0
CLTT
EV
Adipo
Ctrl
H2O2
C
SOD
##
##
SOD mRNA levels / GAPDH
2.5
2.0
1.5
1.0
0.5
0.0
CLTT
EV
Adipo
Ctrl
H2O2
D
catalase
##
**
catalase mRNA levels / GAPDH
2.0
1.5
1.0
0.5
0.0
CLTT
EV
Adipo
Ctrl
H2O2

Fig. 5

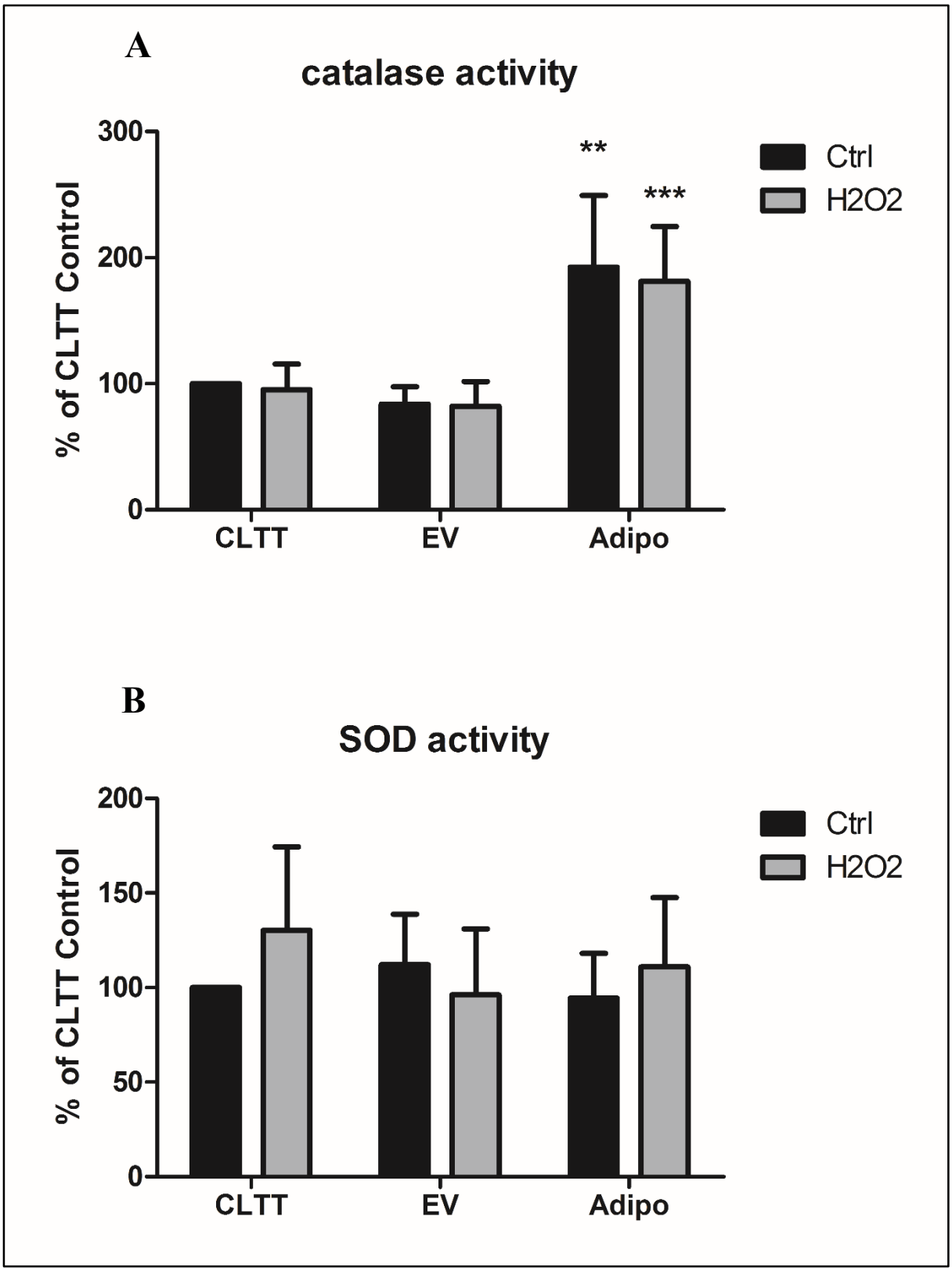
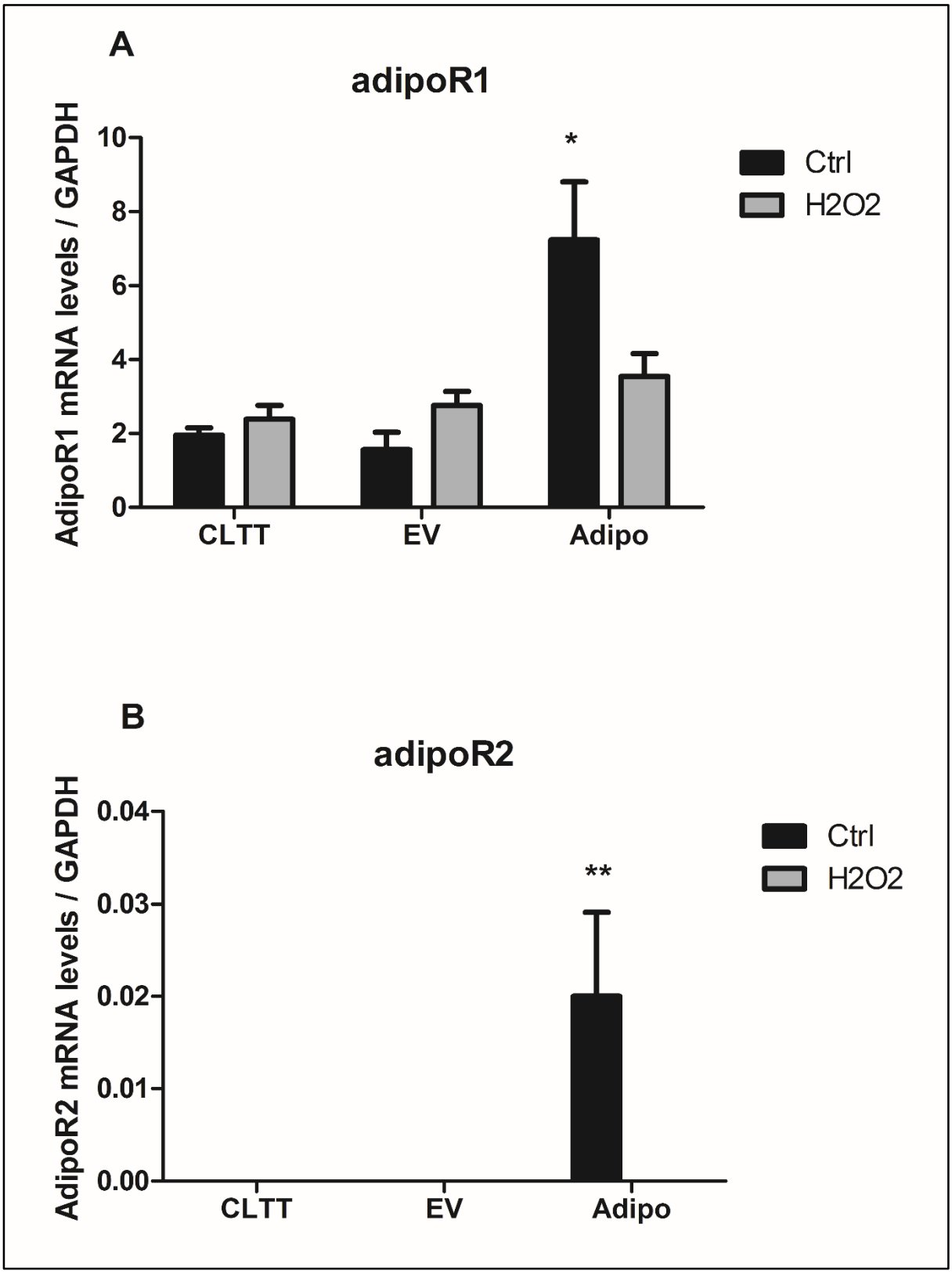

A
catalase activity
% of CLTT Control
300
200
100
0
**
***
Ctrl
H2O2
CLTT
EV
Adipo
B
SOD activity
% of CLTT Control
200
150
100
50
0
Ctrl
H2O2
CLTT
EV
Adipo

Fig. 6


A
adipoR1
AdipoR1 mRNA levels / GAPDH
10
8
6
4
2
0
*
Ctrl
H2O2
CLTT
EV
Adipo
B
adipoR2
AdipoR2 mRNA levels / GAPDH
0.04
0.03
0.02
0.01
0.00
**
Ctrl
H2O2
CLTT
EV
Adipo

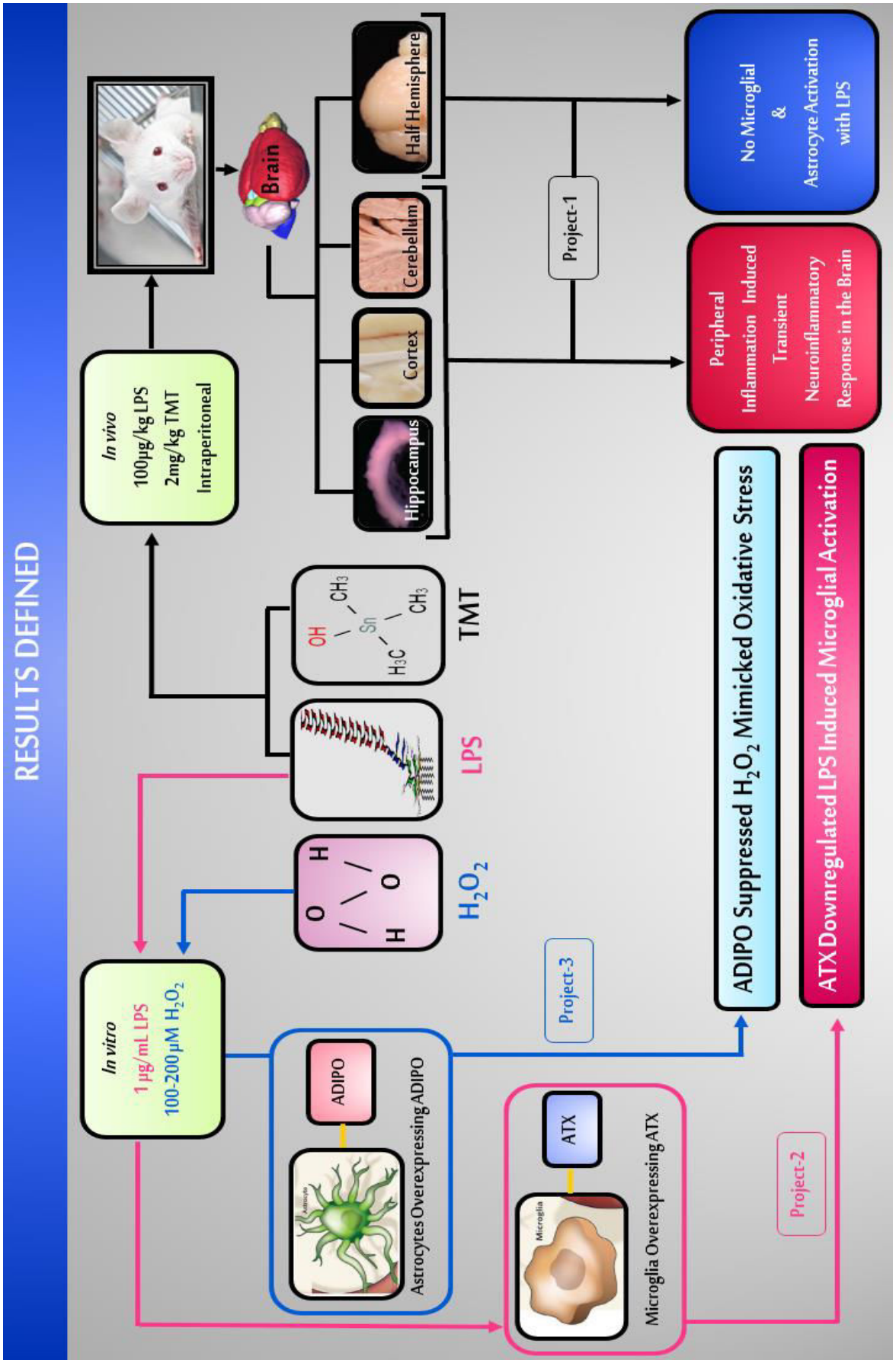


FIGURE R-1 **Results Defined**

DISCUSSION

Global Discussion of Thesis:

This section discusses and emphasizes the key findings of the thesis. A more detailed elucidation of the results and further information on the association between adipocytokines and its impact on central nervous system can be found in the corresponding articles.

The impact of adipose tissue had gained significant importance during early 1990's with the discovery of leptin. This had made the researchers to ponder about the impact of adipocytokines in health and disease. Substantial data in the arena of adipose tissue biology strongly affirms that obesity is one of the crucial factor and is responsible for the onset of various pathophysiological diseases including insulin resistance (Kahn and Flier, 2000), T2DM (Eckel et al., 2011), CVD and atherosclerosis (Rocha and Libby, 2009). But the recent advances in neuroimmunological research and as well multiple clinical studies reveals the detrimental effects of adiposity on neurodegenerative diseases. For example, one of the first and foremost scientific report on the association between adiposity (higher BMI) and Alzheimer's disease were published by Deborah Gustafson on 2003, who stated that "*Overweight at high ages is a risk factor for dementia, particularly AD, in women*" (Gustafson et al., 2003). Following this research, several other studies reported the potential link between obesity and neurodegeneration (Bruce-Keller et al., 2009; Debette et al., 2010). These significant discoveries for nearly two decades had buildup to lay even more concrete foundation in understanding the relation between adipocytokines and central nervous system.

Control of energy homeostasis requires communication between brain and adipose tissue (Turtzo and Lane, 2002). Many chronic neurodegenerative diseases such as Alzheimer's and Parkinson's disease have been associated with physiological processes of inflammation and oxidative stress in the CNS in which glial cells such as microglia and astrocytes were found to be the key players involved in neurodegeneration. Besides the active involvement of these glial cells in repairing the CNS, microglia and astrocytes were found to be the richest sources of cytokines and adipocytokines (Leptin, ADIPO and ATX) found in the brain. It is also worth noticing that these adipocytokines and their receptors are not only expressed in the adipose tissue but well expressed in the CNS. Despite of the accumulating information indicating the involvement of adipocytokines on CNS vulnerability, the precise mechanistic pathways responsible for the onset of variable changes in the CNS remains uncertain.

Henceforth, we aimed to investigate the properties (inflammatory and oxidative stress) of the factors produced by the adipose tissue (Autotaxin and Adiponectin) and their potential implication in neuroinflammation and neurodegeneration. The potential of these two factors were evaluated, both *in vitro* in glial cell lines (microglia and astrocytes) and *in vivo* in neuroinflammatory, acute hippocampal neurodegenerative mice models. In order to accomplish this task, we designed our experimental setup into three individual projects. The results thus obtained from these three projects were conglomerated to have an overall opinion that should fetch us a rational conclusion on how these factors secreted by the adipose tissue are acting on the brain glial cells both *in vivo* and *in vitro*.

Adiponectin, Resistin and Autotaxin Expression in Neuroinflammation and Neurodegeneration

The current study was undertaken to characterize the gene expression of inflammatory mediators generated in response to defined intraperitoneal LPS and TMT stimulations *in vivo* in mice. In addition to this, we also examined microglial activation and reactive astrogliosis with respect to LPS stimulus that should lead us to comprehend the mechanisms underlying the nexus between immune system activation towards brain signalling following peripheral inflammation. Various neuroimmunologists had doubted the fact that peripheral immune system could signal the brain? This is majorly due to the assumption that cytokines could not cross the bloodbrain barrier. BBB exceptionally refines all the biomolecules such as (glucose, amino acids, soluble lipids), gases such as (O_2, CO_2) water, hormones, cytokines (McLay et al., 1997) and selective antibiotics (Raza et al., 2005) that allows to pass through the brain. Under compromised conditions, the cells lining BBB will lose its selective permeability in allowing these molecules, thereby microscopic infectious agents such as bacteria, virus and various pathogenic agents diffuses into the brain which ultimately leads to vulnerable brain status. However, recent works have demonstrated that there are several routes by which peripheral cytokines can either directly cross the BBB or indirectly signal the brain through other informational substances (iNOS, IL-1) or via the leaky regions of endothelia under compromised brain status (Kronfol and Remick, 2000).

Growing body of evidences on immune to brain interactions suggests that, activated peripheral immune system in response to exogenous or endogenous insults cross talk with the CNS mediated via cytokine signalling. For example, peripheral cellular immune activation and cytokine response to low grade inflammation (LPS-100µg/kg) and their potential impact towards brain can be justified from the works of Teeling and his team. In this work, the authors had demonstrated that the expression and secretion of cytokine signalling factor IL-1β was found to be significantly upregulated in peripheral organs such as liver and spleen with respect to LPS treatment. Cells expressing IL-1β were morphologically recognized to be Kupffer cells and neutrophils in the liver, and dendritic cells and neutrophils in spleen (Teeling et al., 2007). Another study from Quan et al in 1997 put forward that peripheral administration of 2.5 mg/kg bwt of LPS into mice induced the upregulation of IkB-α mRNA. IkB controls the activity of NFkB, which regulates the transcription of many immune signal molecules. IkBα thus produced were found to be highly localized especially in endothelial cells, astrocytes rather than microglia or perivascular monocytes. This data represents that cytokines signals had reached brain parenchyma and importantly, the responsive astrocytes are probably producing centrally derived cytokines (Quan et al., 1997).

LPS is the outermost layer of gram negative bacteria which elicits strong innate immune response both in animals and humans. Gram negative bacteria are responsible for mounting various kinds of inflammation related infectious disorders in the brain such as meningitis. Since LPS produces pronounced inflammatory effects in both animals and humans, interaction between LPS and inflammation related toll like receptor (TLR) has been widely used to study inflammation related disorders both *in vitro* and *in vivo*. Peripheral infection with LPS triggers

transcriptional activation of genes that could induce a transient neuroinflammatory response in the brain mediated via microglia, astrocytes in the brain parenchyma. This reaction involves inflammatory and oxidative stress cytokines and is highly dose and time dependent. This wide spread reactivity mediated by these cells mimics the expression of genes, in which some genes are induced rapidly (early mediators of inflammation and oxidative stress - TNF-α, iNOS, Adiponectin, IL-1β, IκBα, CD14) whereas others will be detected from hours to days (late mediators of inflammation and oxidative stress - members of the complement family, COX, IL-6). TMT is a derivative of tin and is used as a plastic stabilizer. It distributes throughout the body and is well known for its toxic effects on CNS that produces sound effect especially on hippocampal neurodegeneration (Reuhl et al., 1983) via TNF-α pathway (Neurodegenerative TMT model).

Acute intraperitoneal injection of mice with LPS and TMT provokes strong inflammatory response accompanied by the activation of glial cells in the CNS (microglial activation and reactive astrogliosis) which are found to be the hallmark features of neurodegenerative disorders (Bruccoleri et al., 1998; Eskes et al., 2003). Reactive microglial cells can be identified by its display of striking features such as cellular hypertrophy, exhibition of remarkable morphological and phenotypic changes, having characteristic bushy appearance because of extended cytoplasmic processes, display of increased phagocytic activity and as well by upregulating the expression of inflammatory and oxidative stress factors. Reactive astrogliosis can be characterized via spectrum of morphological, molecular, cellular and functional changes, significant upregulation of astrocyte-specific structural intermediate filamentous protein, glial fibrillary acidic protein, cellular proliferation, scar formation and cellular hypertrophic reactions. As a result of this, these glial cells will promote the extensive upregulation of expressing the inflammatory cytokines that will have a greater impact on the CNS.

Despite of the accumulating data suggesting the involvement of cytokines in regulating neuroinflammation, the precise pathways responsible for the onset of these inflammatory changes in the CNS remains uncertain. Henceforth, we set up this project in order to comprehend the interactions between immune system and CNS vulnerability.

Here in our current study, low grade peripheral inflammation was induced in mice using 100 µg/kg LPS to mimic aspects of gram negative bacterial infection (Neuroinflammatory LPS model). Apart from neuroinflammatory LPS model, we used organometal TMT (2mg/kg bwt) as a chemically induced potential neurotoxicant to induce both neuroinflammatory and neurodegenerative response in the mouse CNS.

Here, we show that i.p injection of LPS in mice, results in an early response of TNFα and iNOS in the hippocampus, cortex and cerebellum with a peak at 2-4 hours, while ADIPO showed its expression peak at 6h, 120h and 24h in hippocampus, cortex and cerebellum respectively, whereas Resistin showed its inflammatory peak at 6h point of time period only in hippocampus, while no significant changes were observed for ATX. We confirmed that an acute ip injection of TMT induces an increase in TNF-α, iNOS mRNA (peak at 24h, 4h

respectively). Elevated ATX and ADIPO mRNA levels in the hippocampus were demonstrated in mice 5 and 8 days respectively following the TMT injection.

Summing up from our experimentational outputs, we demonstrated that LPS and TMT could induce a transient neuroinflammatory response in three distinct regions of the brain (HIP-COR-CER) that involved inflammatory (TNF-α, Adiponectin, and Resistin) and oxidative stress mediators (iNOS). Besides the absence of microglial and astrocyte activation suggests that low grade inflammation induced by infection, will not induce neurodegeneration (unless a massive infection) but could prime the glial cells making them to be more responsive for further stimulation. This particular dosage of LPS (100μg/Kg bwt) is sufficient enough to trigger transient neuroinflammatory response in the brain, but not to induce the glial cell activation. Higher LPS dosage (1 mg/kg bwt), such as described in other studies demonstrated LPS-induced microglial activation and reactive astrogliosis in the hippocampus 3 days post intraperitoneal LPS treatment (Okuyama et al., 2013).

Conclusively our results are in strong agreement with the hypothesis that peripheral immune system could signal the brain via cytokine signalling and there exists a relationship between immune system activation and neuroinflammatory response in the CNS.

Autotaxin Downregulates LPS-Induced Microglia Activation and Pro-Inflammatory Cytokines Production

In the current study, we investigated the impact of ATX's interaction in microglial CNS cells under inflammation settings. Long been the CNS was considered an immune privilege organ because of its isolation from the immune system by the blood brain barrier, and as well by the inability of the glial cells (especially microglia) to initiate an immunological reaction leading to the process of neuroinflammation. But this misconception was dramatically altered with the various scientific documentations unravelling the facts that peripheral immune cells can cross the intact BBB and glial cells can actively regulate CNS interactions (Carson et al., 2006).

Microglia make up the innate immune system of the CNS and are the key cellular mediators of neuroinflammatory processes and plays an active role in both acute and chronic neuroinflammatory responses (Roy et al., 2008). Acute neuroinflammation includes immediate response of early glial cells, including microglia which is the first and foremost defensive reaction that prepares the repairing mechanism. Whereas chronic inflammation persists when the inflammatory trigger or stimulus persists. The concept of inflammation is more pertinent in the context of the understanding of brain neurodegenerative disorders that involve some degree of chronicity. Microglia are the major effector cells of the CNS that initiates an immune response following CNS trauma. Microglia in possession of its immunocompetent phagocytic activity serves as sentinels after infection, injury, ischemia and process of neurodegeneration. It is also involved in the clearance of cellular debris after CNS trauma. In addition to this, several data in the literature suggest a beneficial role of microglia following a neurodegenerative episode (Gemma and Bachstetter, 2013).

Autotaxin is a secreted Lysophospholipase D responsible for the synthesis of lysophosphatidic acid (LPA). ATX by means of the LPA and LPA1 receptor present on the surface of the microglia could participate in several physiological activities (Kanda et al., 2008). The role of ATX in inflammation is controversial. ATX or LPA receptor expression and/or secretion were elevated as demonstrated in the patients suffering from inflammatory disorders including rheumatoid arthritis, multiple sclerosis and others. Besides this, LPA inhibition of LPS induced inflammation in macrophages demonstrates the anti-inflammatory properties of ATX in mice.

However, the role of ATX in microglial CNS cells under inflammation has never been studied before. Here in the current study, we proposed to use lipopolysaccharide (LPS) to mimic inflammatory signalling in microglial cells. LPS stimulates the transcriptional activity of inflammatory genes in brain parenchymal microglia. Indeed, LPS causes a sharp increase in terms of the transmembrane receptors CD14 and TLR4 on the surface of these microglial cells. These receptors form a complex with LPS and My88 protein which initiates NF-kB signaling that triggers the transcription of pro-inflammatory genes such as TNF-α.

First of all, we stimulated microglia with two different concentrations of LPS (10ng-1μg/mL) and then we investigated the effects of LPS on TNF-α, IL-10 and ATX time course expression. We observed that LPS augmented TNF-α, ATX mRNA levels and diminished the IL-10 levels.

We then transfected the microglial cells with a plasmid vector containing ATX gene. Following post transfection, mRNA expression levels of the ATX, LPA1 and LPA production were determined. LPS heightened the production of LPA. Besides this, we also confirmed that microglia express LPA1 receptor and the over expression of ATX significantly upregulated this receptor expression which suggests that LPA is responsible for most of the biological effects caused by ATX mediated via the activation of G protein-coupled receptor.

From this study we observed that ATX prevented microglial activation which can be supported from the data that LPS induction of mRNA and protein levels of pro-inflammatory cytokines TNF-α and IL-6 were significantly inhibited in Autotaxin transfected cells, while the anti-inflammatory cytokine IL-10 was elevated in these cells. Inhibition of NF-kB may be partly responsible for the inhibition of these inflammatory cytokines, as shown in the conditioning effect of microglial overexpressing ATX transfected cells. The effect of the conditioned medium was similar to the effect LPA in the presence of LPS. Besides this, different markers were used to monitor microglial activation. CD11b, CD14 are the markers of activation well known for microglia. B7.1 (CD80) and B7.2 (CD86) are costimulatory molecules of the family of glycoproteins and found that the expression of these molecules was reduced in cells overexpressing ATX, indicating not only regulation of innate immunity microglia, but also their adaptive immune functions.

With these results we show for the first time, an anti-inflammatory role of ATX in microglia cells subjected to inflammation. Summarizing the results, we have shown that LPS induced the production of the ATX in microglia and that overexpression of autotaxin inhibits inflammation induced by LPS and as well inhibits NF-kB activation by suppressing the expression of TNF-α and IL-6. Besides this we had also demonstrated that ATX blocks the induction of CD11b, CD14, B7.1 and B7.2 induced by LPS at the surface of microglia. Finally, our results strongly suggest the ATX downregulates LPS induced microglial activation and pro-inflammatory cytokine production. Conclusively, our results are consistent with the work of Fan et al. on macrophages suggesting a beneficial role of ATX in the CNS injury by protecting the microglial cells and downregulating their activation under inflammatory conditions. These potential beneficial actions could open new therapeutic perspectives for ATX or LPA.

Role Of Adiponectin On Astrocytes Under Oxidative Stress Situation

The present study investigated the role of Adiponectin in astrocytic CNS cells subjected to oxidative stress situations. Astrocytes have first been considered just as glue and as a provider of physical support to neurons. This simplistic view is now obsolete with a variety of functions attributed to astrocytes. It has been demonstrated that astrocytes have a greater impact in CNS development, homeostasis and CNS vulnerability and pathology (Aschner et al., 1999). The complex biology of astrocytes and the reciprocal communicating networks between astrocytes, neurons and other cell types have made these cells the focus for studying the neurodegenerative diseases. Astrocytes constitutes about 50-90% of all the brain cells and displays a manifold of immune and other assorted functions in which these star shaped cells plays a critical role in the brain antioxidant defense. Recently, it was found that implication of astrocytes and microglia in the brain are very much important to determine the optimal functioning and communication of neurons (Norden et al., 2014). So the malfunctioning of these brain resident astrocytes eventually leads to a process called reactive astrogliosis which could subsequently lead to various neurodegenerative brain diseases, including Alzheimer's disease, Parkinson's disease, and various forms of dementia.

This remarkable glial cell activation is induced by LPS as well by IFNs and involves various inflammatory molecules including receptor (TLR), transcription factor (NF-kB), signalling pathway (STATs). STATs function as sensors of cellular stress, including oxidative stress. Although antioxidant enzyme systems in the brain especially in astrocytes are well developed, there is a tipping point in the redox balance beyond which it is virtually impossible for the brain to recover from oxidative injuries. In this context, it is critical for the brain to be able to detect ROS rapidly enough to mount an antioxidant response capable of maintaining redox homeostasis (Park et al., 2012).

Adiponectin (Adipo) is a 30kDa adipocyte secretory protein (Pajvani and Scherer, 2003) (adipocytokine) with direct anti-diabetic and anti-inflammatory properties (Yamauchi et al., 2001). Adiponectin has the potentiality to cross the blood-CSF-barrier (BCB) and the blood-brain barrier (BBB) and there are substantial evidences which suggests that adiponectin receptors are expressed widely in the brain. Adiponectin receptors expression has been detected in whole brain and pituitary extracts, in specific regions such as hypothalamus, brainstem, and in cell types (cortical neurons and endothelial cells) (Thundyil et al., 2012a). Adiponectin apart from its defined role in metabolic disorders such as obesity and T2DM, also plays a contributing role in neurodegenerative disorders including Alzheimer's disease (Song and Lee, 2013). Despite of these considerations, the role of ADIPO in oxidative stress conditions still remains debatable. Besides this, Adiponectin also plays a promising role in immune system in the CNS where adiponectin decreases the expression of pro-inflammatory cytokines such as tumor necrosis factor-α (TNF-α), increases the expression of anti-inflammatory molecules such as interleukin (IL)-10 and IL-1 receptor antagonist (Wolf et al., 2004) and decreases the activation of the pro-inflammatory signal pathway such as nuclear factor-κB pathway (NF-κB).

Various studies in the central nervous system suggest the neuroprotective actions of adiponectin (Jung et al., 2006). One includes protection of hippocampal neurons against kainic acid-induced excitotoxicity (Jeon et al., 2009) and another one includes regulation of severe brain inflammation in mild cognitive impairment and Alzheimer's disease (Hivert et al., 2008; Forlenza et al., 2009).

However, the potential role of ADIPO in astrocyte CNS cells under oxidative stress conditions has never been studied before. In this regard, we were curious in understanding the mechanisms underlying the impact of adiponectin in CNS astrocytes under oxidative stress conditions. Here in the current study, we proposed to use hydrogen peroxide (H_2O_2) to mimic oxidative stress signalling in astrocytes. H_2O_2 stimulates the transcriptional activity of oxidative stress genes in which astrocytes sense H_2O_2 by rapidly phosphorylating the transcription factor STAT6. STAT6 phosphorylation can be induced by generators of other reactive oxygen species (ROS) and reactive nitrogen species. This can be justified from the reports of Soo Jung Park et al., in which they had demonstrated that ROS-induced STAT6 phosphorylation in astrocytes can modulate the functions of neighboring cells, including microglia, through cyclooxygenase-2 induction and subsequent release of PGs (Park et al., 2012).

In order to investigate the potential effects of adiponectin in astrocytic cells under H_2O_2 mediated oxidative stress, we sub cloned the complementary DNA of adiponectin in eukaryotic expression vectors and stably transfected it in mouse CLTT astrocyte cells. Overexpressing adiponectin and non-transfected cells were treated with hydrogen peroxide (50-1000μM H_2O_2). Relative toxicity was determined using trypan blue and MTT assays. Protein oxidation and ROS levels were quantified by measuring carbonyl content and rates of DCF oxidation, respectively. CLTT astrocyte cell expression of adiponectin receptors (AdipoR1, AdipoR2), SOD, iNOS, COX-2 and Catalase expression were determined by qRT-PCR.

Here we successfully showed that adiponectin overexpression in CLTT cells protects from oxidative stress induced cellular damages, improves cell viability and reduces intracellular ROS formation and carbonylated protein accumulation. We observed that ADIPO controlled the oxidative stress situation in astrocytes which can be supported by the data that H_2O_2 stimulated induction of mRNA levels of pro-oxidative factors iNOS, COX-2 were significantly inhibited in Adiponectin transfected cells, while the anti-oxidative factors SOD, CAT were significantly elevated in these ADIPO overexpressing cells. Besides the protective effects of ADIPO, it is interesting to note that wan et al in 2014 reported the pro-inflammatory effects of gAd in human astrocytic (U373 MG) cells in which gAd induced secretion of IL-6 and MCP-1, and gene expression of IL-6, MCP-1, IL-1β and IL-8 (Wan et al., 2014). This depicts the varied and vast effects of Adiponectin. So deepening on the nature of the target cell, nature of produced cytokines, timing, sequence of cytokine action and even the experimental model are the parameters that greatly influences the action of cytokine properties (Cavaillon, 2001).

To our knowledge, this is the first report to demonstrate that ADIPO exerted its protective anti-oxidative effects in astrocyte cells under oxidative stress situation. In summary, we have

shown the expression of adiponectin, adiponectin receptors R1 and R2 in murine CLTT cell line. Overexpression of adiponectin induced a significantly higher cell survival rate in OS states in comparison with EV or CLTT cells. Besides this, our results demonstrated that treatment with H_2O_2 significantly increased ROS generation in CLTT cells as well as in cells transfected with the EV cells whereas the overexpression of adiponectin regulates ROS generation as compared to control after H_2O_2 treatment. In addition to this, a decrease in protein oxidation was noticed in adiponectin overexpressing transfected clones when compared to the non-transfected CLTT and CLTT-EV. After H_2O_2 treatment cells overexpressing adiponectin had also maintained a lower amount of oxidized proteins than the corresponding control cells. Conclusively our results suggest that adiponectin set up in the CNS could represent a mechanism to protect central nervous system against oxidative stress which could lead to the novel therapeutic strategies of Adiponectin in treating neurodegenerative disorders.

CONCLUSIONS AND FUTURE VISION

Conclusions And Future Vision

The current study was undertaken to address the anti-inflammatory properties of factors produced by the fat tissue and its potential implications towards neurodegenerative process. In order to accomplish this hypothesized task we had generated neuroinflammatory LPS mice model and neurodegenerative TMT model to investigate the assumed postulations that, peripheral inflammation could induce CNS vulnerability *in vivo*. Besides this, we also made our experimental setup *in vitro* on immortalized glial cell lines transfected with adipocytes produced factors (ATX and ADIPO) and the stable transfectant clones thus obtained were analyzed under both inflammatory and oxidative stress situations respectively.

Based on our research investigations, we made evident that activated peripheral immune system in response to infection or inflammation could signal the CNS via cytokine signalling and depending on the intensity of the stimulus the response could trigger glial cell activation. Under *in vitro* settings, our results authenticate the anti-inflammatory properties of ATX in microglial cell line under inflammatory settings, whereas ADIPO performed its anti-oxidative role in astrocyte cell line under oxidative stress context - that justified the thesis title.

Table CON-1	**Statements Drawn From Thesis**
Fat Tissue, Inflammatory And Oxidative Stress Factors Were Expressed *in vivo* In The Brain.	
Autotaxin Exhibited Anti-Inflammatory Actions In Microglia Under Inflammatory Settings.	
Adiponectin Showcased Anti-Oxidative Effects In Astrocytes Under Oxidative Stress.	

The results procured through our *in vivo* investigations are quite promising that LPS and TMT can induce neuroinflammatory response in the different localities of the brain that involved inflammation and oxidative stress cytokines. Henceforth, we planned our further investigations on the treatment of mice with adipocyte specific factors Lysophosphatidic Acid, Recombinant Adiponectin) on neuroinflammatory and neurodegenerative mice models to advance on this project. This should give us better insight into the molecular mechanisms of brain inflammation regulation.

From our *in vitro* observations, we put forward the anti-inflammatory properties of ATX in microglial cells under inflammatory settings and anti-oxidant role of ADIPO in astrocyte cells in oxidative stress situations. In conjunction with our microglial and astrocyte *in vitro* studies, it will be pertinent and interesting to understand the inter-related signal transducing mechanisms of neuronal and endothelial transfection studies with adipocytokines that should bring about novel outputs to comprehend the link between adipose tissue and CNS vulnerability.

Beyond any doubt with the accumulating evidences on the relation between obesity and CNS research, now it is certain that adipose tissue can affect human physiology through its secreted products (adipocytokines-Leptin, ADIPO and ATX) which mediate complex crosstalk between various organs including CNS. But still the pathophysiological significance of these adipocytokines towards CNS vulnerability remains poorly portrayed.

So a prudent approach is thus warranted and much more research should be appreciated on these specific areas to address obesity induced neurodegenerative research that might bring about novel therapies by exploiting adipocyte specific factors (ATX, ADIPO) and glial cells (Microglia, Astrocytes) as potential therapeutic hotspots to reduce the risk and to treat obesity induced neurodegenerative disorders.

ACKNOWLEDGEMENTS

Acknowledging People For Their Credits

Finally the time has come to write a page full of acknowledging the people who were literally involved to make this thesis happened.

First of all I would like to express my sincere thought to my thesis director ***Prof.Christian Lefebvre d'Hellencourt*** *- for having the faith and belief in me that I could be a part of your research activities and as well for considering me as a Ph.D researcher in your Neuroteam. In this regard, I would like express my heartfelt thanks for being my principal investigator in directing my doctoral thesis. The amount of research freedom you gave and the way you let me to think by myself in taking critical decisions had led me to stretch my wings a bit further and to tackle the toughest situations in the laboratory life, especially when you was at the work in National Institutes of Health (NIH). Besides your resonant expertise on neurosciences and professional life, your wittiness and sense of humor are the most captivating things which I commend a lot.*

Lakshman *- I think you are the only one in this entire world to whom I can be myself and connect very easily. Words are not enough to convey the time we spent together since our kindergarten. It's far beyond the materialistic world. You will remain in me forever.*

Guna Anna *- I take it an occasion to convey my message that - Without your support my journey towards research would be directionless. Just a simple thanks wouldn't serve the purpose. We know the relation we share. I am extremely grateful for all your priceless suggestions and words in and out of science.*

Annayya (Sunil) *- All I can say is - "You Are The Reason For My Beingness -* (*Raison d'être*)"

Vishnu Anna *- I would also like to thank you so much in delivering your precious thoughts and words during my Ph.D trials.*

Wildriss Viranaicken *- your support to the emerging researchers at the platform of CYROI is really admirable and noteworthy. For me you are one of the most inspiring researchers whom I met in these years.*

Nicolas Diotel - you are one of the most active persons I have met at this place. Thank you so much for your support and as well for the scientific and paper discussions we shared during my Ph.D journey.

I would also like to thank my thesis committee members ***Phillippe Rondeau, Chaker El kalamouni,*** *and* ***Jean Jacques Hoareau*** *for a great collaboration and as well for being the part of my every year's research evaluation.*

I also wanted to thank ***Prof. Dora Brites, HDR.Fabien Gosselet and MC. Marjolaine Roche*** *for their critical reading of manuscript and for their valuable suggestions.*

I express my sincere thanks to my Master's degree supervisors ***Asst.Prof. Balaji, Prof. Thyagaraju Kedam, Prof. Ramgopal Ghanta*** *and* ***Prof. Vijaya Sarathi Obulam*** *for their valuable advises during my master's study.*

To My Lovely Friends whom I met here:
Mekala, Somasekhar, Shiv Raj Mishra and his family from Bombay, Mahesh, Li Peng, Q Li, Max, Shanu, Julien, Jennifer, Perrine, Karen, Valentine, Hubert Brugada, Ashoka Sreedhara, Ajay Narendra, Ranga Rao (India) *and many others. Besides our everyday experimenting lives, I cherish our regular talks on the toughest times of our laboratory life, paper discussions, news across the world, our outings, parties and all the best times we had together.*

Friends and colleagues of DETROI Team: **A Big Thanks To you All!!!**
Cynthia Planesse, Aurelie Catan, Nekita Ramlagan, Jennifer Baraka Vidot, Angelique Gajahi, Giovedie Stansislas, Brice Nativel, Mery Marimoutou, Sarah Hatia, Etienne Frumence, Latufa Youssouf, Florian Guibbal, Miora Ralambon, Julien Melade, Johan Cretiaux, Pierre Giraud, Maya Cesari, Marjolaine Roche, Emmanuel Bourdon, Jessica patche, Joel Couprie, Christine Robert, Pascal Baret, Marie-Paul Gonthier, Lynda Saminadin, Geraldine Zampiero, Marie Dijoux, Shiril, Ravi, Ravanan *and to all the members of the DETROI team and people at CYROI research platform for an interesting everyday Faire la Bise, scientific discussions and as well for all the good times we had together at the lab.*

Prof. Olivier Meilhac, the Director of DETROI, INSERM unit 1188, and to all the members of GEICO team - Congratulations!!! for all your efforts towards the transformation of GEICO to newly born DETROI Baby. Wishing this Baby to reach greater scientific heights towards the journey of benefitting mankind.

I would also like to thank for the countless cordless talks that we shared together (things happening around the world) from the wonderful people in my life: Pooja-Bharath, Naveen-Anusha, Suresh-Gopi, Asha-Vinay, Asha, Yashu-Akhil, Meena, Ranga Rajan, Naik, Lokesh-Lalu, Chitra, Raju, Purushottam and Shiva Thej Naidu.
You loaded my life with such an unforgettable and sweet memories for my life's time.

My deep sense of respect and heartfelt reverence to Balayya Babai, Gangaiah Mavayya, Desamma Aunty, and Velu Mama. Babai if you were there at this point of time, we might have seen another phase of life. Thank you so much for giving me such an immense and awesome memories.

To the most beautiful women in my life Keerthy Sindhia and Thulasi Duddela.
Thank you so much for being my past, present and future.

Last but not least, this thesis wouldn't have been possible without my Super Dad Sudhakar Babu, my Sweetest Mom Sukanya Alias Thulasi, my Greatest Brother Sunil Kumar, RamaRajyam-Balakrishna, Preethi, and Indhumathi-Madhu Sudhan Rao. Your consistent love, support and understanding boosted me up in executing this task fruitfully. I would like to express my love in the name of Avinash to Mr. & Mrs. Sukanya Sudhakar Babu for giving me such a fantastic birth and I can't ask u for more.

Finally I would like to end up this acknowledging task by remembering myself and my thoughts that involved sheer determination coupled with focusing attitude which finally culminated to accomplish an international doctoral degree in the arena of Brain Research at the age of 27, which is an unforgettable chapter in my life.

This Thesis Was Supported By The Grants From Regional Reunion Council, France, Europe.

CITATIONS

Citations

1924. What is meant by third element of the nerve centers. Bol de la Soc esp de biol 11: 33.

1924, 1925. Glia and the third element of the nerve centers. Bull de la Soc de Sc méd et biol de Montpellier 10.

1927. Basic lesions of the nervous centers. Rev méd de Barcelona 8:36.

1920. The microglia and its transformation into cells and granule-adipose swab bodies. Trab del Lab de invest biol 18: 37.

1921. Histogenesis and Evolution regular exodus and regional distribution of microglia. Mem de la Soc esp de hist nat, 11: 213.

(WHO) WHO. 2012. Dementia. http://wwwwhoint/mediacentre/factsheets/fs362/en/.

(WHO) WHO. 2015a. Diabetes. http://wwwwhoint/mediacentre/factsheets/fs312/en/.

(WHO) WHO. 2015b. Obesity and overweight. http://wwwwhoint/mediacentre/factsheets/fs311/en/.

A. B. 1991. Glial cells in the central nervous system. Discussions in neuroscience. Amsterdam. Elsevier:1-45.

Aarsland A, Chinkes D, Wolfe RR. 1997. Hepatic and whole-body fat synthesis in humans during carbohydrate overfeeding. The American journal of clinical nutrition 65(6):1774-1782.

Abbas, Abul. 2006. Basic Immunology. Elsevier.

Abramov AY, Jacobson J, Wientjes F, Hothersall J, Canevari L, Duchen MR. 2005. Expression and modulation of an NADPH oxidase in mammalian astrocytes. The Journal of neuroscience : the official journal of the Society for Neuroscience 25(40):9176-9184.

Adamczak M, Wiecek A. 2013. The adipose tissue as an endocrine organ. Seminars in nephrology 33(1):2-13.

Agarwal A, Prabakaran SA. 2005. Mechanism, measurement, and prevention of oxidative stress in male reproductive physiology. Indian Journal of Experimental Biology 43: 963-974.

Ahima RS, Saper CB, Flier JS, Elmquist JK. 2000. Leptin regulation of neuroendocrine systems. Front Neuroendocrinol 21(3):263-307.

Akiyama H, Arai T, Kondo H, Tanno E, Haga C, Ikeda K. 2000. Cell mediators of inflammation in the Alzheimer disease brain. Alzheimer disease and associated disorders 14 Suppl 1:S47-53.

Alberti KG, Zimmet PZ. 1998. Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. Diabetic medicine : a journal of the British Diabetic Association 15(7):539-553.

Allen NJ. 2014. Astrocyte regulation of synaptic behavior. Annual review of cell and developmental biology 30:439-463.

Allen NJ, Barres BA. 2009. Neuroscience: Glia [mdash] more than just brain glue. Nature 457(7230):675-677.

Allison DB, Fontaine KR, Manson JE, Stevens J, VanItallie TB. 1999. Annual deaths attributable to obesity in the United States. Jama 282(16):1530-1538.

Aloisi F. 2001. Immune function of microglia. Glia 36(2):165-179.

Althaus D. 2013. Mexico takes title of "most obese" from America. Global Post, CBS Interactive Inc.

Amor S, Puentes F, Baker D, van der Valk P. 2010. Inflammation in neurodegenerative diseases. Immunology 129(2):154-169.

An S, Bleu T, Hallmark OG, Goetzl EJ. 1998. Characterization of a novel subtype of human G protein-coupled receptor for lysophosphatidic acid. The Journal of biological chemistry 273(14):7906-7910.
Andrade Júnior DRd, Souza RBd, Santos SAd, Andrade DRd. 2005. Os radicais livres de oxigênio e as doenças pulmonares. Jornal Brasileiro de Pneumologia 31:60-68.
Andreyev AY, Kushnareva YE, Starkov AA. 2005. Mitochondrial metabolism of reactive oxygen species. Biochemistry Biokhimiia 70(2):200-214.
Anthony DC, Ferguson B, Matyzak MK, Miller KM, Esiri MM, Perry VH. 1997. Differential matrix metalloproteinase expression in cases of multiple sclerosis and stroke. Neuropathology and applied neurobiology 23(5):406-415.
Aoki J. 2004. Mechanisms of lysophosphatidic acid production. Seminars in cell & developmental biology 15(5):477-489.
Aquilano K, Baldelli S, Ciriolo MR. 2014. Glutathione: new roles in redox signaling for an old antioxidant. Frontiers in Pharmacology 5:196.
Araki E, Forster C, Dubinsky JM, Ross ME, Iadecola C. 2001. Cyclooxygenase-2 inhibitor ns-398 protects neuronal cultures from lipopolysaccharide-induced neurotoxicity. Stroke 32(10):2370-2375.
Araujo DM, Cotman CW. 1992. Basic FGF in astroglial, microglial, and neuronal cultures: characterization of binding sites and modulation of release by lymphokines and trophic factors. The Journal of neuroscience : the official journal of the Society for Neuroscience 12(5):1668-1678.
Ardan T, Kovaceva J, Cejkova J. 2004. Comparative histochemical and immunohistochemical study on xanthine oxidoreductase/xanthine oxidase in mammalian corneal epithelium. Acta histochemica 106(1):69-75.
Arnoldussen IA, Kiliaan AJ, Gustafson DR. 2014. Obesity and dementia: Adipokines interact with the brain. European neuropsychopharmacology : the journal of the European College of Neuropsychopharmacology.
Aschner M, Allen JW, Kimelberg HK, LoPachin RM, Streit WJ. 1999. Glial cells in neurotoxicity development. Annual review of pharmacology and toxicology 39:151-173.
Aschner M, Kimelberg HK. 1991. The use of astrocytes in culture as model systems for evaluating neurotoxic-induced-injury. Neurotoxicology 12(3):505-517.
Ashcroft GS. 1999. Bidirectional regulation of macrophage function by TGF-beta. Microbes and infection / Institut Pasteur 1(15):1275-1282.
Ashcroft GS, Yang X, Glick AB, Weinstein M, Letterio JJ, Mizel DE, Anzano M, Greenwell-Wild T, Wahl SM, Deng C, Roberts AB. 1999. Mice lacking Smad3 show accelerated wound healing and an impaired local inflammatory response. Nat Cell Biol 1(5):260-266.
Atlas AB. 2015. Sagital View of Mice Brain. Allens Mouse Brain Atlas.
Awada R, Parimisetty A, d'Hellencourt CL. 2013a. Influence of Obesity on Neurodegenerative Diseases.
Awada R, Parimisetty A, Lefebvre d'Hellencourt C. 2013b. Influence of obesity on neurodegenerative diseases. Neurodegenerative Diseases Chapter 16:381-401.
Awada R, Rondeau P, Gres S, Saulnier-Blache JS, Lefebvre d'Hellencourt C, Bourdon E. 2012. Autotaxin protects microglial cells against oxidative stress. Free radical biology & medicine 52(2):516-526.
Awada R, Saulnier-Blache JS, Gres S, Bourdon E, Rondeau P, Parimisetty A, Orihuela R, Harry GJ, d'Hellencourt CL. 2014. Autotaxin downregulates LPS-induced microglia activation and pro-inflammatory cytokines production. Journal of cellular biochemistry 115(12):2123-2132.

Aydin S, Kuloglu T, Aydin S. 2013. Copeptin, adropin and irisin concentrations in breast milk and plasma of healthy women and those with gestational diabetes mellitus. Peptides 47:66-70.

Babior BM. 2004. NADPH oxidase. Current opinion in immunology 16(1):42-47.

Babior BM, Kipnes RS, Curnutte JT. 1973. Biological Defense Mechanisms. THE PRODUCTION BY LEUKOCYTES OF SUPEROXIDE, A POTENTIAL BACTERICIDAL AGENT. The Journal of clinical investigation 52(3):741-744.

Bachner D, Ahrens M, Betat N, Schroder D, Gross G. 1999. Developmental expression analysis of murine autotaxin (ATX). Mech Dev 84(1-2):121-125.

Bailey SL, Carpentier PA, McMahon EJ, Begolka WS, Miller SD. 2006. Innate and Adaptive Immune Responses of the Central Nervous System. 26(2):149-188.

Baker DL, Fujiwara Y, Pigg KR, Tsukahara R, Kobayashi S, Murofushi H, Uchiyama A, Murakami-Murofushi K, Koh E, Bandle RW, Byun HS, Bittman R, Fan D, Murph M, Mills GB, Tigyi G. 2006. Carba analogs of cyclic phosphatidic acid are selective inhibitors of autotaxin and cancer cell invasion and metastasis. The Journal of biological chemistry 281(32):22786-22793.

Baldwin AS, Jr. 1996. The NF-kappa B and I kappa B proteins: new discoveries and insights. Annual review of immunology 14:649-683.

Baldwin AS, Jr. 2001. Series introduction: the transcription factor NF-kappaB and human disease. The Journal of clinical investigation 107(1):3-6.

Bamberger ME, Landreth GE. 2001. Microglial interaction with beta-amyloid: implications for the pathogenesis of Alzheimer's disease. Microscopy research and technique 54(2):59-70.

Bambrick L, Kristian T, Fiskum G. 2004. Astrocyte mitochondrial mechanisms of ischemic brain injury and neuroprotection. Neurochemical research 29(3):601-608.

Bandoh K, Aoki J, Hosono H, Kobayashi S, Kobayashi T, Murakami-Murofushi K, Tsujimoto M, Arai H, Inoue K. 1999. Molecular cloning and characterization of a novel human G-protein-coupled receptor, EDG7, for lysophosphatidic acid. The Journal of biological chemistry 274(39):27776-27785.

Banks WA, Clever CM, Farrell CL. 2000. Partial saturation and regional variation in the blood-to-brain transport of leptin in normal weight mice. American journal of physiology Endocrinology and metabolism 278(6):E1158-1165.

Banks WA, Kastin AJ, Huang W, Jaspan JB, Maness LM. 1996. Leptin enters the brain by a saturable system independent of insulin. Peptides 17(2):305-311.

Barclay AN, Wright GJ, Brooke G, Brown MH. 2002. CD200 and membrane protein interactions in the control of myeloid cells. Trends in immunology 23(6):285-290.

Barger SW, Horster D, Furukawa K, Goodman Y, Krieglstein J, Mattson MP. 1995. Tumor necrosis factors alpha and beta protect neurons against amyloid beta-peptide toxicity: evidence for involvement of a kappa B-binding factor and attenuation of peroxide and Ca2+ accumulation. Proceedings of the National Academy of Sciences of the United States of America 92(20):9328-9332.

Barness LA, Opitz JM, Gilbert-Barness E. 2007. Obesity: genetic, molecular, and environmental aspects. American journal of medical genetics Part A 143a(24):3016-3034.

Barreto GE, Gonzalez J, Capani F, Morales L. 2011. Role of Astrocytes in Neurodegenerative Diseases. Neurodegenerative Diseases - Processes, Prevention, Protection and Monitoring, Dr Raymond Chuen-Chung Chang (Ed), InTech

Baskin DG, Hahn TM, Schwartz MW. 1999. Leptin sensitive neurons in the hypothalamus. Hormone and metabolic research = Hormon- und Stoffwechselforschung = Hormones et metabolisme 31(5):345-350.

Bauche IB, El Mkadem SA, Pottier AM, Senou M, Many MC, Rezsohazy R, Penicaud L, Maeda N, Funahashi T, Brichard SM. 2007. Overexpression of adiponectin targeted to adipose tissue in transgenic mice: impaired adipocyte differentiation. Endocrinology 148(4):1539-1549.

Baune BT, Wiede F, Braun A, Golledge J, Arolt V, Koerner H. 2008. Cognitive dysfunction in mice deficient for TNF- and its receptors. American journal of medical genetics Part B, Neuropsychiatric genetics : the official publication of the International Society of Psychiatric Genetics 147B(7):1056-1064.

Beattie EC, Stellwagen D, Morishita W, Bresnahan JC, Ha BK, Von Zastrow M, Beattie MS, Malenka RC. 2002. Control of synaptic strength by glial TNFalpha. Science 295(5563):2282-2285.

Befit. 2015. Free Radicals And Our Health. Be Fit, Scientific Article.

Belanger M, Magistretti PJ. 2009. The role of astroglia in neuroprotection. Dialogues in clinical neuroscience 11(3):281-295.

Benediktsson AM, Marrs GS, Tu JC, Worley PF, Rothstein JD, Bergles DE, Dailey ME. 2012. Neuronal activity regulates glutamate transporter dynamics in developing astrocytes. Glia 60(2):175-188.

Benomar Y, Gertler A, De Lacy P, Crepin D, Ould Hamouda H, Riffault L, Taouis M. 2013. Central resistin overexposure induces insulin resistance through Toll-like receptor 4. Diabetes 62(1):102-114.

Benveniste EN, Nguyen VT, O'Keefe GM. 2001. Immunological aspects of microglia: relevance to Alzheimer's disease. Neurochemistry international 39(5-6):381-391.

Berg AH, Combs TP, Du X, Brownlee M, Scherer PE. 2001. The adipocyte-secreted protein Acrp30 enhances hepatic insulin action. Nature medicine 7(8):947-953.

Berg AH, Combs TP, Scherer PE. 2002. ACRP30/adiponectin: an adipokine regulating glucose and lipid metabolism. Trends in endocrinology and metabolism: TEM 13(2):84-89.

Berg AH, Scherer PE. 2005. Adipose tissue, inflammation, and cardiovascular disease. Circulation research 96(9):939-949.

Berman NE, Marcario JK, Yong C, Raghavan R, Raymond LA, Joag SV, Narayan O, Cheney PD. 1999. Microglial activation and neurological symptoms in the SIV model of NeuroAIDS: association of MHC-II and MMP-9 expression with behavioral deficits and evoked potential changes. Neurobiology of disease 6(6):486-498.

Berridge MJ, Lipp P, Bootman MD. 2000. The versatility and universality of calcium signalling. Nat Rev Mol Cell Biol 1(1):11-21.

Betteridge DJ. 2000. What is oxidative stress? Metabolism 49(2 Suppl 1):3-8.

Beydoun MA, Beydoun HA, Wang Y. 2008. Obesity and central obesity as risk factors for incident dementia and its subtypes: a systematic review and meta-analysis. Obesity reviews : an official journal of the International Association for the Study of Obesity 9(3):204-218.

Bhakar AL, Tannis LL, Zeindler C, Russo MP, Jobin C, Park DS, MacPherson S, Barker PA. 2002. Constitutive nuclear factor-kappa B activity is required for central neuron survival. The Journal of neuroscience : the official journal of the Society for Neuroscience 22(19):8466-8475.

Bhattacharjee J, Sanyal S. 1975. Developmental origin and early differentiation of retinal Muller cells in mice. Journal of anatomy 120(Pt 2):367-372.

Biessels GJ, Deary IJ, Ryan CM. 2008. Cognition and diabetes: a lifespan perspective. Lancet Neurol 7(2):184-190.

Bigal ME, Lipton RB. 2008. Obesity and chronic daily headache. Current pain and headache reports 12(1):56-61.

Black RA, Rauch CT, Kozlosky CJ, Peschon JJ, Slack JL, Wolfson MF, Castner BJ, Stocking KL, Reddy P, Srinivasan S, Nelson N, Boiani N, Schooley KA, Gerhart M, Davis R, Fitzner JN, Johnson RS, Paxton RJ, March CJ, Cerretti DP. 1997. A metalloproteinase disintegrin that releases tumour-necrosis factor-alpha from cells. Nature 385(6618):729-733.

Block ML, Zecca L, Hong JS. 2007. Microglia-mediated neurotoxicity: uncovering the molecular mechanisms. Nat Rev Neurosci 8(1):57-69.

Bluher M. 2012. Clinical relevance of adipokines. Diabetes & metabolism journal 36(5):317-327.

Bluher M. 2013. Adipose tissue dysfunction contributes to obesity related metabolic diseases. Best practice & research Clinical endocrinology & metabolism 27(2):163-177.

Bluher M. 2014. Adipokines - removing road blocks to obesity and diabetes therapy. Molecular metabolism 3(3):230-240.

Bluher M, Mantzoros CS. 2015. From leptin to other adipokines in health and disease: facts and expectations at the beginning of the 21st century. Metabolism 64(1):131-145.

Blüher M, Mantzoros CS. 2015. From leptin to other adipokines in health and disease: Facts and expectations at the beginning of the 21st century. Metabolism 64(1):131-145.

Boje KM, Arora PK. 1992. Microglial-produced nitric oxide and reactive nitrogen oxides mediate neuronal cell death. Brain research 587(2):250-256.

Bokarewa M, Nagaev I, Dahlberg L, Smith U, Tarkowski A. 2005. Resistin, an adipokine with potent pro-inflammatory properties. Journal of immunology 174(9):5789-5795.

Bollen M, Gijsbers R, Ceulemans H, Stalmans W, Stefan C. 2000. Nucleotide pyrophosphatases/phosphodiesterases on the move. Critical reviews in biochemistry and molecular biology 35(6):393-432.

Bonda DJ, Stone JG, Torres SL, Siedlak SL, Perry G, Kryscio R, Jicha G, Casadesus G, Smith MA, Zhu X, Lee HG. 2014. Dysregulation of leptin signaling in Alzheimer disease: evidence for neuronal leptin resistance. Journal of neurochemistry 128(1):162-172.

Bonda DJ, Wang X, Perry G, Nunomura A, Tabaton M, Zhu X, Smith MA. 2010. Oxidative stress in Alzheimer disease: a possibility for prevention. Neuropharmacology 59(4-5):290-294.

Boucher J, Quilliot D, Praderes JP, Simon MF, Gres S, Guigne C, Prevot D, Ferry G, Boutin JA, Carpene C, Valet P, Saulnier-Blache JS. 2005. Potential involvement of adipocyte insulin resistance in obesity-associated up-regulation of adipocyte lysophospholipase D/autotaxin expression. Diabetologia 48(3):569-577.

Bouret SG. 2010. Neurodevelopmental actions of leptin. Brain research 1350:2-9.

Bousquet M, St-Amour I, Vandal M, Julien P, Cicchetti F, Calon F. 2012. High-fat diet exacerbates MPTP-induced dopaminergic degeneration in mice. Neurobiology of disease 45(1):529-538.

Bowman GL, Kaye JA, Moore M, Waichunas D, Carlson NE, Quinn JF. 2007. Blood-brain barrier impairment in Alzheimer disease: stability and functional significance. Neurology 68(21):1809-1814.

Broglio C, Gómez A, Durán E, Ocaña FM, Jiménez-Moya F, Rodríguez F, Salas C. 2005. Hallmarks of a common forebrain vertebrate plan: specialized pallial areas for spatial, temporal and emotional memory in actinopterygian fish. Brain research bulletin 66(4-6):277-281.

Bronstein DM, Perez-Otano I, Sun V, Mullis Sawin SB, Chan J, Wu GC, Hudson PM, Kong LY, Hong JS, McMillian MK. 1995. Glia-dependent neurotoxicity and neuroprotection in mesencephalic cultures. Brain research 704(1):112-116.

Brook FA, Gardner RL. 1997. The origin and efficient derivation of embryonic stem cells in the mouse. Proc Natl Acad Sci U S A 94(11):5709-5712.

Brown R, Imran SA, Belsham DD, Ur E, Wilkinson M. 2007. Adipokine gene expression in a novel hypothalamic neuronal cell line: resistin-dependent regulation of fasting-induced adipose factor and SOCS-3. Neuroendocrinology 85(4):232-241.

Brown R, Thompson HJ, Imran SA, Ur E, Wilkinson M. 2008. Traumatic brain injury induces adipokine gene expression in rat brain. Neuroscience letters 432(1):73-78.

Brown R, Wiesner G, Ur E, Wilkinson M. 2005. Pituitary resistin gene expression is upregulated in vitro and in vivo by dexamethasone but is unaffected by rosiglitazone. Neuroendocrinology 81(1):41-48.

Bruccoleri, Brown, Harry. 1998. Cellular localization and temporal elevation of tumor necrosis factoralpha, interleukin-1alpha, and transforming growth factor-beta mRNA in hippocampal injury response induced by trimethyltin. Journal of neurochemistry.

Bruce-Keller AJ, Keller JN, Morrison CD. 2009. Obesity and vulnerability of the CNS. Biochimica et biophysica acta 1792(5):395-400.

Bruno Dubuc HL. 2015. Brain from top to bottom. Brain from top to bottom Blog.

Buechler C, Wanninger J, Neumeier M. 2010a. Adiponectin receptor binding proteins--recent advances in elucidating adiponectin signalling pathways. FEBS letters 584(20):4280-4286.

Buechler C, Wanninger J, Neumeier M. 2010b. Adiponectin receptor binding proteins – recent advances in elucidating adiponectin signalling pathways. FEBS letters 584(20):4280-4286.

Burguera B, Couce ME, Long J, Lamsam J, Laakso K, Jensen MD, Parisi JE, Lloyd RV. 2000. The long form of the leptin receptor (OB-Rb) is widely expressed in the human brain. Neuroendocrinology 71(3):187-195.

Bushong EA, Martone ME, Jones YZ, Ellisman MH. 2002. Protoplasmic astrocytes in CA1 stratum radiatum occupy separate anatomical domains. The Journal of neuroscience : the official journal of the Society for Neuroscience 22(1):183-192.

Butler MP, O'Connor JJ, Moynagh PN. 2004. Dissection of tumor-necrosis factor-alpha inhibition of long-term potentiation (LTP) reveals a p38 mitogen-activated protein kinase-dependent mechanism which maps to early-but not late-phase LTP. Neuroscience 124(2):319-326.

Byrd T, Zinser P. 2001. Tuberculosis Meningitis. Current treatment options in neurology 3(5):427-432.

Cabiscol E, Piulats E, Echave P, Herrero E, Ros J. 2000. Oxidative stress promotes specific protein damage in Saccharomyces cerevisiae. The Journal of biological chemistry 275(35):27393-27398.

Cadenas E, Davies KJ. 2000. Mitochondrial free radical generation, oxidative stress, and aging. Free radical biology & medicine 29(3-4):222-230.

Cagnin A, Brooks DJ, Kennedy AM, Gunn RN, Myers R, Turkheimer FE, Jones T, Banati RB. 2001. In-vivo measurement of activated microglia in dementia. Lancet 358(9280):461-467.

Cajal R. 1897. Histology of the Nervous System of Man and Vertebrates. . Oxford University Press; Oxford.

Calingasan NY, Chun WJ, Park LC, Uchida K, Gibson GE. 1999. Oxidative stress is associated with region-specific neuronal death during thiamine deficiency. Journal of neuropathology and experimental neurology 58(9):946-958.

Campfield LA, Smith FJ, Guisez Y, Devos R, Burn P. 1995. Recombinant mouse OB protein: evidence for a peripheral signal linking adiposity and central neural networks. Science 269(5223):546-549.

Cani PD, Bibiloni R, Knauf C, Waget A, Neyrinck AM, Delzenne NM, Burcelin R. 2008. Changes in gut microbiota control metabolic endotoxemia-induced inflammation in high-fat diet-induced obesity and diabetes in mice. Diabetes 57(6):1470-1481.
Cannon B, Nedergaard J. 2004. Brown adipose tissue: function and physiological significance. Physiological reviews 84(1):277-359.
Carson MJ, Doose JM, Melchior B, Schmid CD, Ploix CC. 2006. CNS immune privilege: hiding in plain sight. Immunol Rev 213:48-65.
Castle BE, Kishimoto K, Stearns C, Brown ML, Kehry MR. 1993. Regulation of expression of the ligand for CD40 on T helper lymphocytes. Journal of immunology (Baltimore, Md : 1950) 151(4):1777-1788.
Catalan V, Gomez-Ambrosi J, Rodriguez A, Salvador J, Fruhbeck G. 2009. Adipokines in the treatment of diabetes mellitus and obesity. Expert opinion on pharmacotherapy 10(2):239-254.
Catli G, Olgac Dundar N, Dundar BN. 2014. Adipokines in breast milk: an update. Journal of clinical research in pediatric endocrinology 6(4):192-201.
Cavaillon JM. 2001. Pro- versus anti-inflammatory cytokines: myth or reality. Cellular and molecular biology (Noisy-le-Grand, France) 47(4):695-702.
Cawthorn WP, Scheller EL, MacDougald OA. 2012. Adipose tissue stem cells meet preadipocyte commitment: going back to the future. Journal of lipid research 53(2):227-246.
Cengiz SE, Cetinkaya E, Altin S, Gunluoglu Z, Demir A, Gunluoglu G, Epozturk K. 2008. Nutritional and prognostic significance of sick euthyroid syndrome in non-small cell lung cancer patients. Internal medicine (Tokyo, Japan) 47(4):211-216.
Central NA. 2015. 100 Fascinating Facts You Never Knew About the Human Brain | Nursing Assistant Central. Nursing Assistant Central.
Chaldakov GN. 2007. The Adipobiology of Disease. Immunology, Endocrine & Metabolic Agents in Medicinal Chemistry (Formerly Current Medicinal Chemistry - Immunology, Endocrine and Metabolic Agents) Volume 7, Number 2, April 2007,: pp. 105-105.
Chan KH, Lam KS, Cheng OY, Kwan JS, Ho PW, Cheng KK, Chung SK, Ho JW, Guo VY, Xu A. 2012. Adiponectin is protective against oxidative stress induced cytotoxicity in amyloid-beta neurotoxicity. PLoS One 7(12):e52354.
Chandran M, Phillips SA, Ciaraldi T, Henry RR. 2003. Adiponectin: more than just another fat cell hormone? Diabetes care 26(8):2442-2450.
Chandrasekar B, Freeman GL. 1997. Induction of nuclear factor kappaB and activation protein 1 in postischemic myocardium. FEBS letters 401(1):30-34.
Chao CC, Hu S, Molitor TW, Shaskan EG, Peterson PK. 1992. Activated microglia mediate neuronal cell injury via a nitric oxide mechanism. Journal of immunology (Baltimore, Md : 1950) 149(8):2736-2741.
Chao CC, Hu S, Sheng WS, Bu D, Bukrinsky MI, Peterson PK. 1996. Cytokine-stimulated astrocytes damage human neurons via a nitric oxide mechanism. Glia 16(3):276-284.
Chaudhari N, Talwar P, Parimisetty A, Lefebvre d'Hellencourt C, Ravanan P. 2014. A Molecular Web: Endoplasmic Reticulum Stress, Inflammation and Oxidative Stress. Frontiers in Cellular Neuroscience 8.
Chaudry A. 2004. Embryonic Stem cells. STEM CELL BIOENGINEERING.
Chen C, Jiang J, Lu JM, Chai H, Wang X, Lin PH, Yao Q. Resistin decreases expression of endothelial nitric oxide synthase through oxidative stress in human coronary artery endothelial cells. Am J Physiol Heart Circ Physiol 299(1):H193-201.
Chen H, Charlat O, Tartaglia LA, Woolf EA, Weng X, Ellis SJ, Lakey ND, Culpepper J, Moore KJ, Breitbart RE, Duyk GM, Tepper RI, Morgenstern JP. 1996. Evidence that the

diabetes gene encodes the leptin receptor: identification of a mutation in the leptin receptor gene in db/db mice. Cell 84(3):491-495.
Chen J, Guan Z, Wang L, Song G, Ma B, Wang Y. 2014. Meta-analysis: overweight, obesity, and Parkinson's disease. International journal of endocrinology 2014:203930.
Chen J, Tan B, Karteris E, Zervou S, Digby J, Hillhouse EW, Vatish M, Randeva HS. 2006a. Secretion of adiponectin by human placenta: differential modulation of adiponectin and its receptors by cytokines. Diabetologia 49(6):1292-1302.
Chen R, Roman J, Guo J, West E, McDyer J, Williams MA, Georas SN. 2006b. Lysophosphatidic acid modulates the activation of human monocyte-derived dendritic cells. Stem cells and development 15(6):797-804.
Chen Y, Ramakrishnan DP, Ren B. 2013. Regulation of angiogenesis by phospholipid lysophosphatidic acid. Frontiers in bioscience 18:852-861.
Chen Y, Swanson RA. 2003. Astrocytes and brain injury. Journal of cerebral blood flow and metabolism : official journal of the International Society of Cerebral Blood Flow and Metabolism 23(2):137-149.
Cheng B, Christakos S, Mattson MP. Tumor necrosis factors protect neurons against metabolic-excitotoxic insults and promote maintenance of calcium homeostasis. Neuron 12(1):139-153.
Cheng G, Huang C, Deng H, Wang H. 2012. Diabetes as a risk factor for dementia and mild cognitive impairment: a meta-analysis of longitudinal studies. Internal medicine journal 42(5):484-491.
Chenn A, Walsh CA. 2002. Regulation of cerebral cortical size by control of cell cycle exit in neural precursors. Science 297(5580):365-369.
Chiang CS, Powell HC, Gold LH, Samimi A, Campbell IL. 1996. Macrophage/microglial-mediated primary demyelination and motor disease induced by the central nervous system production of interleukin-3 in transgenic mice. The Journal of clinical investigation 97(6):1512-1524.
Chinta SJ, Andersen JK. 2008. Redox imbalance in Parkinson's disease. Biochimica et biophysica acta 1780(11):1362-1367.
Choi JW, Herr DR, Noguchi K, Yung YC, Lee CW, Mutoh T, Lin ME, Teo ST, Park KE, Mosley AN, Chun J. 2010. LPA receptors: subtypes and biological actions. Annual review of pharmacology and toxicology 50:157-186.
Chung IY, Benveniste EN. 1990. Tumor necrosis factor-alpha production by astrocytes. Induction by lipopolysaccharide, IFN-gamma, and IL-1 beta. Journal of immunology 144(8):2999-3007.
Cicchetti F, Brownell AL, Williams K, Chen YI, Livni E, Isacson O. 2002. Neuroinflammation of the nigrostriatal pathway during progressive 6-OHDA dopamine degeneration in rats monitored by immunohistochemistry and PET imaging. The European journal of neuroscience 15(6):991-998.
Cinti S. 2006. The role of brown adipose tissue in human obesity. Nutrition, metabolism, and cardiovascular diseases : NMCD 16(8):569-574.
Clair T, Koh E, Ptaszynska M, Bandle RW, Liotta LA, Schiffmann E, Stracke ML. 2005. L-histidine inhibits production of lysophosphatidic acid by the tumor-associated cytokine, autotaxin. Lipids in health and disease 4:5.
Clair T, Lee HY, Liotta LA, Stracke ML. 1997. Autotaxin is an exoenzyme possessing 5'-nucleotide phosphodiesterase/ATP pyrophosphatase and ATPase activities. The Journal of biological chemistry 272(2):996-1001.
Clement K, Viguerie N, Poitou C, Carette C, Pelloux V, Curat CA, Sicard A, Rome S, Benis A, Zucker JD, Vidal H, Laville M, Barsh GS, Basdevant A, Stich V, Cancello R, Langin

D. 2004. Weight loss regulates inflammation-related genes in white adipose tissue of obese subjects. FASEB J 18(14):1657-1669.
Cohen O, Reichenberg A, Perry C, Ginzberg D, Pollmacher T, Soreq H, Yirmiya R. 2003. Endotoxin-induced changes in human working and declarative memory associate with cleavage of plasma "readthrough" acetylcholinesterase. Journal of molecular neuroscience : MN 21(3):199-212.
Cohen P et al. MM, Socci ND, Hagge-Greenberg A, Liedtke W. 2002. Role for stearoyl-CoA desaturase-1 in leptin-mediated weight loss. Science 297:240.
Cohen P, Friedman JM. 2004. Leptin and the control of metabolism: Role for stearoyl-CoA desaturase-1 (SCD-1). Journal of Nutrition 134(9):2455S-2463S.
Coleman DL et al. HK. 1969. Effects of parabiosis of normal with genetically diabetic mice. Am J Physiol 217:1298.
Collins, Cotran, Kumar. 1998. Robbins Pathologic Basis of Disease. Philadelphia: WB Saunders Company.
Colombo C, Cutson JJ, Yamauchi T, Vinson C, Kadowaki T, Gavrilova O, Reitman ML. 2002. Transplantation of adipose tissue lacking leptin is unable to reverse the metabolic abnormalities associated with lipoatrophy. Diabetes 51(9):2727-2733.
Colton CA, Chernyshev ON, Gilbert DL, Vitek MP. 2000. Microglial contribution to oxidative stress in Alzheimer's disease. Annals of the New York Academy of Sciences 899:292-307.
Colton CA, Gilbert DL. 1987. Production of superoxide anions by a CNS macrophage, the microglia. FEBS letters 223(2):284-288.
Combs TP, Berg AH, Rajala MW, Klebanov S, Iyengar P, Jimenez-Chillaron JC, Patti ME, Klein SL, Weinstein RS, Scherer PE. 2003. Sexual differentiation, pregnancy, calorie restriction, and aging affect the adipocyte-specific secretory protein adiponectin. Diabetes 52(2):268-276.
Compston A, Zajicek J, Sussman J, Webb A, Hall G, Muir D, Shaw C, Wood A, Scolding N. 1997. Glial lineages and myelination in the central nervous system. Journal of anatomy 190 (Pt 2):161-200.
Contos JJ, Ishii I, Chun J. 2000. Lysophosphatidic acid receptors. Molecular pharmacology 58(6):1188-1196.
Cotran; Kumar C. 1998. Robbins Pathologic Basis of Disease. WB Saunders Company, Philadelphia.
Cournot M, Marquie JC, Ansiau D, Martinaud C, Fonds H, Ferrieres J, Ruidavets JB. 2006. Relation between body mass index and cognitive function in healthy middle-aged men and women. Neurology 67(7):1208-1214.
Cowley MA, Smart JL, Rubinstein M, Cerdan MG, Diano S, Horvath TL, Cone RD, Low MJ. 2001. Leptin activates anorexigenic POMC neurons through a neural network in the arcuate nucleus. Nature 411(6836):480-484.
Craft S, Asthana S, Newcomer JW, Wilkinson CW, Matos IT, Baker LD, Cherrier M, Lofgreen C, Latendresse S, Petrova A, Plymate S, Raskind M, Grimwood K, Veith RC. 1999a. Enhancement of memory in Alzheimer disease with insulin and somatostatin, but not glucose. Archives of general psychiatry 56(12):1135-1140.
Craft S, Asthana S, Schellenberg G, Cherrier M, Baker LD, Newcomer J, Plymate S, Latendresse S, Petrova A, Raskind M, Peskind E, Lofgreen C, Grimwood K. 1999b. Insulin metabolism in Alzheimer's disease differs according to apolipoprotein E genotype and gender. Neuroendocrinology 70(2):146-152.
Cross AK, Woodroofe MN. 1999. Chemokine modulation of matrix metalloproteinase and TIMP production in adult rat brain microglia and a human microglial cell line in vitro. Glia 28(3):183-189.

Cummings B. 2004. Mid-Sagittal Section of Brain. Gross Anatomy of the Brain.
Cunningham AJ, Murray CA, O'Neill LA, Lynch MA, O'Connor JJ. 1996. Interleukin-1 beta (IL-1 beta) and tumour necrosis factor (TNF) inhibit long-term potentiation in the rat dentate gyrus in vitro. Neuroscience letters 203(1):17-20.
Czlonkowska A, Kohutnicka M, Kurkowska-Jastrzebska I, Czlonkowski A. 1996. Microglial reaction in MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) induced Parkinson's disease mice model. Neurodegeneration : a journal for neurodegenerative disorders, neuroprotection, and neuroregeneration 5(2):137-143.
Dalamaga M, Diakopoulos KN, Mantzoros CS. 2012. The role of adiponectin in cancer: a review of current evidence. Endocrine reviews 33(4):547-594.
Damier P, Hirsch EC, Zhang P, Agid Y, Javoy-Agid F. 1993. Glutathione peroxidase, glial cells and Parkinson's disease. Neuroscience 52(1):1-6.
Dantzer R, Konsman JP, Bluthe RM, Kelley KW. 2000. Neural and humoral pathways of communication from the immune system to the brain: parallel or convergent? Autonomic neuroscience : basic & clinical 85(1-3):60-65.
Davis EJ, Foster TD, Thomas WE. 1994. Cellular forms and functions of brain microglia. Brain research bulletin 34(1):73-78.
Davis R. J et al. 2000. Signal transduction by the JNK group of MAP kinases. Cell 103(2):239-252.
De Benedetti F et al. AT, Moretta A, Lazzaro D, Costa P. 1997. Interleukin 6 causes growth impairment in transgenic mice through a decrease in insulin-like growth factor-I. A model for stunted growth in children with chronic inflammation. J Clin Invest 99:643.
Debette S, Beiser A, Hoffmann U, Decarli C, O'Donnell CJ, Massaro JM, Au R, Himali JJ, Wolf PA, Fox CS, Seshadri S. 2010. Visceral fat is associated with lower brain volume in healthy middle-aged adults. Annals of neurology 68(2):136-144.
Degawa-Yamauchi M, Bovenkerk JE, Juliar BE, Watson W, Kerr K, Jones R, Zhu Q, Considine RV. 2003. Serum resistin (FIZZ3) protein is increased in obese humans. The Journal of clinical endocrinology and metabolism 88(11):5452-5455.
DeKosky ST, Styren SD, O'Malley ME, Goss JR, Kochanek P, Marion D, Evans CH, Robbins PD. 1996. Interleukin-1 receptor antagonist suppresses neurotrophin response in injured rat brain. Annals of neurology 39(1):123-127.
Desagher S, Glowinski J, Premont J. 1996. Astrocytes protect neurons from hydrogen peroxide toxicity. The Journal of neuroscience : the official journal of the Society for Neuroscience 16(8):2553-2562.
Deverman BE, Patterson PH. 2009. Cytokines and CNS development. Neuron 64(1):61-78.
Devi L, Raghavendran V, Prabhu BM, Avadhani NG, Anandatheerthavarada HK. 2008. Mitochondrial import and accumulation of alpha-synuclein impair complex I in human dopaminergic neuronal cultures and Parkinson disease brain. The Journal of biological chemistry 283(14):9089-9100.
Devos R, Richards JG, Campfield LA, Tartaglia LA, Guisez Y, van der Heyden J, Travernier J, Plaetinck G, Burn P. 1996. OB protein binds specifically to the choroid plexus of mice and rats. Proceedings of the National Academy of Sciences of the United States of America 93(11):5668-5673.
DiBaise JK, Zhang H, Crowell MD, Krajmalnik-Brown R, Decker GA, Rittmann BE. 2008. Gut microbiota and its possible relationship with obesity. Mayo Clinic proceedings 83(4):460-469.
Dickinson ME, Krumlauf R, McMahon AP. 1994. Evidence for a mitogenic effect of Wnt-1 in the developing mammalian central nervous system. Development 120(6):1453-1471.

Diez JJ, Iglesias P. 2003. The role of the novel adipocyte-derived hormone adiponectin in human disease. European journal of endocrinology / European Federation of Endocrine Societies 148(3):293-300.
Dinarello CA. 2000. Pro-inflammatory cytokines*. Chest 118(2):503-508.
Dinarello CA. 2007. Historical insights into cytokines. European journal of immunology 37 Suppl 1:S34-45.
Diniz BS, Teixeira AL, Ojopi EB, Talib LL, Mendonca VA, Gattaz WF, Forlenza OV. 2010. Higher serum sTNFR1 level predicts conversion from mild cognitive impairment to Alzheimer's disease. Journal of Alzheimer's disease : JAD 22(4):1305-1311.
Dong Y, Benveniste EN. 2001. Immune function of astrocytes. Glia 36(2):180-190.
Dormandy T. 2006. The worst of evils: man's fight against pain. . New Haven, Conn: Yale University Press:p. 22. ISBN 20-300-11322-11326.
Drexel Uo. 2015. Contributing factors to overweight and obesity.
Dringen R. 2000. Metabolism and functions of glutathione in brain. Progress in neurobiology 62(6):649-671.
Droge W. 2002. Free radicals in the physiological control of cell function. Physiological reviews 82(1):47-95.
Du Y, Ma Z, Lin S, Dodel RC, Gao F, Bales KR, Triarhou LC, Chernet E, Perry KW, Nelson DL, Luecke S, Phebus LA, Bymaster FP, Paul SM. 2001. Minocycline prevents nigrostriatal dopaminergic neurodegeneration in the MPTP model of Parkinson's disease. Proc Natl Acad Sci U S A 98(25):14669-14674.
Dubin AE, Herr DR, Chun J. 2010. Diversity of lysophosphatidic acid receptor-mediated intracellular calcium signaling in early cortical neurogenesis. The Journal of neuroscience : the official journal of the Society for Neuroscience 30(21):7300-7309.
Eckel RH, Grundy SM, Zimmet PZ. 2005. The metabolic syndrome. Lancet 365(9468):1415-1428.
Eckel RH, Kahn SE, Ferrannini E, Goldfine AB, Nathan DM, Schwartz MW, Smith RJ, Smith SR. 2011. Obesity and type 2 diabetes: what can be unified and what needs to be individualized? The Journal of clinical endocrinology and metabolism 96(6):1654-1663.
Edlund T, Jessell TM. 1999. Progression from extrinsic to intrinsic signaling in cell fate specification: a view from the nervous system. Cell 96(2):211-224.
Edmondson DE, Binda C, Wang J, Upadhyay AK, Mattevi A. 2009. Molecular and mechanistic properties of the membrane-bound mitochondrial monoamine oxidases. Biochemistry 48(20):4220-4230.
Eitzman DT et al. WR, Xu Z, Tyson J, Ginsburg D. 2000. Plasminogen activator inhibitor-1 deficiency protects against atherosclerosis progression in the mouse carotid artery. Blood 96:4212.
EkDahl CT, Claasen JH, Bonde S, Kokaia Z, Lindvall O. 2003. Inflammation is detrimental for neurogenesis in adult brain. Proc Natl Acad Sci U S A 100(23):13632-13637.
Elkabes S, DiCicco-Bloom EM, Black IB. 1996. Brain microglia/macrophages express neurotrophins that selectively regulate microglial proliferation and function. The Journal of neuroscience : the official journal of the Society for Neuroscience 16(8):2508-2521.
Emerit J, Edeas M, Bricaire F. 2004. Neurodegenerative diseases and oxidative stress. Biomed Pharmacother 58(1):39-46.
Eming SA, Krieg T, Davidson JM. 2007. Inflammation in wound repair: molecular and cellular mechanisms. The Journal of investigative dermatology 127(3):514-525.
Enciu AM, Gherghiceanu M, Popescu BO. 2013. Triggers and effectors of oxidative stress at blood-brain barrier level: relevance for brain ageing and neurodegeneration. Oxid Med Cell Longev 2013:297512.

Eng LF, Ghirnikar RS, Lee YL. 2000. Glial fibrillary acidic protein: GFAP-thirty-one years (1969-2000). Neurochemical research 25(9-10):1439-1451.

Eng LF, Vanderhaeghen JJ, Bignami A, Gerstl B. 1971. An acidic protein isolated from fibrous astrocytes. Brain Res 28(2):351-354.

Enriori PJ, Evans AE, Sinnayah P, Jobst EE, Tonelli-Lemos L, Billes SK, Glavas MM, Grayson BE, Perello M, Nillni EA, Grove KL, Cowley MA. 2007. Diet-induced obesity causes severe but reversible leptin resistance in arcuate melanocortin neurons. Cell metabolism 5(3):181-194.

Eren M, Painter CA, Atkinson JB, Declerck PJ, Vaughan DE. 2002. Age-dependent spontaneous coronary arterial thrombosis in transgenic mice that express a stable form of human plasminogen activator inhibitor-1. Circulation 106(4):491-496.

Eskes C, Juillerat-Jeanneret L, Leuba G, Honegger P, Monnet-Tschudi F. 2003. Involvement of microglia-neuron interactions in the tumor necrosis factor-alpha release, microglial activation, and neurodegeneration induced by trimethyltin. Journal of neuroscience research 71(4):583-590.

Esposito K, Ciotola M, Schisano B, Misso L, Giannetti G, Ceriello A, Giugliano D. 2006. Oxidative stress in the metabolic syndrome. J Endocrinol Invest 29(9):791-795.

Estivill-Torrus G, Llebrez-Zayas P, Matas-Rico E, Santin L, Pedraza C, De Diego I, Del Arco I, Fernandez-Llebrez P, Chun J, De Fonseca FR. 2008. Absence of LPA1 signaling results in defective cortical development. Cerebral cortex 18(4):938-950.

Euer N, Schwirzke M, Evtimova V, Burtscher H, Jarsch M, Tarin D, Weidle UH. 2002. Identification of genes associated with metastasis of mammary carcinoma in metastatic versus non-metastatic cell lines. Anticancer research 22(2a):733-740.

Evans MJ, Kaufman MH. 1981. Establishment in culture of pluripotential cells from mouse embryos. Nature 292(5819):154-156.

F V, N H, C D, S L, V D. 2002. Single-nucleotide polymorphism haplotypes in the both proximal promoter and exon 3 of the APM1 gene modulate adipocyte-secreted adiponectin hormone levels and contribute to the genetic risk for type 2 diabetes in French Caucasians. Hum Mol Genet 11:2607.

Fain JN, Cheema PS, Bahouth SW, Hiler ML. 2003. Resistin release by human adipose tissue explants in primary culture. Biochemical and biophysical research communications 300(3):674-678.

Farina C, Aloisi F, Meinl E. 2007. Astrocytes are active players in cerebral innate immunity. Trends in immunology 28(3):138-145.

Faris Q. B. Alenzi AHB. 2011. Stem cells: Biology and clinical potential African Journal of Biotechnology 10(86):19929-19940.

Farooqi IS et al. ORS. 2005. Monogenic obesity in humans. Annu Rev Med 56:443.

Farooqi IS, Matarese G, Lord GM, Keogh JM, Lawrence E, Agwu C, Sanna V, Jebb SA, Perna F, Fontana S, Lechler RI, DePaoli AM, O'Rahilly S. 2002. Beneficial effects of leptin on obesity, T cell hyporesponsiveness, and neuroendocrine/metabolic dysfunction of human congenital leptin deficiency. Journal of Clinical Investigation 110(8):1093-1103.

Federico L, Pamuklar Z, Smyth SS, Morris AJ. 2008. Therapeutic potential of autotaxin/lysophospholipase d inhibitors. Current drug targets 9(8):698-708.

Feghali CA, Wright TM. 1997. Cytokines in acute and chronic inflammation. Front Biosci 2(1):d12-d26.

Fernandez-Sanchez A, Madrigal-Santillan E, Bautista M, Esquivel-Soto J, Morales-Gonzalez A, Esquivel-Chirino C, Durante-Montiel I, Sanchez-Rivera G, Valadez-Vega C, Morales-Gonzalez JA. 2011. Inflammation, oxidative stress, and obesity. International journal of molecular sciences 12(5):3117-3132.

Ferry G, Tellier E, Try A, Gres S, Naime I, Simon MF, Rodriguez M, Boucher J, Tack I, Gesta S, Chomarat P, Dieu M, Raes M, Galizzi JP, Valet P, Boutin JA, Saulnier-Blache JS. 2003. Autotaxin is released from adipocytes, catalyzes lysophosphatidic acid synthesis, and activates preadipocyte proliferation. Up-regulated expression with adipocyte differentiation and obesity. The Journal of biological chemistry 278(20):18162-18169.

Filipovic R, Jakovcevski I, Zecevic N. 2003. GRO-alpha and CXCR2 in the human fetal brain and multiple sclerosis lesions. Developmental neuroscience 25(2-4):279-290.

Fischer HG, Reichmann G. 2001. Brain dendritic cells and macrophages/microglia in central nervous system inflammation. Journal of immunology (Baltimore, Md : 1950) 166(4):2717-2726.

Fitzpatrick AL, Kuller LH, Lopez OL, Diehr P, O'Meara ES, Longstreth WT, Jr., Luchsinger JA. 2009. Midlife and late-life obesity and the risk of dementia: cardiovascular health study. Archives of neurology 66(3):336-342.

Fleisch AF, Agarwal N, Roberts MD, Han JC, Theim KR, Vexler A, Troendle J, Yanovski SZ, Yanovski JA. 2007. Influence of serum leptin on weight and body fat growth in children at high risk for adult obesity. The Journal of clinical endocrinology and metabolism 92(3):948-954.

Fonseca-Alaniz MH, Takada J, Alonso-Vale MI, Lima FB. 2007. Adipose tissue as an endocrine organ: from theory to practice. Jornal de pediatria 83(5 Suppl):S192-203.

Forlenza OV, Diniz BS, Talib LL, Mendonca VA, Ojopi EB, Gattaz WF, Teixeira AL. 2009. Increased serum IL-1beta level in Alzheimer's disease and mild cognitive impairment. Dementia and geriatric cognitive disorders 28(6):507-512.

Frade JM, Barde YA. 1998. Microglia-derived nerve growth factor causes cell death in the developing retina. Neuron 20(1):35-41.

Freeman LR, Keller JN. 2012. Oxidative stress and cerebral endothelial cells: regulation of the blood-brain-barrier and antioxidant based interventions. Biochimica et biophysica acta 1822(5):822-829.

Frei B, England L, Ames BN. 1989. Ascorbate is an outstanding antioxidant in human blood plasma. Proc Natl Acad Sci U S A 86(16):6377-6381.

Freidin M, Bennett MV, Kessler JA. 1992. Cultured sympathetic neurons synthesize and release the cytokine interleukin 1 beta. Proc Natl Acad Sci U S A 89(21):10440-10443.

Friedman J. 2014. 20 years of leptin: leptin at 20: an overview. The Journal of endocrinology 223(1):T1-8.

Friedman JM, Halaas JL. 1998. Leptin and the regulation of body weight in mammals. Nature 395(6704):763-770.

Friedman JM, Mantzoros CS. 2015. 20 years of leptin: from the discovery of the leptin gene to leptin in our therapeutic armamentarium. Metabolism 64(1):1-4.

Frisca F, Sabbadini RA, Goldshmit Y, Pebay A. 2012. Biological effects of lysophosphatidic acid in the nervous system. International review of cell and molecular biology 296:273-322.

Frugier T, Crombie D, Conquest A, Tjhong F, Taylor C, Kulkarni T, McLean C, Pebay A. 2011. Modulation of LPA receptor expression in the human brain following neurotrauma. Cellular and molecular neurobiology 31(4):569-577.

Fruhbeck G, Becerril S, Sainz N, Garrastachu P, Garcia-Velloso MJ. 2009. BAT: a new target for human obesity? Trends in pharmacological sciences 30(8):387-396.

Fry M, Smith PM, Hoyda TD, Duncan M, Ahima RS, Sharkey KA, Ferguson AV. 2006. Area postrema neurons are modulated by the adipocyte hormone adiponectin. The Journal of neuroscience : the official journal of the Society for Neuroscience 26(38):9695-9702.

Fu R, Shen Q, Xu P, Luo JJ, Tang Y. 2014. Phagocytosis of microglia in the central nervous system diseases. Molecular neurobiology 49(3):1422-1434.

Fujita R, Ma Y, Ueda H. 2008. Lysophosphatidic acid-induced membrane ruffling and brain-derived neurotrophic factor gene expression are mediated by ATP release in primary microglia. Journal of neurochemistry 107(1):152-160.
Fujita T, Tozaki-Saitoh H, Inoue K. 2009. P2Y1 receptor signaling enhances neuroprotection by astrocytes against oxidative stress via IL-6 release in hippocampal cultures. Glia 57(3):244-257.
Fukushima N. 2004. LPA in neural cell development. Journal of cellular biochemistry 92(5):993-1003.
Fukushima N, Ishii I, Contos JJ, Weiner JA, Chun J. 2001. Lysophospholipid receptors. Annual review of pharmacology and toxicology 41:507-534.
Fukushima N, Shano S, Moriyama R, Chun J. 2007. Lysophosphatidic acid stimulates neuronal differentiation of cortical neuroblasts through the LPA1-G(i/o) pathway. Neurochemistry international 50(2):302-307.
Fukushima N, Ye X, Chun J. 2002. Neurobiology of lysophosphatidic acid signaling. The Neuroscientist : a review journal bringing neurobiology, neurology and psychiatry 8(6):540-550.
Furukawa S, Fujita T, Shimabukuro M, Iwaki M, Yamada Y, Nakajima Y, Nakayama O, Makishima M, Matsuda M, Shimomura I. 2004. Increased oxidative stress in obesity and its impact on metabolic syndrome. J Clin Invest 114(12):1752-1761.
Gale SM, Castracane VD, Mantzoros CS. 2004. Energy homeostasis, obesity and eating disorders: recent advances in endocrinology. The Journal of nutrition 134(2):295-298.
Galli F, Piroddi M, Annetti C, Aisa C, Floridi E, Floridi A. 2005. Oxidative stress and reactive oxygen species. Contributions to nephrology 149:240-260.
Gandhi S, Abramov AY. 2012. Mechanism of oxidative stress in neurodegeneration. Oxidative medicine and cellular longevity 2012:428010.
Gao HM, Jiang J, Wilson B, Zhang W, Hong JS, Liu B. 2002. Microglial activation-mediated delayed and progressive degeneration of rat nigral dopaminergic neurons: relevance to Parkinson's disease. Journal of neurochemistry 81(6):1285-1297.
Gao HM, Liu B, Zhang W, Hong JS. 2003. Critical role of microglial NADPH oxidase-derived free radicals in the in vitro MPTP model of Parkinson's disease. FASEB journal : official publication of the Federation of American Societies for Experimental Biology 17(13):1954-1956.
Gegg ME, Beltran B, Salas-Pino S, Bolanos JP, Clark JB, Moncada S, Heales SJ. 2003. Differential effect of nitric oxide on glutathione metabolism and mitochondrial function in astrocytes and neurones: implications for neuroprotection/neurodegeneration? Journal of neurochemistry 86(1):228-237.
Gehrmann J. 1996. Microglia: a sensor to threats in the nervous system? Research in virology 147(2-3):79-88.
Gehrmann J, Matsumoto Y, Kreutzberg GW. 1995. Microglia: intrinsic immuneffector cell of the brain. Brain research Brain research reviews 20(3):269-287.
Gemma C, Bachstetter AD. 2013. The role of microglia in adult hippocampal neurogenesis. Frontiers in Cellular Neuroscience 7:229.
Gems D, Partridge L. Stress-Response Hormesis and Aging: “That which Does Not Kill Us Makes Us Stronger”. Cell metabolism 7(3):200-203.
Gems D, Partridge L. 2008. Stress-response hormesis and aging: "that which does not kill us makes us stronger". Cell metabolism 7(3):200-203.
George E. Barreto JG, Francisco Capani and Ludis Morales. December, 2011. Role of Astrocytes in Neurodegenerative Diseases, Neurodegenerative Diseases - Processes, Prevention, Protection and Monitoring. INTECH open minds - Neurodegenerative Diseases

Gesta S, Simon MF, Rey A, Sibrac D, Girard A, Lafontan M, Valet P, Saulnier-Blache JS. 2002. Secretion of a lysophospholipase D activity by adipocytes: involvement in lysophosphatidic acid synthesis. Journal of lipid research 43(6):904-910.

Gesta S, Tseng Y-H, Kahn CR. 2007. Developmental Origin of Fat: Tracking Obesity to Its Source. Cell 131(2):242-256.

Ghorpade A, Persidskaia R, Suryadevara R, Che M, Liu XJ, Persidsky Y, Gendelman HE. 2001. Mononuclear phagocyte differentiation, activation, and viral infection regulate matrix metalloproteinase expression: implications for human immunodeficiency virus type 1-associated dementia. Journal of virology 75(14):6572-6583.

Gianfrancesco MA, Acuna B, Shen L, Briggs FB, Quach H, Bellesis KH, Bernstein A, Hedstrom AK, Kockum I, Alfredsson L, Olsson T, Schaefer C, Barcellos LF. 2014. Obesity during childhood and adolescence increases susceptibility to multiple sclerosis after accounting for established genetic and environmental risk factors. Obesity research & clinical practice 8(5):e435-447.

Giganti A, Rodriguez M, Fould B, Moulharat N, Coge F, Chomarat P, Galizzi JP, Valet P, Saulnier-Blache JS, Boutin JA, Ferry G. 2008. Murine and human autotaxin alpha, beta, and gamma isoforms: gene organization, tissue distribution, and biochemical characterization. The Journal of biological chemistry 283(12):7776-7789.

Gilmore TD. 2006. Introduction to NF-[kappa]B: players, pathways, perspectives. Oncogene 25(51):6680-6684.

Giordano G, Kavanagh TJ, Costa LG. 2009. Mouse cerebellar astrocytes protect cerebellar granule neurons against toxicity of the polybrominated diphenyl ether (PBDE) mixture DE-71. Neurotoxicology 30(2):326-329.

Giralt M, Villarroya F. 2013. White, brown, beige/brite: different adipose cells for different functions? Endocrinology 154(9):2992-3000.

Giulian D, Corpuz M, Chapman S, Mansouri M, Robertson C. 1993. Reactive mononuclear phagocytes release neurotoxins after ischemic and traumatic injury to the central nervous system. Journal of neuroscience research 36(6):681-693.

Glenda Chidrawi MR, Stephanie Hollis. 2008. Biology in focus: HSC course

Goding JW, Grobben B, Slegers H. 2003. Physiological and pathophysiological functions of the ecto-nucleotide pyrophosphatase/phosphodiesterase family. Biochimica et biophysica acta 1638(1):1-19.

Goetzl EJ, Kong Y, Mei B. 1999. Lysophosphatidic acid and sphingosine 1-phosphate protection of T cells from apoptosis in association with suppression of Bax. Journal of immunology (Baltimore, Md : 1950) 162(4):2049-2056.

Goetzl EJ, Kong Y, Voice JK. 2000. Cutting edge: differential constitutive expression of functional receptors for lysophosphatidic acid by human blood lymphocytes. Journal of immunology (Baltimore, Md : 1950) 164(10):4996-4999.

Goldstein BJ, Scalia RG, Ma XL. 2009a. Protective vascular and myocardial effects of adiponectin. Nature clinical practice Cardiovascular medicine 6(1):27-35.

Goldstein BJ, Scalia RG, Ma XL. 2009b. Protective vascular and myocardial effects of adiponectin. Nature clinical practice Cardiovascular medicine 6(1):27-35.

Gonzalez-Scarano F, Baltuch G. 1999. Microglia as mediators of inflammatory and degenerative diseases. Annu Rev Neurosci 22:219-240.

Goodsell D. 2004. "Catalase". Molecule of the Month. RCSB Protein Data Bank.

Gorska E, Popko K, Stelmaszczyk-Emmel A, Ciepiela O, Kucharska A, Wasik M. 2010. Leptin receptors. European journal of medical research 15 Suppl 2:50-54.

Gottschall PE, Deb S. 1996. Regulation of matrix metalloproteinase expressions in astrocytes, microglia and neurons. Neuroimmunomodulation 3(2-3):69-75.

Gottschall PE, Yu X, Bing B. 1995. Increased production of gelatinase B (matrix metalloproteinase-9) and interleukin-6 by activated rat microglia in culture. Journal of neuroscience research 42(3):335-342.
Grakoui A, Bromley SK, Sumen C, Davis MM, Shaw AS, Allen PM, Dustin ML. 1999. The immunological synapse: a molecular machine controlling T cell activation. Science 285(5425):221-227.
Gras G, Chretien F, Vallat-Decouvelaere AV, Le Pavec G, Porcheray F, Bossuet C, Leone C, Mialocq P, Dereuddre-Bosquet N, Clayette P, Le Grand R, Creminon C, Dormont D, Rimaniol AC, Gray F. 2003. Regulated expression of sodium-dependent glutamate transporters and synthetase: a neuroprotective role for activated microglia and macrophages in HIV infection? Brain pathology (Zurich, Switzerland) 13(2):211-222.
Green SJ, Scheller LF, Marletta MA, Seguin MC, Klotz FW, Slayter M, Nelson BJ, Nacy CA. 1994. Nitric oxide: cytokine-regulation of nitric oxide in host resistance to intracellular pathogens. Immunology letters 43(1-2):87-94.
Greenberg AS, Obin MS. 2006. Obesity and the role of adipose tissue in inflammation and metabolism. Am J Clin Nutr 83(2):461S-465S.
Greenhalgh DG. 1998. The role of apoptosis in wound healing. The international journal of biochemistry & cell biology 30(9):1019-1030.
Greenlee JE, Rose JW. 2000. Controversies in neurological infectious diseases. Seminars in neurology 20(3):375-386.
Gregor MF, Hotamisligil GS. 2011. Inflammatory mechanisms in obesity. Annual review of immunology 29:415-445.
Grusby MJ, Glimcher LH. 1995. Immune responses in MHC class II-deficient mice. Annual review of immunology 13:417-435.
GS H, DL M, LN C, BM S. 1994. Tumor necrosis factor α inhibits signaling from the insulin receptor. Proc Natl Acad Sci USA 91:4854.
Guetens G, De Boeck G, Highley M, van Oosterom AT, de Bruijn EA. 2002. Oxidative DNA damage: biological significance and methods of analysis. Critical reviews in clinical laboratory sciences 39(4-5):331-457.
Gustafson D. Adiposity indices and dementia. The Lancet Neurology 5(8):713-720.
Gustafson D. 2006. Adiposity indices and dementia. Lancet Neurol 5(8):713-720.
Gustafson D, Lissner L, Bengtsson C, Bjorkelund C, Skoog I. 2004. A 24-year follow-up of body mass index and cerebral atrophy. Neurology 63(10):1876-1881.
Gustafson D, Rothenberg E, Blennow K, Steen B, Skoog I. 2003. An 18-year follow-up of overweight and risk of Alzheimer disease. Arch Intern Med 163(13):1524-1528.
Gustafson DR, Backman K, Waern M, Ostling S, Guo X, Zandi P, Mielke MM, Bengtsson C, Skoog I. 2009. Adiposity indicators and dementia over 32 years in Sweden. Neurology 73(19):1559-1566.
Gustafson DR, Karlsson C, Skoog I, Rosengren L, Lissner L, Blennow K. 2007. Mid-life adiposity factors relate to blood-brain barrier integrity in late life. J Intern Med 262(6):643-650.
Gutteridge JM, Halliwell B. 2010. Antioxidants: Molecules, medicines, and myths. Biochemical and biophysical research communications 393(4):561-564.
Haan MN. 2006. Therapy Insight: type 2 diabetes mellitus and the risk of late-onset Alzheimer's disease. Nature clinical practice Neurology 2(3):159-166.
Haigis MC, Yankner BA. The Aging Stress Response. Molecular Cell 40(2):333-344.
Hailer NP, Wirjatijasa F, Roser N, Hischebeth GT, Korf HW, Dehghani F. 2001. Astrocytic factors protect neuronal integrity and reduce microglial activation in an in vitro model of N-methyl-D-aspartate-induced excitotoxic injury in organotypic hippocampal slice cultures. The European journal of neuroscience 14(2):315-326.

Halaas JL, Gajiwala KS, Maffei M, Cohen SL, Chait BT, Rabinowitz D, Lallone RL, Burley SK, Friedman JM. 1995. Weight-reducing effects of the plasma protein encoded by the obese gene. Science 269(5223):543-546.
Halliday G, Robinson SR, Shepherd C, Kril J. 2000. Alzheimer's disease and inflammation: a review of cellular and therapeutic mechanisms. Clinical and experimental pharmacology & physiology 27(1-2):1-8.
Halliday GM, Stevens CH. 2011. Glia: initiators and progressors of pathology in Parkinson's disease. Movement disorders : official journal of the Movement Disorder Society 26(1):6-17.
Halliwell B. 2007. Oxidative stress and cancer: have we moved forward? The Biochemical journal 401(1):1-11.
Halliwell B, Cross CE. 1994. Oxygen-derived species: their relation to human disease and environmental stress. Environmental health perspectives 102 Suppl 10:5-12.
Halliwell B G. 2007. Free Radicals in Biology and Medicine. JMC, Oxford, UK: Oxford University Press 4th edition.
Halliwell B, Gutteridge JM, Cross CE. 1992. Free radicals, antioxidants, and human disease: where are we now? The Journal of laboratory and clinical medicine 119(6):598-620.
Hammack BN, Fung KY, Hunsucker SW, Duncan MW, Burgoon MP, Owens GP, Gilden DH. 2004. Proteomic analysis of multiple sclerosis cerebrospinal fluid. Multiple sclerosis (Houndmills, Basingstoke, England) 10(3):245-260.
Hanisch UK. 2002. Microglia as a source and target of cytokines. Glia 40(2):140-155.
Hansson E. 1988. Astroglia from defined brain regions as studied with primary cultures. Progress in neurobiology 30(5):369-397.
Hara K, Boutin P, Mori Y, Tobe K, Dina C, Yasuda K, Yamauchi T, Otabe S, Okada T, Eto K, Kadowaki H, Hagura R, Akanuma Y, Yazaki Y, Nagai R, Taniyama M, Matsubara K, Yoda M, Nakano Y, Tomita M, Kimura S, Ito C, Froguel P, Kadowaki T. 2002. Genetic variation in the gene encoding adiponectin is associated with an increased risk of type 2 diabetes in the Japanese population. Diabetes 51(2):536-540.
Harms M, Seale P. 2013. Brown and beige fat: development, function and therapeutic potential. Nature medicine 19(10):1252-1263.
Harris JJ, Jolivet R, Attwell D. 2012. Synaptic energy use and supply. Neuron 75(5):762-777.
Harrison DE, Cailliet R, Harrison DD, Troyanovich SJ, Harrison SO. 1999. A review of biomechanics of the central nervous system--Part III: spinal cord stresses from postural loads and their neurologic effects. Journal of manipulative and physiological therapeutics 22(6):399-410.
Harrison JK, Jiang Y, Chen S, Xia Y, Maciejewski D, McNamara RK, Streit WJ, Salafranca MN, Adhikari S, Thompson DA, Botti P, Bacon KB, Feng L. 1998. Role for neuronally derived fractalkine in mediating interactions between neurons and CX3CR1-expressing microglia. Proc Natl Acad Sci U S A 95(18):10896-10901.
Harrison R. 2002. Structure and function of xanthine oxidoreductase: where are we now? Free radical biology & medicine 33(6):774-797.
Harry GJ, McPherson CA, Wine RN, Atkinson K, Lefebvre d'Hellencourt C. 2004. Trimethyltin-induced neurogenesis in the murine hippocampus. Neurotoxicity research 5(8):623-627.
Haslam DW, James WPT. Obesity. The Lancet 366(9492):1197-1209.
Hatton GI. 1988. Pituicytes, glia and control of terminal secretion. The Journal of experimental biology 139:67-79.
Hawkes J, Bryan DL, Gibson R. 2004. Cells from mature human milk are capable of cytokine production following in vitro stimulation. Advances in experimental medicine and biology 554:467-470.

Hayden KM, Zandi PP, Lyketsos CG, Khachaturian AS, Bastian LA, Charoonruk G, Tschanz JT, Norton MC, Pieper CF, Munger RG, Breitner JC, Welsh-Bohmer KA. 2006. Vascular risk factors for incident Alzheimer disease and vascular dementia: the Cache County study. Alzheimer disease and associated disorders 20(2):93-100.

Heese K, Fiebich BL, Bauer J, Otten U. 1998a. NF-kappaB modulates lipopolysaccharide-induced microglial nerve growth factor expression. Glia 22(4):401-407.

Heese K, Hock C, Otten U. 1998b. Inflammatory signals induce neurotrophin expression in human microglial cells. Journal of neurochemistry 70(2):699-707.

Heldmann U, Thored P, Claasen JH, Arvidsson A, Kokaia Z, Lindvall O. 2005. TNF-alpha antibody infusion impairs survival of stroke-generated neuroblasts in adult rat brain. Experimental neurology 196(1):204-208.

Heneka MT, Carson MJ, Khoury JE, Landreth GE, Brosseron F, Feinstein DL, Jacobs AH, Wyss-Coray T, Vitorica J, Ransohoff RM, Herrup K, Frautschy SA, Finsen B, Brown GC, Verkhratsky A, Yamanaka K, Koistinaho J, Latz E, Halle A, Petzold GC, Town T, Morgan D, Shinohara ML, Perry VH, Holmes C, Bazan NG, Brooks DJ, Hunot S, Joseph B, Deigendesch N, Garaschuk O, Boddeke E, Dinarello CA, Breitner JC, Cole GM, Golenbock DT, Kummer MP. 2015. Neuroinflammation in Alzheimer's disease. The Lancet Neurology 14(4):388-405.

Henrich-Noack P, Prehn JH, Krieglstein J. 1996. TGF-beta 1 protects hippocampal neurons against degeneration caused by transient global ischemia. Dose-response relationship and potential neuroprotective mechanisms. Stroke 27(9):1609-1614; discussion 1615.

Hensley K, Butterfield DA, Hall N, Cole P, Subramaniam R, Mark R, Mattson MP, Markesbery WR, Harris ME, Aksenov M, et al. 1996. Reactive oxygen species as causal agents in the neurotoxicity of the Alzheimer's disease-associated amyloid beta peptide. Annals of the New York Academy of Sciences 786:120-134.

Hensley K, Robinson KA, Gabbita SP, Salsman S, Floyd RA. 2000. Reactive oxygen species, cell signaling, and cell injury. Free radical biology & medicine 28(10):1456-1462.

Herder C, Baumert J, Thorand B, Koenig W, de Jager W, Meisinger C, Illig T, Martin S, Kolb H. 2006. Chemokines as risk factors for type 2 diabetes: results from the MONICA/KORA Augsburg study, 1984-2002. Diabetologia 49(5):921-929.

Hermann GE, Rogers RC, Bresnahan JC, Beattie MS. 2001. Tumor necrosis factor-alpha induces cFOS and strongly potentiates glutamate-mediated cell death in the rat spinal cord. Neurobiology of disease 8(4):590-599.

Herrmann M, Vos P, Wunderlich MT, de Bruijn CH, Lamers KJ. 2000. Release of glial tissue-specific proteins after acute stroke: A comparative analysis of serum concentrations of protein S-100B and glial fibrillary acidic protein. Stroke 31(11):2670-2677.

Herx LM, Rivest S, Yong VW. 2000. Central nervous system-initiated inflammation and neurotrophism in trauma: IL-1 beta is required for the production of ciliary neurotrophic factor. Journal of immunology (Baltimore, Md : 1950) 165(4):2232-2239.

Herx LM, Yong VW. 2001. Interleukin-1 beta is required for the early evolution of reactive astrogliosis following CNS lesion. Journal of neuropathology and experimental neurology 60(10):961-971.

Heslehurst N, Simpson H, Ells LJ, Rankin J, Wilkinson J, Lang R, Brown TJ, Summerbell CD. 2008. The impact of maternal BMI status on pregnancy outcomes with immediate short-term obstetric resource implications: a meta-analysis. Obesity reviews : an official journal of the International Association for the Study of Obesity 9(6):635-683.

Hickey WF. 1991. Migration of hematogenous cells through the blood-brain barrier and the initiation of CNS inflammation. Brain pathology (Zurich, Switzerland) 1(2):97-105.

Hillenbrand A, Knippschild U, Weiss M, Schrezenmeier H, Henne-Bruns D, Huber-Lang M, Wolf AM. 2010. Sepsis induced changes of adipokines and cytokines - septic patients compared to morbidly obese patients. BMC surgery 10:26.

Hillenbrand A, Weiss M, Knippschild U, Wolf AM, Huber-Lang M. 2012. Sepsis-Induced Adipokine Change with regard to Insulin Resistance. International journal of inflammation 2012:972368.

Himms-Hagen J. 1990. Brown adipose tissue thermogenesis: interdisciplinary studies. FASEB journal : official publication of the Federation of American Societies for Experimental Biology 4(11):2890-2898.

Hines T. 2013. Anatomy of the brain. Mayfield Clinic and spine institute

Hiramitsu S, Ishiguro Y, Matsuyama H, Yamada K, Kato K, Noba M, Uemura A, Yoshida S, Matsubara Y, Kani A, Hasegawa K, Hishida H, Ozaki Y. 2010. The effects of ezetimibe on surrogate markers of cholesterol absorption and synthesis in Japanese patients with dyslipidemia. Journal of atherosclerosis and thrombosis 17(1):106-114.

Hirsch EC, Breidert T, Rousselet E, Hunot S, Hartmann A, Michel PP. 2003. The role of glial reaction and inflammation in Parkinson's disease. Annals of the New York Academy of Sciences 991:214-228.

Hirschberg DL, Schwartz M. 1995. Macrophage recruitment to acutely injured central nervous system is inhibited by a resident factor: a basis for an immune-brain barrier. Journal of neuroimmunology 61(1):89-96.

Hitoshi S, Alexson T, Tropepe V, Donoviel D, Elia AJ, Nye JS, Conlon RA, Mak TW, Bernstein A, van der Kooy D. 2002. Notch pathway molecules are essential for the maintenance, but not the generation, of mammalian neural stem cells. Genes Dev 16(7):846-858.

Hivert MF, Sullivan LM, Fox CS, Nathan DM, D'Agostino RB, Sr., Wilson PW, Meigs JB. 2008. Associations of adiponectin, resistin, and tumor necrosis factor-alpha with insulin resistance. The Journal of clinical endocrinology and metabolism 93(8):3165-3172.

Ho AJ, Stein JL, Hua X, Lee S, Hibar DP, Leow AD, Dinov ID, Toga AW, Saykin AJ, Shen L, Foroud T, Pankratz N, Huentelman MJ, Craig DW, Gerber JD, Allen AN, Corneveaux JJ, Stephan DA, DeCarli CS, DeChairo BM, Potkin SG, Jack CR, Jr., Weiner MW, Raji CA, Lopez OL, Becker JT, Carmichael OT, Thompson PM. A commonly carried allele of the obesity-related FTO gene is associated with reduced brain volume in the healthy elderly. Proc Natl Acad Sci U S A 107(18):8404-8409.

Ho AJ, Stein JL, Hua X, Lee S, Hibar DP, Leow AD, Dinov ID, Toga AW, Saykin AJ, Shen L, Foroud T, Pankratz N, Huentelman MJ, Craig DW, Gerber JD, Allen AN, Corneveaux JJ, Stephan DA, DeCarli CS, DeChairo BM, Potkin SG, Jack CR, Jr., Weiner MW, Raji CA, Lopez OL, Becker JT, Carmichael OT, Thompson PM. 2010a. A commonly carried allele of the obesity-related FTO gene is associated with reduced brain volume in the healthy elderly. Proceedings of the National Academy of Sciences of the United States of America 107(18):8404-8409.

Ho AJ, Stein JL, Hua X, Lee S, Hibar DP, Leow AD, Dinov ID, Toga AW, Saykin AJ, Shen L, Foroud T, Pankratz N, Huentelman MJ, Craig DW, Gerber JD, Allen AN, Corneveaux JJ, Stephan DA, DeCarli CS, DeChairo BM, Potkin SG, Jack CR, Weiner MW, Raji CA, Lopez OL, Becker JT, Carmichael OT, Thompson PM, Initiative tAsDN. 2010b. A commonly carried allele of the obesity-related FTO gene is associated with reduced brain volume in the healthy elderly. Proceedings of the National Academy of Sciences 107(18):8404-8409.

Ho N, Sommers MS, Lucki I. 2013. Effects of diabetes on hippocampal neurogenesis: links to cognition and depression. Neuroscience and biobehavioral reviews 37(8):1346-1362.

Hochstim C, Deneen B, Lukaszewicz A, Zhou Q, Anderson DJ. 2008. Identification of positionally distinct astrocyte subtypes whose identities are specified by a homeodomain code. Cell 133(3):510-522.
Hoek RM, Ruuls SR, Murphy CA, Wright GJ, Goddard R, Zurawski SM, Blom B, Homola ME, Streit WJ, Brown MH, Barclay AN, Sedgwick JD. 2000. Down-regulation of the macrophage lineage through interaction with OX2 (CD200). Science 290(5497):1768-1771.
Holley AE, Cheeseman KH. 1993. Measuring free radical reactions in vivo. British medical bulletin 49(3):494-505.
Holtsberg FW, Steiner MR, Keller JN, Mark RJ, Mattson MP, Steiner SM. 1998. Lysophosphatidic acid induces necrosis and apoptosis in hippocampal neurons. Journal of neurochemistry 70(1):66-76.
Hotamisligil GS, Shargill NS, Spiegelman BM. 1993. Adipose expression of tumor necrosis factor-alpha: direct role in obesity-linked insulin resistance. Science 259(5091):87-91.
Houben AJ, van Wijk XM, van Meeteren LA, van Zeijl L, van de Westerlo EM, Hausmann J, Fish A, Perrakis A, van Kuppevelt TH, Moolenaar WH. 2013. The polybasic insertion in autotaxin alpha confers specific binding to heparin and cell surface heparan sulfate proteoglycans. The Journal of biological chemistry 288(1):510-519.
Hoyda TD, Fry M, Ahima RS, Ferguson AV. 2007. Adiponectin selectively inhibits oxytocin neurons of the paraventricular nucleus of the hypothalamus. The Journal of physiology 585(Pt 3):805-816.
Hsiao H-Y, Chen Y-C, Chen H-M, Tu P-H, Chern Y. 2013. A critical role of astrocyte-mediated nuclear factor-κB-dependent inflammation in Huntington's disease. Human Molecular Genetics 22(9):1826-1842.
Hu J, Akama KT, Krafft GA, Chromy BA, Van Eldik LJ. 1998. Amyloid-beta peptide activates cultured astrocytes: morphological alterations, cytokine induction and nitric oxide release. Brain research 785(2):195-206.
Huang YL, Sheu JY, Lin TH. 1999. Association between oxidative stress and changes of trace elements in patients with breast cancer. Clinical biochemistry 32(2):131-136.
Ikeya M, Lee SM, Johnson JE, McMahon AP, Takada S. 1997. Wnt signalling required for expansion of neural crest and CNS progenitors. Nature 389(6654):966-970.
in t' Veld BA, Ruitenberg A, Hofman A, Launer LJ, van Duijn CM, Stijnen T, Breteler MM, Stricker BH. 2001. Nonsteroidal anti-inflammatory drugs and the risk of Alzheimer's disease. The New England journal of medicine 345(21):1515-1521.
Inoue M, Rashid MH, Fujita R, Contos JJ, Chun J, Ueda H. 2004. Initiation of neuropathic pain requires lysophosphatidic acid receptor signaling. Nature medicine 10(7):712-718.
Iosif RE, EkDahl CT, Ahlenius H, Pronk CJ, Bonde S, Kokaia Z, Jacobsen SE, Lindvall O. 2006. Tumor necrosis factor receptor 1 is a negative regulator of progenitor proliferation in adult hippocampal neurogenesis. The Journal of neuroscience : the official journal of the Society for Neuroscience 26(38):9703-9712.
Ishii I, Friedman B, Ye X, Kawamura S, McGiffert C, Contos JJ, Kingsbury MA, Zhang G, Brown JH, Chun J. 2001. Selective loss of sphingosine 1-phosphate signaling with no obvious phenotypic abnormality in mice lacking its G protein-coupled receptor, LP(B3)/EDG-3. The Journal of biological chemistry 276(36):33697-33704.
Ishii I, Fukushima N, Ye X, Chun J. 2004. Lysophospholipid receptors: signaling and biology. Annual review of biochemistry 73:321-354.
Ivacko JA, Sun R, Silverstein FS. 1996. Hypoxic-ischemic brain injury induces an acute microglial reaction in perinatal rats. Pediatric research 39(1):39-47.

Iwaki M, Matsuda M, Maeda N, Funahashi T, Matsuzawa Y, Makishima M, Shimomura I. 2003. Induction of adiponectin, a fat-derived antidiabetic and antiatherogenic factor, by nuclear receptors. Diabetes 52(7):1655-1663.

Izcue A, Coombes JL, Powrie F. 2009. Regulatory lymphocytes and intestinal inflammation. Annual review of immunology 27:313-338.

Jain V, Langham MC, Wehrli FW. 2010. MRI estimation of global brain oxygen consumption rate. Journal of Cerebral Blood Flow and Metabolism: Official Journal of the International Society of Cerebral Blood Flow and Metabolism 30(9):1598-1607.

Jansen S, Andries M, Vekemans K, Vanbilloen H, Verbruggen A, Bollen M. 2009. Rapid clearance of the circulating metastatic factor autotaxin by the scavenger receptors of liver sinusoidal endothelial cells. Cancer letters 284(2):216-221.

Jensen PK. 1966. Antimycin-insensitive oxidation of succinate and reduced nicotinamide-adenine dinucleotide in electron-transport particles. I. pH dependency and hydrogen peroxide formation. Biochimica et biophysica acta 122(2):157-166.

Jeon BT, Shin HJ, Kim JB, Kim YK, Lee DH, Kim KH, Kim HJ, Kang SS, Cho GJ, Choi WS, Roh GS. 2009. Adiponectin protects hippocampal neurons against kainic acid-induced excitotoxicity. Brain research reviews 61(2):81-88.

Johansson JU, Woodling NS, Wang Q, Panchal M, Liang X, Trueba-Saiz A, Brown HD, Mhatre SD, Loui T, Andreasson KI. 2015. Prostaglandin signaling suppresses beneficial microglial function in Alzheimer's disease models. The Journal of clinical investigation 125(1):350-364.

John GR, Lee SC, Brosnan CF. 2003a. Cytokines: powerful regulators of glial cell activation. The Neuroscientist : a review journal bringing neurobiology, neurology and psychiatry 9(1):10-22.

John GR, Lee SC, Brosnan CF. 2003b. Cytokines: Powerful Regulators of Glial Cell Activation. The Neuroscientist 9(1):10-22.

Jung TW, Lee JY, Shim WS, Kang ES, Kim JS, Ahn CW, Lee HC, Cha BS. 2006. Adiponectin protects human neuroblastoma SH-SY5Y cells against acetaldehyde-induced cytotoxicity. Biochem Pharmacol 72(5):616-623.

Juric DM, Carman-Krzan M. 2001. Interleukin-1 beta, but not IL-1 alpha, mediates nerve growth factor secretion from rat astrocytes via type I IL-1 receptor. International journal of developmental neuroscience : the official journal of the International Society for Developmental Neuroscience 19(7):675-683.

Kadowaki T, Yamauchi T, Kubota N, Hara K, Ueki K, Tobe K. 2006. Adiponectin and adiponectin receptors in insulin resistance, diabetes, and the metabolic syndrome. Journal of Clinical Investigation 116(7):1784-1792.

Kahn BB, Flier JS. 2000. Obesity and insulin resistance. Journal of Clinical Investigation 106(4):473-481.

Kanda H, Newton R, Klein R, Morita Y, Gunn MD, Rosen SD. 2008. Autotaxin, an ectoenzyme that produces lysophosphatidic acid, promotes the entry of lymphocytes into secondary lymphoid organs. Nature immunology 9(4):415-423.

Kanoski SE, Zhang Y, Zheng W, Davidson TL. 2010. The effects of a high-energy diet on hippocampal function and blood-brain barrier integrity in the rat. Journal of Alzheimer's disease : JAD 21(1):207-219.

Kawaguchi M, Okabe T, Okudaira S, Nishimasu H, Ishitani R, Kojima H, Nureki O, Aoki J, Nagano T. 2013. Screening and X-ray crystal structure-based optimization of autotaxin (ENPP2) inhibitors, using a newly developed fluorescence probe. ACS chemical biology 8(8):1713-1721.

Kehlen A, Englert N, Seifert A, Klonisch T, Dralle H, Langner J, Hoang-Vu C. 2004. Expression, regulation and function of autotaxin in thyroid carcinomas. International journal of cancer Journal international du cancer 109(6):833-838.
Kelesidis T, Kelesidis I, Chou S, Mantzoros CS. 2010. Narrative review: the role of leptin in human physiology: emerging clinical applications. Annals of internal medicine 152(2):93-100.
Kelesidis T, Mantzoros CS. 2006. The emerging role of leptin in humans. Pediatric endocrinology reviews : PER 3(3):239-248.
Keller JN, Pang Z, Geddes JW, Begley JG, Germeyer A, Waeg G, Mattson MP. 1997a. Impairment of glucose and glutamate transport and induction of mitochondrial oxidative stress and dysfunction in synaptosomes by amyloid beta-peptide: role of the lipid peroxidation product 4-hydroxynonenal. Journal of neurochemistry 69(1):273-284.
Keller JN, Steiner MR, Holtsberg FW, Mattson MP, Steiner SM. 1997b. Lysophosphatidic acid-induced proliferation-related signals in astrocytes. Journal of neurochemistry 69(3):1073-1084.
Kern W, Peters A, Fruehwald-Schultes B, Deininger E, Born J, Fehm HL. 2001. Improving influence of insulin on cognitive functions in humans. Neuroendocrinology 74(4):270-280.
Kettenmann H, Verkhratsky A. 2011. [Neuroglia--living nerve glue]. Fortschritte der Neurologie-Psychiatrie 79(10):588-597.
Khandelwal PJ, Herman AM, Moussa CE. 2011. Inflammation in the early stages of neurodegenerative pathology. Journal of neuroimmunology 238(1-2):1-11.
Kiliaan AJ, Arnoldussen IA, Gustafson DR. 2014. Adipokines: a link between obesity and dementia? The Lancet Neurology 13(9):913-923.
Kim S, Moustaid-Moussa N. 2000. Secretory, endocrine and autocrine/paracrine function of the adipocyte. The Journal of nutrition 130(12):3110s-3115s.
Kim SU, de Vellis J. 2005. Microglia in health and disease. Journal of neuroscience research 81(3):302-313.
Kimelberg HK, Nedergaard M. 2010. Functions of astrocytes and their potential as therapeutic targets. Neurotherapeutics : the journal of the American Society for Experimental NeuroTherapeutics 7(4):338-353.
Kingsbury MA, Rehen SK, Contos JJ, Higgins CM, Chun J. 2003. Non-proliferative effects of lysophosphatidic acid enhance cortical growth and folding. Nature neuroscience 6(12):1292-1299.
Kivipelto M, Ngandu T, Fratiglioni L, Viitanen M, Kareholt I, Winblad B, Helkala EL, Tuomilehto J, Soininen H, Nissinen A. 2005. Obesity and vascular risk factors at midlife and the risk of dementia and Alzheimer disease. Archives of neurology 62(10):1556-1560.
Klesney-Tait J, Turnbull IR, Colonna M. 2006. The TREM receptor family and signal integration. Nature immunology 7(12):1266-1273.
Koizumi S, Shigemoto-Mogami Y, Nasu-Tada K, Shinozaki Y, Ohsawa K, Tsuda M, Joshi BV, Jacobson KA, Kohsaka S, Inoue K. 2007. UDP acting at P2Y6 receptors is a mediator of microglial phagocytosis. Nature 446(7139):1091-1095.
Kos K, Harte AL, Silva NFd, Tonchev A, Chaldakov G, James S, Snead DR, Hoggart B, O'Hare JP, McTernan PG, Kumar S. 2007. Adiponectin and Resistin in Human Cerebrospinal Fluid and Expression of Adiponectin Receptors in the Human Hypothalamus. The Journal of Clinical Endocrinology & Metabolism 92(3):1129-1136.

Kosari S, Camera DM, Hawley JA, Stebbing M, Badoer E. 2013. ERK1/2 in the brain mediates the effects of central resistin on reducing thermogenesis in brown adipose tissue. International journal of physiology, pathophysiology and pharmacology 5(3):184-189.
Kosari S, Rathner JA, Badoer E. 2012. Central resistin enhances renal sympathetic nerve activity via phosphatidylinositol 3-kinase but reduces the activity to brown adipose tissue via extracellular signal-regulated kinase 1/2. Journal of neuroendocrinology 24(11):1432-1439.
Kotarsky K, Boketoft A, Bristulf J, Nilsson NE, Norberg A, Hansson S, Owman C, Sillard R, Leeb-Lundberg LM, Olde B. 2006. Lysophosphatidic acid binds to and activates GPR92, a G protein-coupled receptor highly expressed in gastrointestinal lymphocytes. The Journal of pharmacology and experimental therapeutics 318(2):619-628.
Koutsilieri E, Scheller C, Tribl F, Riederer P. 2002. Degeneration of neuronal cells due to oxidative stress--microglial contribution. Parkinsonism & related disorders 8(6):401-406.
Kozak W, Soszynski D, Rudolph K, Conn CA, Kluger MJ. 1997. Dietary n-3 fatty acids differentially affect sickness behavior in mice during local and systemic inflammation. The American journal of physiology 272(4 Pt 2):R1298-1307.
Kraft AD, Harry GJ. 2011. Features of microglia and neuroinflammation relevant to environmental exposure and neurotoxicity. International journal of environmental research and public health 8(7):2980-3018.
Kreutzberg GW. 1995. Microglia, the first line of defence in brain pathologies. Arzneimittel-Forschung 45(3a):357-360.
Kreutzberg GW. 1996. Microglia: a sensor for pathological events in the CNS. Trends in neurosciences 19(8):312-318.
Kriegler M, Perez C, DeFay K, Albert I, Lu SD. 1988. A novel form of TNF/cachectin is a cell surface cytotoxic transmembrane protein: ramifications for the complex physiology of TNF. Cell 53(1):45-53.
Kronfol Z, Remick DG. 2000. Cytokines and the brain: implications for clinical psychiatry. The American journal of psychiatry 157(5):683-694.
Ku IA, Farzaneh-Far R, Vittinghoff E, Zhang MH, Na B, Whooley MA. 2011. Association of low leptin with cardiovascular events and mortality in patients with stable coronary artery disease: the Heart and Soul Study. Atherosclerosis 217(2):503-508.
Kubota N, Yano W, Kubota T, Yamauchi T, Itoh S, Kumagai H, Kozono H, Takamoto I, Okamoto S, Shiuchi T, Suzuki R, Satoh H, Tsuchida A, Moroi M, Sugi K, Noda T, Ebinuma H, Ueta Y, Kondo T, Araki E, Ezaki O, Nagai R, Tobe K, Terauchi Y, Ueki K, Minokoshi Y, Kadowaki T. 2007. Adiponectin stimulates AMP-activated protein kinase in the hypothalamus and increases food intake. Cell metabolism 6(1):55-68.
Kumar C. 1998. Robbins Pathologic Basis of Disease. WB Saunders Company, Philadelphia, ISBN 0-7216-7335-X.
Kusminski CM, McTernan PG, Schraw T, Kos K, O'Hare JP, Ahima R, Kumar S, Scherer PE. 2007a. Adiponectin complexes in human cerebrospinal fluid: distinct complex distribution from serum. Diabetologia 50(3):634-642.
Kusminski CM, McTernan PG, Schraw T, Kos K, O'Hare JP, Ahima R, Kumar S, Scherer PE. 2007b. Adiponectin complexes in human cerebrospinal fluid: distinct complex distribution from serum. Diabetologia 50(3):634-642.
LA T, M D, X W, N D, J C. 1995. Identification and expression cloning of a leptin receptor, OB-R. Cell 83:1263.
Lacy P, Stow JL. 2011. Cytokine release from innate immune cells: association with diverse membrane trafficking pathways. Blood 118(1):9-18.

Lafortuna CL, Agosti F, Proietti M, Adorni F, Sartorio A. 2006. The combined effect of adiposity, fat distribution and age on cardiovascular risk factors and motor disability in a cohort of obese women (aged 18-83). Journal of endocrinological investigation 29(10):905-912.

Lai CQ, Parnell LD, Arnett DK, Garcia-Bailo B, Tsai MY, Kabagambe EK, Straka RJ, Province MA, An P, Borecki IB, Tucker KL, Ordovas JM. 2009. WDTC1, the ortholog of Drosophila adipose gene, associates with human obesity, modulated by MUFA intake. Obesity (Silver Spring, Md) 17(3):593-600.

Lam Kee OEP. 2014. Sources of Free Radicals. Mangosteen.

Lan X, Chen Q, Wang Y, Jia B, Sun L, Zheng J, Peng H. 2012. TNF-alpha affects human cortical neural progenitor cell differentiation through the autocrine secretion of leukemia inhibitory factor. PloS one 7(12):e50783.

Langeveld CH, Jongenelen CA, Schepens E, Stoof JC, Bast A, Drukarch B. 1995. Cultured rat striatal and cortical astrocytes protect mesencephalic dopaminergic neurons against hydrogen peroxide toxicity independent of their effect on neuronal development. Neuroscience letters 192(1):13-16.

Langlet F, Mullier A, Bouret SG, Prevot V, Dehouck B. 2013. Tanycyte-like cells form a blood-cerebrospinal fluid barrier in the circumventricular organs of the mouse brain. The Journal of comparative neurology 521(15):3389-3405.

Lanzavecchia A. 1997. Understanding the mechanisms of sustained signaling and T cell activation. The Journal of experimental medicine 185(10):1717-1719.

Larner AJ, Johnson AR, Keynes RJ. 1995. Regeneration in the vertebrate central nervous system: phylogeny, ontogeny, and mechanisms. Biological reviews of the Cambridge Philosophical Society 70(4):597-619.

Larsen GL, Henson PM. 1983. Mediators of inflammation. Annual review of immunology 1:335-359.

Lau DC, Douketis JD, Morrison KM, Hramiak IM, Sharma AM, Ur E. 2007a. 2006 Canadian clinical practice guidelines on the management and prevention of obesity in adults and children [summary]. CMAJ : Canadian Medical Association journal = journal de l'Association medicale canadienne 176(8):S1-13.

Lau FC, Shukitt-Hale B, Joseph JA. 2007b. Nutritional intervention in brain aging: reducing the effects of inflammation and oxidative stress. Subcell Biochem 42:299-318.

Laurikka A, Vuolteenaho K, Toikkanen V, Rinne T, Leppanen T, Tarkka M, Laurikka J, Moilanen E. 2014. Adipocytokine resistin correlates with oxidative stress and myocardial injury in patients undergoing cardiac surgery. European journal of cardio-thoracic surgery : official journal of the European Association for Cardio-thoracic Surgery 46(4):729-736.

Lawrence CB, Allan SM, Rothwell NJ. 1998. Interleukin-1beta and the interleukin-1 receptor antagonist act in the striatum to modify excitotoxic brain damage in the rat. The European journal of neuroscience 10(3):1188-1195.

Lawrence T. 2009. The Nuclear Factor NF-κB Pathway in Inflammation. Cold Spring Harbor perspectives in biology 1(6):a001651.

Le W, Rowe D, Xie W, Ortiz I, He Y, Appel SH. 2001. Microglial activation and dopaminergic cell injury: an in vitro model relevant to Parkinson's disease. The Journal of neuroscience : the official journal of the Society for Neuroscience 21(21):8447-8455.

Leal Vde O, Mafra D. 2013. Adipokines in obesity. Clinica chimica acta; international journal of clinical chemistry 419:87-94.

Leclerc C, Moreau M, Néant I. 2012. The calcium: An early signal that initiate the formation of the nervous system during embryogenesis. Frontiers in Molecular Neuroscience 5.

Lee BP, Imhof BA. 2008. Lymphocyte transmigration in the brain: a new way of thinking. Nature immunology 9(2):117-118.

Lee EB. 2011. Obesity, leptin, and Alzheimer's disease. Annals of the New York Academy of Sciences 1243:15-29.
Lee G-H, Proenca R, Montez JM, Carroll KM, Darvishzadeh JG, Lee JI, Friedman JM. 1996a. Abnormal splicing of the leptin receptor in diabetic mice. Nature 379(6566):632-635.
Lee HY, Clair T, Mulvaney PT, Woodhouse EC, Aznavoorian S, Liotta LA, Stracke ML. 1996b. Stimulation of tumor cell motility linked to phosphodiesterase catalytic site of autotaxin. The Journal of biological chemistry 271(40):24408-24412.
Lee JH, Bullen JW, Jr., Stoyneva VL, Mantzoros CS. 2005. Circulating resistin in lean, obese, and insulin-resistant mouse models: lack of association with insulinemia and glycemia. American journal of physiology Endocrinology and metabolism 288(3):E625-632.
Lee MH, Klein RL, El-Shewy HM, Luttrell DK, Luttrell LM. 2008. The adiponectin receptors AdipoR1 and AdipoR2 activate ERK1/2 through a Src/Ras-dependent pathway and stimulate cell growth. Biochemistry 47(44):11682-11692.
Lee SM, Tole S, Grove E, McMahon AP. 2000. A local Wnt-3a signal is required for development of the mammalian hippocampus. Development 127(3):457-467.
Lehr S, Hartwig S, Sell H. 2012. Adipokines: a treasure trove for the discovery of biomarkers for metabolic disorders. Proteomics Clinical applications 6(1-2):91-101.
Lenschow DJ, Walunas TL, Bluestone JA. 1996. CD28/B7 system of T cell costimulation. Annual review of immunology 14:233-258.
Letra L, Santana I, Seica R. 2014. Obesity as a risk factor for Alzheimer's disease: the role of adipocytokines. Metabolic brain disease 29(3):563-568.
Leveille C, Chandad F, Al-Daccak R, Mourad W. 1999. CD40 associates with the MHC class II molecules on human B cells. European journal of immunology 29(11):3516-3526.
Levison SW, Goldman JE. 1993. Both oligodendrocytes and astrocytes develop from progenitors in the subventricular zone of postnatal rat forebrain. Neuron 10(2):201-212.
Li W, Prakash R, Chawla D, Du W, Didion SP, Filosa JA, Zhang Q, Brann DW, Lima VV, Tostes RC, Ergul A. 2013. Early effects of high-fat diet on neurovascular function and focal ischemic brain injury. American journal of physiology Regulatory, integrative and comparative physiology 304(11):R1001-1008.
Li Y, Tan M-S, Jiang T, Tan L. 2014. Microglia in Alzheimer’s Disease. BioMed Research International 2014:7.
Liang SL, Carlson GC, Coulter DA. 2006. Dynamic regulation of synaptic GABA release by the glutamate-glutamine cycle in hippocampal area CA1. The Journal of neuroscience : the official journal of the Society for Neuroscience 26(33):8537-8548.
Lieberman AP, Pitha PM, Shin HS, Shin ML. 1989. Production of tumor necrosis factor and other cytokines by astrocytes stimulated with lipopolysaccharide or a neurotropic virus. Proceedings of the National Academy of Sciences of the United States of America 86(16):6348-6352.
Lindsay RS, Funahashi T, Hanson RL, Matsuzawa Y, Tanaka S, Tataranni PA, Knowler WC, Krakoff J. 2002. Adiponectin and development of type 2 diabetes in the Pima Indian population. Lancet 360(9326):57-58.
Liu B, Gao HM, Hong JS. 2003a. Parkinson's disease and exposure to infectious agents and pesticides and the occurrence of brain injuries: role of neuroinflammation. Environmental health perspectives 111(8):1065-1073.
Liu B, Gao HM, Wang JY, Jeohn GH, Cooper CL, Hong JS. 2002. Role of nitric oxide in inflammation-mediated neurodegeneration. Annals of the New York Academy of Sciences 962:318-331.

Liu B, Hong JS. 2003. Role of microglia in inflammation-mediated neurodegenerative diseases: mechanisms and strategies for therapeutic intervention. The Journal of pharmacology and experimental therapeutics 304(1):1-7.
Liu T, Gao YJ, Ji RR. Emerging role of Toll-like receptors in the control of pain and itch. Neurosci Bull 28(2):131-144.
Liu Y, Qin L, Li G, Zhang W, An L, Liu B, Hong JS. 2003b. Dextromethorphan protects dopaminergic neurons against inflammation-mediated degeneration through inhibition of microglial activation. The Journal of pharmacology and experimental therapeutics 305(1):212-218.
Lochhead JJ, McCaffrey G, Quigley CE, Finch J, DeMarco KM, Nametz N, Davis TP. 2010. Oxidative stress increases blood-brain barrier permeability and induces alterations in occludin during hypoxia-reoxygenation. Journal of cerebral blood flow and metabolism : official journal of the International Society of Cerebral Blood Flow and Metabolism 30(9):1625-1636.
Lokken KL, Boeka AG, Austin HM, Gunstad J, Harmon CM. 2009. Evidence of executive dysfunction in extremely obese adolescents: a pilot study. Surgery for obesity and related diseases : official journal of the American Society for Bariatric Surgery 5(5):547-552.
Loos RJ, Yeo GS. 2014. The bigger picture of FTO: the first GWAS-identified obesity gene. Nature reviews Endocrinology 10(1):51-61.
Lopaczynski W ZS. 2007. Antioxidants, programmed cell death, and cancer. . Nutr Res 21:295–307.
Lopez-Bayghen E, Ortega A. 2011. Glial glutamate transporters: new actors in brain signaling. IUBMB Life 63(10):816-823.
Loschen G, Flohe L, Chance B. 1971. Respiratory chain linked H(2)O(2) production in pigeon heart mitochondria. FEBS letters 18(2):261-264.
Lovell MA, Ehmann WD, Butler SM, Markesbery WR. 1995. Elevated thiobarbituric acid-reactive substances and antioxidant enzyme activity in the brain in Alzheimer's disease. Neurology 45(8):1594-1601.
Lucas S-M, Rothwell NJ, Gibson RM. 2006a. The role of inflammation in CNS injury and disease. British Journal of Pharmacology 147(Suppl 1):S232-S240.
Lucas SM, Rothwell NJ, Gibson RM. 2006b. The role of inflammation in CNS injury and disease. Br J Pharmacol 147 Suppl 1:S232-240.
Lucius R, Sievers J. 1996. Postnatal retinal ganglion cells in vitro: protection against reactive oxygen species (ROS)-induced axonal degeneration by cocultured astrocytes. Brain research 743(1-2):56-62.
Lull ME, Block ML. 2010. Microglial Activation & Chronic Neurodegeneration. Neurotherapeutics : the journal of the American Society for Experimental NeuroTherapeutics 7(4):354-365.
Lumeng CN, Bodzin JL, Saltiel AR. 2007. Obesity induces a phenotypic switch in adipose tissue macrophage polarization. The Journal of clinical investigation 117(1):175-184.
Luth HJ, Munch G, Arendt T. 2002. Aberrant expression of NOS isoforms in Alzheimer's disease is structurally related to nitrotyrosine formation. Brain research 953(1-2):135-143.
Lynch CM, Kinzenbaw DA, Chen X, Zhan S, Mezzetti E, Filosa J, Ergul A, Faulkner JL, Faraci FM, Didion SP. 2013. Nox2-derived superoxide contributes to cerebral vascular dysfunction in diet-induced obesity. Stroke 44(11):3195-3201.
Lyons A, Downer EJ, Crotty S, Nolan YM, Mills KH, Lynch MA. 2007. CD200 ligand receptor interaction modulates microglial activation in vivo and in vitro: a role for IL-4. The

Journal of neuroscience : the official journal of the Society for Neuroscience 27(31):8309-8313.
MacKnight C, Rockwood K, Awalt E, McDowell I. 2002. Diabetes mellitus and the risk of dementia, Alzheimer's disease and vascular cognitive impairment in the Canadian Study of Health and Aging. Dementia and geriatric cognitive disorders 14(2):77-83.
Maddineni S, Metzger S, Ocon O, Hendricks G, 3rd, Ramachandran R. 2005. Adiponectin gene is expressed in multiple tissues in the chicken: food deprivation influences adiponectin messenger ribonucleic acid expression. Endocrinology 146(10):4250-4256.
Maeda N, Shimomura I, Kishida K, Nishizawa H, Matsuda M, Nagaretani H, Furuyama N, Kondo H, Takahashi M, Arita Y, Komuro R, Ouchi N, Kihara S, Tochino Y, Okutomi K, Horie M, Takeda S, Aoyama T, Funahashi T, Matsuzawa Y. 2002. Diet-induced insulin resistance in mice lacking adiponectin/ACRP30. Nature medicine 8(7):731-737.
Maier CM, Chan PH. 2002. Role of superoxide dismutases in oxidative damage and neurodegenerative disorders. The Neuroscientist : a review journal bringing neurobiology, neurology and psychiatry 8(4):323-334.
Maillard G, Charles MA, Thibult N, Forhan A, Sermet C, Basdevant A, Eschwege E. 1999. Trends in the prevalence of obesity in the French adult population between 1980 and 1991. International journal of obesity and related metabolic disorders : journal of the International Association for the Study of Obesity 23(4):389-394.
Mallat M, Houlgatte R, Brachet P, Prochiantz A. 1989. Lipopolysaccharide-stimulated rat brain macrophages release NGF in vitro. Developmental biology 133(1):309-311.
Maragakis NJ, Rothstein JD. 2006. Mechanisms of Disease: astrocytes in neurodegenerative disease. Nature clinical practice Neurology 2(12):679-689.
Marchetti L, Klein M, Schlett K, Pfizenmaier K, Eisel UL. 2004. Tumor necrosis factor (TNF)-mediated neuroprotection against glutamate-induced excitotoxicity is enhanced by N-methyl-D-aspartate receptor activation. Essential role of a TNF receptor 2-mediated phosphatidylinositol 3-kinase-dependent NF-kappa B pathway. The Journal of biological chemistry 279(31):32869-32881.
Marin-Teva JL, Dusart I, Colin C, Gervais A, van Rooijen N, Mallat M. 2004. Microglia promote the death of developing Purkinje cells. Neuron 41(4):535-547.
Martin GR. 1981. Isolation of a pluripotent cell line from early mouse embryos cultured in medium conditioned by teratocarcinoma stem cells. Proc Natl Acad Sci U S A 78(12):7634-7638.
Martin LJ, Pan Y, Price AC, Sterling W, Copeland NG, Jenkins NA, Price DL, Lee MK. 2006. Parkinson's disease alpha-synuclein transgenic mice develop neuronal mitochondrial degeneration and cell death. The Journal of neuroscience : the official journal of the Society for Neuroscience 26(1):41-50.
Mason JL, Suzuki K, Chaplin DD, Matsushima GK. 2001. Interleukin-1beta promotes repair of the CNS. The Journal of neuroscience : the official journal of the Society for Neuroscience 21(18):7046-7052.
Masuda A, Nakamura K, Izutsu K, Igarashi K, Ohkawa R, Jona M, Higashi K, Yokota H, Okudaira S, Kishimoto T, Watanabe T, Koike Y, Ikeda H, Kozai Y, Kurokawa M, Aoki J, Yatomi Y. 2008. Serum autotaxin measurement in haematological malignancies: a promising marker for follicular lymphoma. British journal of haematology 143(1):60-70.
Matas-Rico E, Garcia-Diaz B, Llebrez-Zayas P, Lopez-Barroso D, Santin L, Pedraza C, Smith-Fernandez A, Fernandez-Llebrez P, Tellez T, Redondo M, Chun J, De Fonseca FR, Estivill-Torrus G. 2008. Deletion of lysophosphatidic acid receptor LPA1 reduces neurogenesis in the mouse dentate gyrus. Molecular and cellular neurosciences 39(3):342-355.

Matsuda M, Shimomura I. 2014. Roles of adiponectin and oxidative stress in obesity-associated metabolic and cardiovascular diseases. Reviews in endocrine & metabolic disorders 15(1):1-10.
Matsunaga E, Nambu S, Oka M, Iriki A. 2013. Differential cadherin expression in the developing postnatal telencephalon of a New World monkey. J Comp Neurol 521(17):4027-4060.
Matsuzawa Y. 2005. Adiponectin: Identification, physiology and clinical relevance in metabolic and vascular disease. Atherosclerosis Supplements 6(2):7-14.
Matsuzawa Y, Funahashi T, Kihara S, Shimomura I. 2004. Adiponectin and metabolic syndrome. Arteriosclerosis, thrombosis, and vascular biology 24(1):29-33.
Mattson MP, Goodman Y, Luo H, Fu W, Furukawa K. 1997. Activation of NF-κB protects hippocampal neurons against oxidative stress-induced apoptosis: Evidence for induction of manganese superoxide dismutase and suppression of peroxynitrite production and protein tyrosine nitration. Journal of neuroscience research 49(6):681-697.
Mauro C, De Rosa V, Marelli-Berg F, Solito E. 2014. Metabolic syndrome and the immunological affair with the blood-brain barrier. Frontiers in immunology 5:677.
Maury E, Brichard SM. 2010. Adipokine dysregulation, adipose tissue inflammation and metabolic syndrome. Molecular and cellular endocrinology 314(1):1-16.
Mayer AM, Hall M, Fay MJ, Lamar P, Pearson C, Prozialeck WC, Lehmann VK, Jacobson PB, Romanic AM, Uz T, Manev H. 2001. Effect of a short-term in vitro exposure to the marine toxin domoic acid on viability, tumor necrosis factor-alpha, matrix metalloproteinase-9 and superoxide anion release by rat neonatal microglia. BMC pharmacology 1:7.
Mazereeuw-Hautier J, Gres S, Fanguin M, Cariven C, Fauvel J, Perret B, Chap H, Salles JP, Saulnier-Blache JS. 2005. Production of lysophosphatidic acid in blister fluid: involvement of a lysophospholipase D activity. The Journal of investigative dermatology 125(3):421-427.
Mazurek T, Zhang L, Zalewski A, Mannion JD, Diehl JT, Arafat H, Sarov-Blat L, O'Brien S, Keiper EA, Johnson AG, Martin J, Goldstein BJ, Shi Y. 2003. Human epicardial adipose tissue is a source of inflammatory mediators. Circulation 108(20):2460-2466.
McCrimmon RJ, Ryan CM, Frier BM. 2012. Diabetes and cognitive dysfunction. Lancet 379(9833):2291-2299.
McLay RN, Kimura M, Banks WA, Kastin AJ. 1997. Granulocyte-macrophage colony-stimulating factor crosses the blood--brain and blood--spinal cord barriers. Brain 120 (Pt 11):2083-2091.
McMahon AP, Joyner AL, Bradley A, McMahon JA. 1992. The midbrain-hindbrain phenotype of Wnt-1-/Wnt-1- mice results from stepwise deletion of engrailed-expressing cells by 9.5 days postcoitum. Cell 69(4):581-595.
McNeilly AD, Williamson R, Sutherland C, Balfour DJ, Stewart CA. 2011. High fat feeding promotes simultaneous decline in insulin sensitivity and cognitive performance in a delayed matching and non-matching to position task. Behavioural brain research 217(1):134-141.
Medzhitov R. 2008. Origin and physiological roles of inflammation. Nature 454(7203):428-435.
Megason SG, McMahon AP. 2002. A mitogen gradient of dorsal midline Wnts organizes growth in the CNS. Development 129(9):2087-2098.
Mei Z, Grummer-Strawn LM, Pietrobelli A, Goulding A, Goran MI, Dietz WH. 2002. Validity of body mass index compared with other body-composition screening indexes for the

assessment of body fatness in children and adolescents. The American journal of clinical nutrition 75(6):978-985.
Mercer JG, Hoggard N, Williams LM, Lawrence CB, Hannah LT, Trayhurn P. 1996. Localization of leptin receptor mRNA and the long form splice variant (Ob-Rb) in mouse hypothalamus and adjacent brain regions by in situ hybridization. FEBS letters 387(2-3):113-116.
Merrill JE. 1992. Pro-inflammatory and anti-inflammatory cytokines in multiple sclerosis and central nervous system acquired immunodeficiency syndrome. Journal of immunotherapy : official journal of the Society for Biological Therapy 12(3):167-170.
Miao L, St Clair DK. 2009. Regulation of superoxide dismutase genes: implications in disease. Free radical biology & medicine 47(4):344-356.
Michiels C, Raes M, Toussaint O, Remacle J. 1994. Importance of Se-glutathione peroxidase, catalase, and Cu/Zn-SOD for cell survival against oxidative stress. Free radical biology & medicine 17(3):235-248.
Milligan ED, Watkins LR. 2009. Pathological and protective roles of glia in chronic pain. Nat Rev Neurosci 10(1):23-36.
Min KJ, Yang MS, Kim SU, Jou I, Joe EH. 2006. Astrocytes induce hemeoxygenase-1 expression in microglia: a feasible mechanism for preventing excessive brain inflammation. The Journal of neuroscience : the official journal of the Society for Neuroscience 26(6):1880-1887.
Miralbell J, Lopez-Cancio E, Lopez-Oloriz J, Arenillas JF, Barrios M, Soriano-Raya JJ, Galan A, Caceres C, Alzamora M, Pera G, Toran P, Davalos A, Mataro M. 2013. Cognitive patterns in relation to biomarkers of cerebrovascular disease and vascular risk factors. Cerebrovascular diseases 36(2):98-105.
Misson JP, Edwards MA, Yamamoto M, Caviness VS, Jr. 1988. Identification of radial glial cells within the developing murine central nervous system: studies based upon a new immunohistochemical marker. Brain research Developmental brain research 44(1):95-108.
Mittelbronn M, Dietz K, Schluesener HJ, Meyermann R. 2001. Local distribution of microglia in the normal adult human central nervous system differs by up to one order of magnitude. Acta neuropathologica 101(3):249-255.
Miwa T, Furukawa S, Nakajima K, Furukawa Y, Kohsaka S. 1997. Lipopolysaccharide enhances synthesis of brain-derived neurotrophic factor in cultured rat microglia. Journal of neuroscience research 50(6):1023-1029.
MokDad AH, Marks JS, Stroup DF, Gerberding JL. 2004. Actual causes of death in the United States, 2000. Jama 291(10):1238-1245.
Moller T, Contos JJ, Musante DB, Chun J, Ransom BR. 2001. Expression and function of lysophosphatidic acid receptors in cultured rodent microglial cells. The Journal of biological chemistry 276(28):25946-25952.
Molofsky AV, Krenick R, Ullian E, Tsai H-h, Deneen B, Richardson WD, Barres BA, Rowitch DH. 2012. Astrocytes and disease: a neurodevelopmental perspective. Genes & Development 26(9):891-907.
Monje ML, Toda H, Palmer TD. 2003. Inflammatory blockade restores adult hippocampal neurogenesis. Science 302(5651):1760-1765.
Monks CR, Freiberg BA, Kupfer H, Sciaky N, Kupfer A. 1998. Three-dimensional segregation of supramolecular activation clusters in T cells. Nature 395(6697):82-86.
Montgomery DL. 1994. Astrocytes: form, functions, and roles in disease. Veterinary pathology 31(2):145-167.
Montgomery SL, Bowers WJ. 2012. Tumor necrosis factor-alpha and the roles it plays in homeostatic and degenerative processes within the central nervous system. Journal of

neuroimmune pharmacology : the official journal of the Society on NeuroImmune Pharmacology 7(1):42-59.
Moolenaar WH, Perrakis A. 2011. Insights into autotaxin: how to produce and present a lipid mediator. Nature reviews Molecular cell biology 12(10):674-679.
Morash B, Li A, Murphy PR, Wilkinson M, Ur E. 1999. Leptin gene expression in the brain and pituitary gland. Endocrinology 140(12):5995-5998.
Morash BA, Willkinson D, Ur E, Wilkinson M. 2002. Resistin expression and regulation in mouse pituitary. FEBS letters 526(1-3):26-30.
Moreira PI, Duarte AI, Santos MS, Rego AC, Oliveira CR. 2009. An integrative view of the role of oxidative stress, mitochondria and insulin in Alzheimer's disease. Journal of Alzheimer's disease : JAD 16(4):741-761.
Morganti-Kossman MC, Lenzlinger PM, Hans V, Stahel P, Csuka E, Ammann E, Stocker R, Trentz O, Kossmann T. 1997. Production of cytokines following brain injury: beneficial and deleterious for the damaged tissue. Molecular psychiatry 2(2):133-136.
Morishige J, Touchika K, Tanaka T, Satouchi K, Fukuzawa K, Tokumura A. 2007. Production of bioactive lysophosphatidic acid by lysophospholipase D in hen egg white. Biochimica et biophysica acta 1771(4):491-499.
Morón ÚM, Castilla-Cortázar I. 2012. Protection Against Oxidative Stress and "IGF-I Deficiency Conditions".
Morris MJ, Beilharz JE, Maniam J, Reichelt AC, Westbrook RF. 2014. Why is obesity such a problem in the 21st century? The intersection of palatable food, cues and reward pathways, stress, and cognition. Neuroscience and biobehavioral reviews.
Moschen AR, Kaser A, Enrich B, Mosheimer B, Theurl M, Niederegger H, Tilg H. 2007. Visfatin, an adipocytokine with pro-inflammatory and immunomodulating properties. J Immunol 178(3):1748-1758.
Moschen AR, Wieser V, Tilg H. 2012. Adiponectin: key player in the adipose tissue-liver crosstalk. Current medicinal chemistry 19(32):5467-5473.
Mosher KI, Wyss-Coray T. 2014. Microglial dysfunction in brain aging and Alzheimer's disease. Biochemical Pharmacology 88(4):594-604.
Mosley RL, Hutter-Saunders JA, Stone DK, Gendelman HE. 2012. Inflammation and Adaptive Immunity in Parkinson's Disease. Cold Spring Harbor Perspectives in Medicine 2(1):a009381.
Motori E, Puyal J, Toni N, Ghanem A, Angeloni C, Malaguti M, Cantelli-Forti G, Berninger B, Conzelmann K-K, Götz M, Winklhofer Konstanze F, Hrelia S, Bergami M. Inflammation-Induced Alteration of Astrocyte Mitochondrial Dynamics Requires Autophagy for Mitochondrial Network Maintenance. Cell metabolism 18(6):844-859.
Mrak RE. 2009. Alzheimer-type neuropathological changes in morbidly obese elderly individuals. Clinical neuropathology 28(1):40-45.
Munger KL, Chitnis T, Ascherio A. 2009. Body size and risk of MS in two cohorts of US women. Neurology 73(19):1543-1550.
Murata J, Lee HY, Clair T, Krutzsch HC, Arestad AA, Sobel ME, Liotta LA, Stracke ML. 1994. cDNA cloning of the human tumor motility-stimulating protein, autotaxin, reveals a homology with phosphodiesterases. The Journal of biological chemistry 269(48):30479-30484.
Muroyama Y, Fujiwara Y, Orkin SH, Rowitch DH. 2005. Specification of astrocytes by bHLH protein SCL in a restricted region of the neural tube. Nature 438(7066):360-363.
Muroyama Y, Kondoh H, Takada S. 2004. Wnt proteins promote neuronal differentiation in neural stem cell culture. Biochemical and biophysical research communications 313(4):915-921.

Murphy LA, Goldstein IJ. 1977. Five alpha-D-galactopyranosyl-binding isolectins from Bandeiraea simplicifolia seeds. The Journal of biological chemistry 252(13):4739-4742.
Murray AJ, Knight NS, Cochlin LE, McAleese S, Deacon RM, Rawlins JN, Clarke K. 2009. Deterioration of physical performance and cognitive function in rats with short-term high-fat feeding. FASEB J 23(12):4353-4360.
Naderali EK, Ratcliffe SH, Dale MC. 2009. Obesity and Alzheimer's disease: a link between body weight and cognitive function in old age. American journal of Alzheimer's disease and other dementias 24(6):445-449.
Nagata K, Takei N, Nakajima K, Saito H, Kohsaka S. 1993. Microglial conditioned medium promotes survival and development of cultured mesencephalic neurons from embryonic rat brain. Journal of neuroscience research 34(3):357-363.
Nakamura K, Igarashi K, Ide K, Ohkawa R, Okubo S, Yokota H, Masuda A, Oshima N, Takeuchi T, Nangaku M, Okudaira S, Arai H, Ikeda H, Aoki J, Yatomi Y. 2008. Validation of an autotaxin enzyme immunoassay in human serum samples and its application to hypoalbuminemia differentiation. Clinica chimica acta; international journal of clinical chemistry 388(1-2):51-58.
Napoli I, Neumann H. 2010. Protective effects of microglia in multiple sclerosis. Experimental neurology 225(1):24-28.
Navarro J, Obrador E, Pellicer JA, Asensi M, Viña J, Estrela JM. 1997. Blood Glutathione as an Index of Radiation-Induced Oxidative Stress in Mice and Humans. Free Radical Biology and Medicine 22(7):1203-1209.
Nayak D, Roth TL, McGavern DB. 2014. Microglia development and function. Annual review of immunology 32:367-402.
Nayak D, Zinselmeyer BH, Corps KN, McGavern DB. 2012. In vivo dynamics of innate immune sentinels in the CNS. Intravital 1(2):95-106.
Nedvidkova J, Smitka K, Kopsky V, Hainer V. 2005. Adiponectin, an adipocyte-derived protein. Physiological research / Academia Scientiarum Bohemoslovaca 54(2):133-140.
Neha V-S, Anuja G. 2012. Astrocyte elevated gene-1 regulates astrocyte responses to neural injury: implications for reactive astrogliosis and neurodegeneration. Journal of Neuroinflammation 9:195-195.
Nelson PT, Soma LA, Lavi E. 2002. Microglia in diseases of the central nervous system. Annals of medicine 34(7-8):491-500.
Neumann H. 2001. Control of glial immune function by neurons. Glia 36(2):191-199.
Neumann H, Kotter MR, Franklin RJ. 2009. Debris clearance by microglia: an essential link between degeneration and regeneration. Brain : a journal of neurology 132(Pt 2):288-295.
Neumeier M, Weigert J, Buettner R, Wanninger J, Schaffler A, Muller AM, Killian S, Sauerbruch S, Schlachetzki F, Steinbrecher A, Aslanidis C, Scholmerich J, Buechler C. 2007. Detection of adiponectin in cerebrospinal fluid in humans. American journal of physiology Endocrinology and metabolism 293(4):E965-969.
Nguyen JC, Killcross AS, Jenkins TA. 2014a. Obesity and cognitive decline: role of inflammation and vascular changes. Frontiers in neuroscience 8:375.
Nguyen MD, J. P. Julien, and S. Rivest. . 2002. . Innate immunity: the missing link in neuroprotection and neurodegeneration? . Nat Rev Neurosci 3:216–227.
Nguyen MD, Julien JP, Rivest S. 2002. Innate immunity: the missing link in neuroprotection and neurodegeneration? Nat Rev Neurosci 3(3):216-227.
Nguyen S, Major K, Demonet JF, Smith C, Rubli E, Humbert M, Bula C. 2014b. [Diabetes and dementia: the dangerous liaisons?]. Revue medicale suisse 10(449):2090-2092, 2094-2096.

NHLBI. 2012. What Causes Overweight and Obesity? National Heart, Lung, and Blood Institute Webpage.
Nicholson JK, Holmes E, Kinross J, Burcelin R, Gibson G, Jia W, Pettersson S. 2012. Host-gut microbiota metabolic interactions. Science 336(6086):1262-1267.
Nicola NA. 1994. Cytokine pleiotropy and redundancy: a view from the receptor. Stem cells (Dayton, Ohio) 12 Suppl 1:3-12; discussion 12-14.
NINDS-NIH. 2014. Brain Basics: Know Your Brain. The National Institute of Neurological Disorders and Stroke, National Institutes of Health.
Niranjan R. 2014. The role of inflammatory and oxidative stress mechanisms in the pathogenesis of Parkinson's disease: focus on astrocytes. Molecular neurobiology 49(1):28-38.
Nishimasu H, Ishitani R, Aoki J, Nureki O. 2012. A 3D view of autotaxin. Trends in pharmacological sciences 33(3):138-145.
Nishimasu H, Okudaira S, Hama K, Mihara E, Dohmae N, Inoue A, Ishitani R, Takagi J, Aoki J, Nureki O. 2011a. Crystal structure of autotaxin and insight into GPCR activation by lipid mediators. Nat Struct Mol Biol 18(2):205-212.
Nishimasu H, Okudaira S, Hama K, Mihara E, Dohmae N, Inoue A, Ishitani R, Takagi J, Aoki J, Nureki O. 2011b. Crystal structure of autotaxin and insight into GPCR activation by lipid mediators. Nat Struct Mol Biol 18(2):205-212.
Nishimura M, Izumiya Y, Higuchi A, Shibata R, Qiu J, Kudo C, Shin HK, Moskowitz MA, Ouchi N. 2008. Adiponectin prevents cerebral ischemic injury through endothelial nitric oxide synthase dependent mechanisms. Circulation 117(2):216-223.
Noguchi K, Herr D, Mutoh T, Chun J. 2009. Lysophosphatidic acid (LPA) and its receptors. Current opinion in pharmacology 9(1):15-23.
Noguchi K, Ishii S, Shimizu T. 2003. Identification of p2y9/GPR23 as a novel G protein-coupled receptor for lysophosphatidic acid, structurally distant from the Edg family. The Journal of biological chemistry 278(28):25600-25606.
Noh KM, Koh JY. 2000. Induction and activation by zinc of NADPH oxidase in cultured cortical neurons and astrocytes. The Journal of neuroscience : the official journal of the Society for Neuroscience 20(23):Rc111.
Norden DM, Muccigrosso MM, Godbout JP. 2014. Microglial priming and enhanced reactivity to secondary insult in aging, and traumatic CNS injury, and neurodegenerative disease. Neuropharmacology(0).
Nylen K, Karlsson JE, Blomstrand C, Tarkowski A, Trysberg E, Rosengren LE. 2002. Cerebrospinal fluid neurofilament and glial fibrillary acidic protein in patients with cerebral vasculitis. J Neurosci Res 67(6):844-851.
O'Keefe GM, Nguyen VT, Benveniste EN. 2002. Regulation and function of class II major histocompatibility complex, CD40, and B7 expression in macrophages and microglia: Implications in neurological diseases. Journal of neurovirology 8(6):496-512.
Oberheim NA, Wang X, Goldman S, Nedergaard M. 2006. Astrocytic complexity distinguishes the human brain. Trends in neurosciences 29(10):547-553.
OECD. 2014. Obesity and the economics of prevention: fit not fat key facts – France, update 2014. Organisation for Economic Co-operation and Development.
Oh DK, Ciaraldi T, Henry RR. 2007. Adiponectin in health and disease. Diabetes, obesity & metabolism 9(3):282-289.
Oh DY, Yoon JM, Moon MJ, Hwang JI, Choe H, Lee JY, Kim JI, Kim S, Rhim H, O'Dell DK, Walker JM, Na HS, Lee MG, Kwon HB, Kim K, Seong JY. 2008. Identification of farnesyl pyrophosphate and N-arachidonylglycine as endogenous ligands for GPR92. The Journal of biological chemistry 283(30):21054-21064.

Ohtsuka T, Sakamoto M, Guillemot F, Kageyama R. 2001. Roles of the basic helix-loop-helix genes Hes1 and Hes5 in expansion of neural stem cells of the developing brain. J Biol Chem 276(32):30467-30474.

Ohtsuki T, Ruetzler CA, Tasaki K, Hallenbeck JM. 1996. Interleukin-1 mediates induction of tolerance to global ischemia in gerbil hippocampal CA1 neurons. Journal of cerebral blood flow and metabolism : official journal of the International Society of Cerebral Blood Flow and Metabolism 16(6):1137-1142.

Okamoto Y, Kihara S, Ouchi N, Nishida M, Arita Y, Kumada M, Ohashi K, Sakai N, Shimomura I, Kobayashi H, Terasaka N, Inaba T, Funahashi T, Matsuzawa Y. 2002. Adiponectin reduces atherosclerosis in apolipoprotein E-deficient mice. Circulation 106(22):2767-2770.

Okuyama S, Makihata N, Yoshimura M, Amakura Y, Yoshida T, Nakajima M, Furukawa Y. 2013. Oenothein B Suppresses Lipopolysaccharide (LPS)-Induced Inflammation in the Mouse Brain. International journal of molecular sciences 14(5):9767-9778.

Olefsky JM, Glass CK. 2010. Macrophages, inflammation, and insulin resistance. Annual review of physiology 72:219-246.

Opara EC. 2006. Oxidative stress. Dis Mon 52(5):183-198.

Oral EA, Simha V, Ruiz E, Andewelt A, Premkumar A, Snell P, Wagner AJ, DePaoli AM, Reitman ML, Taylor SI, Gorden P, Garg A. 2002. Leptin-replacement therapy for lipodystrophy. The New England journal of medicine 346(8):570-578.

Ouchi N, Kihara S, Funahashi T, Matsuzawa Y, Walsh K. 2003. Obesity, adiponectin and vascular inflammatory disease. Current opinion in lipidology 14(6):561-566.

Ouchi N, Ohashi K, Shibata R, Murohara T. 2012. Adipocytokines and obesity-linked disorders. Nagoya journal of medical science 74(1-2):19-30.

Ouchi N, Parker JL, Lugus JJ, Walsh K. 2011. Adipokines in inflammation and metabolic disease. Nature reviews Immunology 11(2):85-97.

Ouchi N, Walsh K. 2008. A novel role for adiponectin in the regulation of inflammation. Arteriosclerosis, thrombosis, and vascular biology 28(7):1219-1221.

Ouyang YB, Xu LJ, Emery JF, Lee AS, Giffard RG. 2011. Overexpressing GRP78 influences Ca2+ handling and function of mitochondria in astrocytes after ischemia-like stress. Mitochondrion 11(2):279-286.

Ozata M, Mergen M, Oktenli C, Aydin A, Sanisoglu SY, Bolu E, Yilmaz MI, Sayal A, Isimer A, Ozdemir IC. 2002. Increased oxidative stress and hypozincemia in male obesity. Clin Biochem 35(8):627-631.

Pajvani UB, Du X, Combs TP, Berg AH, Rajala MW, Schulthess T, Engel J, Brownlee M, Scherer PE. 2003. Structure-function studies of the adipocyte-secreted hormone Acrp30/adiponectin. Implications fpr metabolic regulation and bioactivity. The Journal of biological chemistry 278(11):9073-9085.

Pajvani UB, Scherer PE. 2003. Adiponectin: systemic contributor to insulin sensitivity. Current diabetes reports 3(3):207-213.

Panther E, Idzko M, Corinti S, Ferrari D, Herouy Y, Mockenhaupt M, Dichmann S, Gebicke-Haerter P, Di Virgilio F, Girolomoni G, Norgauer J. 2002. The influence of lysophosphatidic acid on the functions of human dendritic cells. Journal of immunology (Baltimore, Md : 1950) 169(8):4129-4135.

Parakrama Chandrasoma CRT. 2005. The Host Response to Injury - The Acute Inflammatory Response, sub-sectionCardinal Clinical Signs". Concise Pathology, Chapter 3. "Part A. General Pathology, Section II. . McGraw-Hill.

Paresce DM, Chung H, Maxfield FR. 1997. Slow degradation of aggregates of the Alzheimer's disease amyloid beta-protein by microglial cells. The Journal of biological chemistry 272(46):29390-29397.

Parimisetty A, Diotel N, Hellencourt CLd. 2015a. Peripheral immune system signals brain via cytokine signalling.
Parimisetty A, Diotel N, Hellencourt CLd. 2015b. Secret talk between adipose tissue and central nervous system.
Park CR, Seeley RJ, Craft S, Woods SC. 2000. Intracerebroventricular insulin enhances memory in a passive-avoidance task. Physiol Behav 68(4):509-514.
Park HK, Ahima RS. 2013. Resistin in rodents and humans. Diabetes & metabolism journal 37(6):404-414.
Park SJ, Lee JH, Kim HY, Choi YH, Park JS, Suh YH, Park SM, Joe EH, Jou I. 2012. Astrocytes, but not microglia, rapidly sense H(2)O(2)via STAT6 phosphorylation, resulting in cyclooxygenase-2 expression and prostaglandin release. Journal of immunology (Baltimore, Md : 1950) 188(10):5132-5141.
Parr BA, Shea MJ, Vassileva G, McMahon AP. 1993. Mouse Wnt genes exhibit discrete domains of expression in the early embryonic CNS and limb buds. Development 119(1):247-261.
Paz-Filho G, Wong ML, Licinio J. 2010a. Leptin levels and Alzheimer disease. JAMA : the journal of the American Medical Association 303(15):1478; author reply 1478-1479.
Paz-Filho G, Wong ML, Licinio J. 2010b. The procognitive effects of leptin in the brain and their clinical implications. International journal of clinical practice 64(13):1808-1812.
Peferoen L, Kipp M, van der Valk P, van Noort JM, Amor S. 2014. Oligodendrocyte-microglia cross-talk in the central nervous system. Immunology 141(3):302-313.
Pekny M, Nilsson M. 2005. Astrocyte activation and reactive gliosis. Glia 50(4):427-434.
Pellerin L, Magistretti PJ. 1994. Glutamate uptake into astrocytes stimulates aerobic glycolysis: a mechanism coupling neuronal activity to glucose utilization. Proc Natl Acad Sci U S A 91(22):10625-10629.
Pelleymounter MA, Cullen MJ, Baker MB, Hecht R, Winters D, Boone T, Collins F. 1995. Effects of the obese gene product on body weight regulation in ob/ob mice. Science 269(5223):540-543.
Perry VH, Teeling J. 2013. Microglia and macrophages of the central nervous system: the contribution of microglia priming and systemic inflammation to chronic neurodegeneration. Seminars in Immunopathology 35(5):601-612.
Peterson LJ, Flood PM. 2012. Oxidative stress and microglial cells in Parkinson's disease. Mediators of inflammation 2012:401264.
Phillips SA, Kung JT. 2010. Mechanisms of adiponectin regulation and use as a pharmacological target. Current opinion in pharmacology 10(6):676-683.
Piani D, Frei K, Do KQ, Cuenod M, Fontana A. 1991. Murine brain macrophages induced NMDA receptor mediated neurotoxicity in vitro by secreting glutamate. Neuroscience letters 133(2):159-162.
Pickering M, Cumiskey D, O'Connor JJ. 2005. Actions of TNF-alpha on glutamatergic synaptic transmission in the central nervous system. Experimental physiology 90(5):663-670.
Pierre J. Magistretti BRR. 2002. ASTROCYTES. Neuropsychopharmacology.
Pierson E, Simmons SB, Castelli L, Goverman JM. 2012. Mechanisms regulating regional localization of inflammation during CNS autoimmunity. Immunological reviews 248(1):205-215.
Pihl E, Zilmer K, Kullisaar T, Kairane C, Magi A, Zilmer M. 2006. Atherogenic inflammatory and oxidative stress markers in relation to overweight values in male former athletes. Int J Obes (Lond) 30(1):141-146.
Pizzi M, Spano P. 2006. Distinct roles of diverse nuclear factor-κB complexes in neuropathological mechanisms. European Journal of Pharmacology 545(1):22-28.

Plaisance EP, Lukasova M, Offermanns S, Zhang Y, Cao G, Judd RL. 2009. Niacin stimulates adiponectin secretion through the GPR109A receptor. American journal of physiology Endocrinology and metabolism 296(3):E549-558.

Plum L, Schubert M, Bruning JC. 2005. The role of insulin receptor signaling in the brain. Trends in endocrinology and metabolism: TEM 16(2):59-65.

Poirier P, Giles TD, Bray GA, Hong Y, Stern JS, Pi-Sunyer FX, Eckel RH. 2006. Obesity and cardiovascular disease: pathophysiology, evaluation, and effect of weight loss. Arteriosclerosis, thrombosis, and vascular biology 26(5):968-976.

Polfliet MMJ, F. van de Veerdonk, E. A. Dopp, E. M. L. van Kesteren- Hendrikx, N. van Rooijen, C. D. Dijkstra, and T. K. van den Berg. . 2002. . The role of perivascular and meningeal macrophages in experimental allergic encephalomyelitis. . J Neuroimmunol 122:1–8.

Polfliet MMJ, P. J. G. Zwijnenburg, A. M. van Furth, T. van der Poll, E. A. Dopp, C. R. de Lavalette, E. M. L. van Kesteren-Hendrikx, N. van Rooijen, C. D. Dijkstra, and T. K. van den Berg. . 2001. . Meningeal and perivascular macrophages of the central nervous system play a progective role during bacterial meningitis. . J Immunol 167:4644–4650.

Porth C. 2007. Essentials of pahtophysiology: concepts of altered health states. Hagerstown, MD: Lippincott Williams & Wilkins p. 270, .

Powell CL, Swenberg JA, Rusyn I. 2005. Expression of base excision DNA repair genes as a biomarker of oxidative DNA damage. Cancer letters 229(1):1-11.

Pradère JP, Tarnus E, Grès S, Valet P, Saulnier-Blache JS. 2007. Secretion and lysophospholipase D activity of autotaxin by adipocytes are controlled by N-glycosylation and signal peptidase. Biochimica et Biophysica Acta (BBA) - Molecular and Cell Biology of Lipids 1771(1):93-102.

Prakash Shetty rSK, Gary Tin-Choi Ko 2008. Waist Circumference and Waist–Hip Ratio. Report of a WHO Expert Consultation Geneva, .

Pratico D. 2008. Evidence of oxidative stress in Alzheimer's disease brain and antioxidant therapy: lights and shadows. Annals of the New York Academy of Sciences 1147:70-78.

Prehn JH, Backhauss C, Krieglstein J. 1993. Transforming growth factor-beta 1 prevents glutamate neurotoxicity in rat neocortical cultures and protects mouse neocortex from ischemic injury in vivo. Journal of cerebral blood flow and metabolism : official journal of the International Society of Cerebral Blood Flow and Metabolism 13(3):521-525.

Prentice AM. 2006. The emerging epidemic of obesity in developing countries. International journal of epidemiology 35(1):93-99.

Prestwich GD, Gajewiak J, Zhang H, Xu X, Yang G, Serban M. 2008. Phosphatase-resistant analogues of lysophosphatidic acid: agonists promote healing, antagonists and autotaxin inhibitors treat cancer. Biochimica et biophysica acta 1781(9):588-594.

Prousek J. 2007. Fenton chemistry in biology and medicine. Pure and applied chemistry 79(12):2325-2338.

Psilopanagioti A, Papadaki H, Kranioti EF, Alexandrides TK, Varakis JN. 2009. Expression of adiponectin and adiponectin receptors in human pituitary gland and brain. Neuroendocrinology 89(1):38-47.

Qi X, Lin W, Wang D, Pan Y, Wang W, Sun M. 2009. A role for the extracellular signal-regulated kinase signal pathway in depressive-like behavior. Behavioural brain research 199(2):203-209.

Qi Y, Takahashi N, Hileman SM, Patel HR, Berg AH, Pajvani UB, Scherer PE, Ahima RS. 2004. Adiponectin acts in the brain to decrease body weight. Nature medicine 10(5):524-529.

Qin L, Liu Y, Wang T, Wei SJ, Block ML, Wilson B, Liu B, Hong JS. 2004. NADPH oxidase mediates lipopolysaccharide-induced neurotoxicity and pro-inflammatory gene expression in activated microglia. The Journal of biological chemistry 279(2):1415-1421.

Qiu G, Wan R, Hu J, Mattson MP, Spangler E, Liu S, Yau SY, Lee TM, Gleichmann M, Ingram DK, So KF, Zou S. 2011. Adiponectin protects rat hippocampal neurons against excitotoxicity. Age 33(2):155-165.

Quan N, Whiteside M, Kim L, Herkenham M. 1997. Induction of inhibitory factor κBα mRNA in the central nervous system after peripheral lipopolysaccharide administration: An in situ hybridization histochemistry study in the rat. Proceedings of the National Academy of Sciences 94(20):10985-10990.

Raichle ME, Gusnard DA. 2002. Appraising the brain's energy budget. Proceedings of the National Academy of Sciences 99(16):10237-10239.

Raji CA, Ho AJ, Parikshak NN, Becker JT, Lopez OL, Kuller LH, Hua X, Leow AD, Toga AW, Thompson PM. Brain structure and obesity. Hum Brain Mapp 31(3):353-364.

Raji CA, Ho AJ, Parikshak NN, Becker JT, Lopez OL, Kuller LH, Hua X, Leow AD, Toga AW, Thompson PM. 2010. Brain structure and obesity. Hum Brain Mapp 31(3):353-364.

Ramaswamy S, Kordower JH. 2009. Are growth factors the answer? Parkinsonism & related disorders 15 Suppl 3:S176-180.

Ranscht B, Dours-Zimmermann MT. 1991. T-cadherin, a novel cadherin cell adhesion molecule in the nervous system lacks the conserved cytoplasmic region. Neuron 7(3):391-402.

Rao TS, Lariosa-Willingham KD, Lin FF, Palfreyman EL, Yu N, Chun J, Webb M. 2003. Pharmacological characterization of lysophospholipid receptor signal transduction pathways in rat cerebrocortical astrocytes. Brain research 990(1-2):182-194.

Rappold PM, Tieu K. 2010a. Astrocytes and therapeutics for Parkinson's disease. Neurotherapeutics : the journal of the American Society for Experimental NeuroTherapeutics 7(4):413-423.

Rappold PM, Tieu K. 2010b. Astrocytes and Therapeutics for Parkinson's Disease. Neurotherapeutics : the journal of the American Society for Experimental NeuroTherapeutics 7(4):413-423.

Rather LJ. 1971. Disturbance of function (functio laesa): the legendary fifth cardinal sign of inflammation, added by Galen to the four cardinal signs of Celsus. Bulletin of the New York Academy of Medicine 47(3):303-322.

Raza MW, Shad A, Pedler SJ, Karamat KA. 2005. Penetration and activity of antibiotics in brain abscess. J Coll Physicians Surg Pak 15(3):165-167.

Reaven GM. 1998. Insulin resistance and human disease: a short history. Journal of basic and clinical physiology and pharmacology 9(2-4):387-406.

Reddy BN. 1998. Body mass index and its association with socioeconomic and behavioral variables among socioeconomically heterogeneous populations of Andhra Pradesh, India. Human biology 70(5):901-917.

Reeves GK, Pirie K, Beral V, Green J, Spencer E, Bull D. 2007. Cancer incidence and mortality in relation to body mass index in the Million Women Study: cohort study. 1134 p.

Reichenberg A, Yirmiya R, Schuld A, et al. 2001. CYtokine-associated emotional and cognitive disturbances in humans. Archives of general psychiatry 58(5):445-452.

Reisberg B, Burns A, Brodaty H, Eastwood R, Rossor M, Sartorius N, Winblad B. 1997. Diagnosis of Alzheimer's disease. Report of an International Psychogeriatric Association Special Meeting Work Group under the cosponsorship of Alzheimer's Disease International, the European Federation of Neurological Societies, the World

Health Organization, and the World Psychiatric Association. International psychogeriatrics / IPA 9 Suppl 1:11-38.
Relton JK, Rothwell NJ. 1992. Interleukin-1 receptor antagonist inhibits ischaemic and excitotoxic neuronal damage in the rat. Brain research bulletin 29(2):243-246.
Remy F, Vayssiere N, Saint-Aubert L, Barbeau E, Pariente J. 2015. White matter disruption at the prodromal stage of Alzheimer's disease: Relationships with hippocampal atrophy and episodic memory performance. NeuroImage Clinical 7:482-492.
Repunte-Canonigo V, Berton F, Cottone P, Reifel-Miller A, Roberts AJ, Morales M, Francesconi W, Sanna PP. 2010. A potential role for adiponectin receptor 2 (AdipoR2) in the regulation of alcohol intake. Brain research 1339:11-17.
Reuhl KR, Smallridge EA, Chang LW, Mackenzie BA. 1983. Developmental effects of trimethyltin intoxication in the neonatal mouse. I. Light microscopic studies. Neurotoxicology 4(1):19-28.
Ricardo Cabezas MFA, Daniel Torrente, Ramon Santos El-Bachá, Ludis Morales, Janneth Gonzalez and George E. Barreto, Dr. Uday Kishore, InTech,. 2013. Astrocytes Role in Parkinson: A Double-Edged Sword, Neurodegenerative Diseases.
Richards AA, Colgrave ML, Zhang J, Webster J, Simpson F, Preston E, Wilks D, Hoehn KL, Stephenson M, Macdonald GA, Prins JB, Cooney GJ, Xu A, Whitehead JP. 2010. Sialic acid modification of adiponectin is not required for multimerization or secretion but determines half-life in circulation. Molecular endocrinology (Baltimore, Md) 24(1):229-239.
Richards AA, Stephens T, Charlton HK, Jones A, Macdonald GA, Prins JB, Whitehead JP. 2006. Adiponectin multimerization is dependent on conserved lysines in the collagenous domain: evidence for regulation of multimerization by alterations in posttranslational modifications. Molecular endocrinology (Baltimore, Md) 20(7):1673-1687.
Richardson KRJaWD. 1997. Glial Cell Development: basic principles and clinical relevance. Oxford Scholarship Online, ISBN-13: 9781872748542.
Ridet JL, Malhotra SK, Privat A, Gage FH. 1997. Reactive astrocytes: cellular and molecular cues to biological function. Trends in neurosciences 20(12):570-577.
Rio-Hortega PD. 1919. The third element of the nerve centers I The microglia in normal microglia II Intervention in Nature III probable pathological processes of microglia. Bol de la Soc esp de biol 9:69-120.
Rio-Hortega PD. 1932a. Microglia. Cytology and Cellular Pathology of the Nervous System, Edited by Penfield W New York: Hoeber:482–1924–1534.
Rio-Hortega PD. 1932b. Microglia. In: Cytology and Cellular Pathology of the Nervous System, Edited by Penfield W. New York: Hoeber.482–1924–1534.
Riquelme R, Miralles CP, De Blas AL. 2002. Bergmann glia GABA(A) receptors concentrate on the glial processes that wrap inhibitory synapses. The Journal of neuroscience : the official journal of the Society for Neuroscience 22(24):10720-10730.
Ritter MR, Banin E, Moreno SK, Aguilar E, Dorrell MI, Friedlander M. 2006. Myeloid progenitors differentiate into microglia and promote vascular repair in a model of ischemic retinopathy. The Journal of clinical investigation 116(12):3266-3276.
Rocha VZ, Libby P. 2009. Obesity, inflammation, and atherosclerosis. Nature reviews Cardiology 6(6):399-409.
Roche R, Hoareau L, Mounet F, Festy F. 2007. Adult stem cells for cardiovascular diseases: the adipose tissue potential. Expert opinion on biological therapy 7(6):791-798.
Rock KL, Kono H. 2008. The inflammatory response to cell death. Annual review of pathology 3:99-126.

Rock RB, Gekker G, Hu S, Sheng WS, Cheeran M, Lokensgard JR, Peterson PK. 2004a. Role of Microglia in Central Nervous System Infections. Clinical microbiology reviews 17(4):942-964.
Rock RB, Gekker G, Hu S, Sheng WS, Cheeran M, Lokensgard JR, Peterson PK. 2004b. Role of microglia in central nervous system infections. Clinical microbiology reviews 17(4):942-964, table of contents.
Rodriguez-Rodriguez A, Egea-Guerrero JJ, Murillo-Cabezas F, Carrillo-Vico A. 2014. Oxidative stress in traumatic brain injury. Current medicinal chemistry 21(10):1201-1211.
Roglic G, Unwin N, Bennett PH, Mathers C, Tuomilehto J, Nag S, Connolly V, King H. 2005. The burden of mortality attributable to diabetes: realistic estimates for the year 2000. Diabetes care 28(9):2130-2135.
Rojo LE, Fernandez JA, Maccioni AA, Jimenez JM, Maccioni RB. 2008. Neuroinflammation: implications for the pathogenesis and molecular diagnosis of Alzheimer's disease. Arch Med Res 39(1):1-16.
Rosenberg GA, Sullivan N, Esiri MM. 2001. White matter damage is associated with matrix metalloproteinases in vascular dementia. Stroke 32(5):1162-1168.
Rosengren LE, Lycke J, Andersen O. 1995. Glial fibrillary acidic protein in CSF of multiple sclerosis patients: relation to neurological deficit. J Neurol Sci 133(1-2):61-65.
Rosengren LE, Wikkelso C, Hagberg L. 1994. A sensitive ELISA for glial fibrillary acidic protein: application in CSF of adults. J Neurosci Methods 51(2):197-204.
Rosenthal TC, Khotianov N. 2003. Managing Alzheimer dementia tomorrow. The Journal of the American Board of Family Practice / American Board of Family Practice 16(5):423-434.
Rowe LA, Degtyareva N, Doetsch PW. 2008. DNA damage-induced reactive oxygen species (ROS) stress response in Saccharomyces cerevisiae. Free radical biology & medicine 45(8):1167-1177.
Roy A, Jana A, Yatish K, Freidt MB, Fung YK, Martinson JA, Pahan K. 2008. Reactive oxygen species up-regulate CD11b in microglia via nitric oxide: Implications for neurodegenerative diseases. Free radical biology & medicine 45(5):686-699.
Rozin P, Kabnick K, Pete E, Fischler C, Shields C. 2003. The ecology of eating: smaller portion sizes in France Than in the United States help explain the French paradox. Psychological science 14(5):450-454.
Ruocco A, Nicole O, Docagne F, Ali C, Chazalviel L, Komesli S, Yablonsky F, Roussel S, MacKenzie ET, Vivien D, Buisson A. 1999. A transforming growth factor-beta antagonist unmasks the neuroprotective role of this endogenous cytokine in excitotoxic and ischemic brain injury. Journal of cerebral blood flow and metabolism : official journal of the International Society of Cerebral Blood Flow and Metabolism 19(12):1345-1353.
Ruszkiewicz J, Albrecht J. 2015. Changes in the mitochondrial antioxidant systems in neurodegenerative diseases and acute brain disorders. Neurochemistry international.
Ryter SW, Kim HP, Hoetzel A, Park JW, Nakahira K, Wang X, Choi AM. 2007. Mechanisms of cell death in oxidative stress. Antioxidants & redox signaling 9(1):49-89.
Sabia S, Kivimaki M, Shipley MJ, Marmot MG, Singh-Manoux A. 2009. Body mass index over the adult life course and cognition in late midlife: the Whitehall II Cohort Study. Am J Clin Nutr 89(2):601-607.
Sahin-Efe A, Katsikeris F, Mantzoros CS. 2012. Advances in adipokines. Metabolism 61(12):1659-1665.

Salbe AD, Weyer C, Lindsay RS, Ravussin E, Tataranni PA. 2002. Assessing risk factors for obesity between childhood and adolescence: I. Birth weight, childhood adiposity, parental obesity, insulin, and leptin. Pediatrics 110(2 Pt 1):299-306.
Saper CB, Breder CD. 1992. Endogenous pyrogens in the CNS: role in the febrile response. Progress in brain research 93:419-428; discussion 428-419.
Sapp E, Kegel KB, Aronin N, Hashikawa T, Uchiyama Y, Tohyama K, Bhide PG, Vonsattel JP, DiFiglia M. 2001. Early and progressive accumulation of reactive microglia in the Huntington disease brain. Journal of neuropathology and experimental neurology 60(2):161-172.
Sato K, Malchinkhuu E, Muraki T, Ishikawa K, Hayashi K, Tosaka M, Mochiduki A, Inoue K, Tomura H, Mogi C, Nochi H, Tamoto K, Okajima F. 2005. Identification of autotaxin as a neurite retraction-inducing factor of PC12 cells in cerebrospinal fluid and its possible sources. Journal of neurochemistry 92(4):904-914.
Sato Y, Ohshima T, Kondo T. 1999. Regulatory role of endogenous interleukin-10 in cutaneous inflammatory response of murine wound healing. Biochemical and biophysical research communications 265(1):194-199.
Saulnier-Blache JS. 2004. [Lysophosphatidic acid: a "bioactive" phospholipid]. Medecine sciences : M/S 20(8-9):799-803.
Savaskan NE, Rocha L, Kotter MR, Baer A, Lubec G, van Meeteren LA, Kishi Y, Aoki J, Moolenaar WH, Nitsch R, Brauer AU. 2007. Autotaxin (NPP-2) in the brain: cell type-specific expression and regulation during development and after neurotrauma. Cellular and molecular life sciences : CMLS 64(2):230-243.
Savino F, Liguori SA. 2008. Update on breast milk hormones: leptin, ghrelin and adiponectin. Clinical nutrition (Edinburgh, Scotland) 27(1):42-47.
Savino F, Liguori SA, Lupica MM. 2010. Adipokines in breast milk and preterm infants. Early human development 86 Suppl 1:77-80.
Scherer PE, Williams S, Fogliano M, Baldini G, Lodish HF. 1995. A novel serum protein similar to C1q, produced exclusively in adipocytes. The Journal of biological chemistry 270(45):26746-26749.
Schilling T, Repp H, Richter H, Koschinski A, Heinemann U, Dreyer F, Eder C. 2002. Lysophospholipids induce membrane hyperpolarization in microglia by activation of IKCa1 Ca(2+)-dependent K(+) channels. Neuroscience 109(4):827-835.
Schilling T, Stock C, Schwab A, Eder C. 2004. Functional importance of Ca2+-activated K+ channels for lysophosphatidic acid-induced microglial migration. The European journal of neuroscience 19(6):1469-1474.
Schleimer RP. 1988. Inflammation: Basic principles and clinical correlates edited by John Gallin, Ira Goldstein and Ralph Snyderman, Raven Press, 1987. $219.00 (xvii + 995 pages) ISBN 0 88167 344 7. Immunol Today 9(10):327.
Schmechel DE, Rakic P. 1979. A Golgi study of radial glial cells in developing monkey telencephalon: morphogenesis and transformation into astrocytes. Anatomy and embryology 156(2):115-152.
Schraw T, Wang ZV, Halberg N, Hawkins M, Scherer PE. 2008. Plasma adiponectin complexes have distinct biochemical characteristics. Endocrinology 149(5):2270-2282.
Segal AW. 2005. How neutrophils kill microbes. Annual review of immunology 23:197-223.
Selmaj K, Raine CS. 1988. Tumor necrosis factor mediates myelin damage in organotypic cultures of nervous tissue. Annals of the New York Academy of Sciences 540:568-570.
Semchyshyn VILaHM. 2012. Oxidative Stress - Molecular Mechanisms and Biological Effects, Introductory Chapter. INTECH open science - open minds ISBN: 978-953-51-0554-1, InTech, DOI: 10.5772/39292.

Serhan CN. 2008. Controlling the resolution of acute inflammation: a new genus of dual anti-inflammatory and proresolving mediators. Journal of periodontology 79(8 Suppl):1520-1526.
Shano S, Moriyama R, Chun J, Fukushima N. 2008. Lysophosphatidic acid stimulates astrocyte proliferation through LPA1. Neurochemistry international 52(1-2):216-220.
Shigetomi E, Bushong EA, Haustein MD, Tong X, Jackson-Weaver O, Kracun S, Xu J, Sofroniew MV, Ellisman MH, Khakh BS. 2013. Imaging calcium microdomains within entire astrocyte territories and endfeet with GCaMPs expressed using adeno-associated viruses. The Journal of general physiology 141(5):633-647.
Shinozaki Y, Nomura M, Iwatsuki K, Moriyama Y, Gachet C, Koizumi S. 2014. Microglia trigger astrocyte-mediated neuroprotection via purinergic gliotransmission. Scientific reports 4:4329.
Sidhu K. 2006. Incidence of Overweight and Obesity among Urban and Rural Males of Amritsar. Journal of Exercise Science and Physiotherapy 2:79-83.
Silswal N, Singh AK, Aruna B, Mukhopadhyay S, Ghosh S, Ehtesham NZ. 2005. Human resistin stimulates the pro-inflammatory cytokines TNF-alpha and IL-12 in macrophages by NF-kappaB-dependent pathway. Biochemical and biophysical research communications 334(4):1092-1101.
Slemmer JE, Shacka JJ, Sweeney MI, Weber JT. 2008. Antioxidants and free radical scavengers for the treatment of stroke, traumatic brain injury and aging. Current medicinal chemistry 15(4):404-414.
Smith ME. 2001. Phagocytic properties of microglia in vitro: implications for a role in multiple sclerosis and EAE. Microscopy research and technique 54(2):81-94.
Snel M, Jonker JT, Schoones J, Lamb H, de Roos A, Pijl H, Smit JW, Meinders AE, Jazet IM. 2012. Ectopic fat and insulin resistance: pathophysiology and effect of diet and lifestyle interventions. International journal of endocrinology 2012:983814.
Sofroniew MV. 2005. Reactive astrocytes in neural repair and protection. The Neuroscientist : a review journal bringing neurobiology, neurology and psychiatry 11(5):400-407.
Sofroniew MV. 2009. Molecular dissection of reactive astrogliosis and glial scar formation. Trends in neurosciences 32(12):638-647.
Sofroniew MV. 2014. Astrogliosis. Cold Spring Harbor perspectives in biology 7(2).
Sofroniew MV, Vinters HV. 2010. Astrocytes: biology and pathology. Acta neuropathologica 119(1):7-35.
Sokolowski JD, Mandell JW. 2011. Phagocytic Clearance in Neurodegeneration. The American journal of pathology 178(4):1416-1428.
Solito E, Sastre M. 2012a. Microglia function in Alzheimer's disease. Frontiers in Pharmacology 3.
Solito E, Sastre M. 2012b. Microglia Function in Alzheimer's Disease. Frontiers in Pharmacology 3:14.
Somogyi V, Gyorffy A, Scalise TJ, Kiss DS, Goszleth G, Bartha T, Frenyo VL, Zsarnovszky A. 2011. Endocrine factors in the hypothalamic regulation of food intake in females: a review of the physiological roles and interactions of ghrelin, leptin, thyroid hormones, oestrogen and insulin. Nutrition research reviews 24(1):132-154.
Song J, Lee JE. 2013. Adiponectin as a new paradigm for approaching Alzheimer's disease. Anatomy & cell biology 46(4):229-234.
Sorensen SD, Nicole O, Peavy RD, Montoya LM, Lee CJ, Murphy TJ, Traynelis SF, Hepler JR. 2003. Common signaling pathways link activation of murine PAR-1, LPA, and S1P receptors to proliferation of astrocytes. Molecular pharmacology 64(5):1199-1209.
Soulet D, Rivest S. 2008a. Microglia. Current biology : CB 18(12):R506-508.
Soulet D, Rivest S. 2008b. Microglia. Current Biology 18(12):R506-R508.

Spranger J, Kroke A, Mohlig M, Bergmann MM, Ristow M, Boeing H, Pfeiffer AF. 2003. Adiponectin and protection against type 2 diabetes mellitus. Lancet 361(9353):226-228.
Spranger J, Verma S, Gohring I, Bobbert T, Seifert J, Sindler AL, Pfeiffer A, Hileman SM, Tschop M, Banks WA. 2006. Adiponectin does not cross the blood-brain barrier but modifies cytokine expression of brain endothelial cells. Diabetes 55(1):141-147.
Stefan N, Stumvoll M. 2002. Adiponectin--its role in metabolism and beyond. Hormone and metabolic research = Hormon- und Stoffwechselforschung = Hormones et metabolisme 34(9):469-474.
Stein J, Schettler T, Rohrer B, Valenti M. 2008. Environmental Threats to Healthy Aging - With a Closer Look at Alzheimer's & Parkinson's Diseases. Greater Boston Physicians for Social Responsibility and Science and Environmental Health Network.
Stitt JT. 1986. Prostaglandin E as the neural mediator of the febrile response. The Yale journal of biology and medicine 59(2):137-149.
Stracke ML, Krutzsch HC, Unsworth EJ, Arestad A, Cioce V, Schiffmann E, Liotta LA. 1992. Identification, purification, and partial sequence analysis of autotaxin, a novel motility-stimulating protein. The Journal of biological chemistry 267(4):2524-2529.
Streit WJ. 1995. Microglial cells. Neuroglia, In H Kettenmann and B R Ransom (ed), Oxford University Press, New York, NY:p. 85–96. .
Streit WJ. 2006. Microglial senescence: does the brain's immune system have an expiration date? Trends in neurosciences 29(9):506-510.
Streit WJ, Sparks DL. 1997. Activation of microglia in the brains of humans with heart disease and hypercholesterolemic rabbits. Journal of molecular medicine (Berlin, Germany) 75(2):130-138.
Strijbos PJ, Rothwell NJ. 1995. Interleukin-1 beta attenuates excitatory amino acid-induced neurodegeneration in vitro: involvement of nerve growth factor. The Journal of neuroscience : the official journal of the Society for Neuroscience 15(5 Pt 1):3468-3474.
Suh JM, Zeve D, McKay R, Seo J, Salo Z, Li R, Wang M, Graff JM. 2007. Adipose is a conserved dosage-sensitive antiobesity gene. Cell metabolism 6(3):195-207.
Sun S, Ji Y, Kersten S, Qi L. 2012. Mechanisms of inflammatory responses in obese adipose tissue. Annual review of nutrition 32:261-286.
Suzuki S, Wilson-Kubalek EM, Wert D, Tsao TS, Lee DH. 2007. The oligomeric structure of high molecular weight adiponectin. FEBS letters 581(5):809-814.
Svetlov SI, Ignatova TN, Wang KK, Hayes RL, English D, Kukekov VG. 2004. Lysophosphatidic acid induces clonal generation of mouse neurospheres via proliferation of Sca-1- and AC133-positive neural progenitors. Stem cells and development 13(6):685-693.
Takeda S, Sato N, Morishita R. 2014. Systemic inflammation, blood-brain barrier vulnerability and cognitive/non-cognitive symptoms in Alzheimer disease: relevance to pathogenesis and therapy. Frontiers in aging neuroscience 6:171.
Taki Y, Kinomura S, Sato K, Inoue K, Goto R, Okada K, Uchida S, Kawashima R, Fukuda H. 2008. Relationship between body mass index and gray matter volume in 1,428 healthy individuals. Obesity (Silver Spring) 16(1):119-124.
Tamatani M, Che YH, Matsuzaki H, Ogawa S, Okado H, Miyake S-i, Mizuno T, Tohyama M. 1999. Tumor Necrosis Factor Induces Bcl-2 and Bcl-x Expression through NFκB Activation in Primary Hippocampal Neurons. Journal of Biological Chemistry 274(13):8531-8538.
Tanaka J, Toku K, Zhang B, Ishihara K, Sakanaka M, Maeda N. 1999. Astrocytes prevent neuronal death induced by reactive oxygen and nitrogen species. Glia 28(2):85-96.

Tanaka M, Okudaira S, Kishi Y, Ohkawa R, Iseki S, Ota M, Noji S, Yatomi Y, Aoki J, Arai H. 2006. Autotaxin stabilizes blood vessels and is required for embryonic vasculature by producing lysophosphatidic acid. The Journal of biological chemistry 281(35):25822-25830.

Tang P, Hung MC, Klostergaard J. 1994. TNF cytotoxicity: effects of HER-2/neu expression and inhibitors of ADP-ribosylation. Lymphokine and cytokine research 13(2):117-123.

Tang YT, Hu T, Arterburn M, Boyle B, Bright JM, Emtage PC, Funk WD. 2005. PAQR proteins: a novel membrane receptor family defined by an ancient 7-transmembrane pass motif. Journal of molecular evolution 61(3):372-380.

Tani H, Dulla Chris G, Farzampour Z, Taylor-Weiner A, Huguenard John R, Reimer Richard J. 2014. A Local Glutamate-Glutamine Cycle Sustains Synaptic Excitatory Transmitter Release. Neuron 81(4):888-900.

Tartaglia LA. 1997. The leptin receptor. The Journal of biological chemistry 272(10):6093-6096.

Tartaglia LA, Dembski M, Weng X, Deng N, Culpepper J, Devos R, Richards GJ, Campfield LA, Clark FT, Deeds J, Muir C, Sanker S, Moriarty A, Moore KJ, Smutko JS, Mays GG, Wool EA, Monroe CA, Tepper RI. 1995a. Identification and expression cloning of a leptin receptor, OB-R. Cell 83(7):1263-1271.

Tartaglia LA, Dembski M, Weng X, Deng N, Culpepper J, Devos R, Richards GJ, Campfield LA, Clark FT, Deeds J, Muir C, Sanker S, Moriarty A, Moore KJ, Smutko JS, Mays GG, Wool EA, Monroe CA, Tepper RI. 1995b. Identification and expression cloning of a leptin receptor, OB-R. Cell 83(7):1263-1271.

Taylor DL, Diemel LT, Pocock JM. 2003. Activation of microglial group III metabotropic glutamate receptors protects neurons against microglial neurotoxicity. The Journal of neuroscience : the official journal of the Society for Neuroscience 23(6):2150-2160.

Teeling JL, Cunningham C, Newman TA, Perry VH. 2010. The effect of non-steroidal anti-inflammatory agents on behavioural changes and cytokine production following systemic inflammation: Implications for a role of COX-1. Brain, behavior, and immunity 24(3):409-419.

Teeling JL, Felton LM, Deacon RM, Cunningham C, Rawlins JN, Perry VH. 2007. Sub-pyrogenic systemic inflammation impacts on brain and behavior, independent of cytokines. Brain, behavior, and immunity 21(6):836-850.

Thaler JP, Yi CX, Schur EA, Guyenet SJ, Hwang BH, Dietrich MO, Zhao X, Sarruf DA, Izgur V, Maravilla KR, Nguyen HT, Fischer JD, Matsen ME, Wisse BE, Morton GJ, Horvath TL, Baskin DG, Tschop MH, Schwartz MW. 2012. Obesity is associated with hypothalamic injury in rodents and humans. The Journal of clinical investigation 122(1):153-162.

Tham CS, Lin FF, Rao TS, Yu N, Webb M. 2003. Microglial activation state and lysophospholipid acid receptor expression. International journal of developmental neuroscience : the official journal of the International Society for Developmental Neuroscience 21(8):431-443.

Thomas CG, Tian H, Diamond JS. 2011. The relative roles of diffusion and uptake in clearing synaptically released glutamate change during early postnatal development. The Journal of neuroscience : the official journal of the Society for Neuroscience 31(12):4743-4754.

Thomas WE. 1999. Brain macrophages: on the role of pericytes and perivascular cells. Brain research Brain research reviews 31(1):42-57.

Thornton P, Pinteaux E, Gibson RM, Allan SM, Rothwell NJ. 2006. Interleukin-1-induced neurotoxicity is mediated by glia and requires caspase activation and free radical release. Journal of neurochemistry 98(1):258-266.

Thundyil J, Pavlovski D, Sobey CG, Arumugam TV. 2012a. Adiponectin receptor signalling in the brain. British Journal of Pharmacology 165(2):313-327.
Thundyil J, Pavlovski D, Sobey CG, Arumugam TV. 2012b. Adiponectin receptor signalling in the brain. Br J Pharmacol 165(2):313-327.
Thundyil J, Tang SC, Okun E, Shah K, Karamyan VT, Li YI, Woodruff TM, Taylor SM, Jo DG, Mattson MP, Arumugam TV. 2010. Evidence that adiponectin receptor 1 activation exacerbates ischemic neuronal death. Experimental & translational stroke medicine 2(1):15.
Tiffany-Castiglion E, Qian Y. 2001. Astroglia as metal depots: molecular mechanisms for metal accumulation, storage and release. Neurotoxicology 22(5):577-592.
Tikka TM, Koistinaho JE. 2001. Minocycline provides neuroprotection against N-methyl-D-aspartate neurotoxicity by inhibiting microglia. Journal of immunology (Baltimore, Md : 1950) 166(12):7527-7533.
Tilg H, Moschen AR. 2006. Adipocytokines: mediators linking adipose tissue, inflammation and immunity. Nat Rev Immunol 6(10):772-783.
Tilg H, Moschen AR. 2008. Role of adiponectin and PBEF/visfatin as regulators of inflammation: involvement in obesity-associated diseases. Clinical science (London, England : 1979) 114(4):275-288.
Tobin MK, Bonds JA, Minshall RD, Pelligrino DA, Testai FD, Lazarov O. 2014. Neurogenesis and inflammation after ischemic stroke: what is known and where we go from here. Journal of cerebral blood flow and metabolism : official journal of the International Society of Cerebral Blood Flow and Metabolism 34(10):1573-1584.
Tokumura A. 2004. Metabolic pathways and physiological and pathological significances of lysolipid phosphate mediators. Journal of cellular biochemistry 92(5):869-881.
Tokumura A, Majima E, Kariya Y, Tominaga K, Kogure K, Yasuda K, Fukuzawa K. 2002. Identification of human plasma lysophospholipase D, a lysophosphatidic acid-producing enzyme, as autotaxin, a multifunctional phosphodiesterase. The Journal of biological chemistry 277(42):39436-39442.
Tower DB NM, Hertz L, and Schousboe A,. 1988. Development of knowledge about astrocytes since Virchow. In: The Biochemical Pathology of Astrocytes,. The Biochemical Pathology of Astrocytes, Alan R Liss, New York, NY, :3-18.
Tran TT, Kahn CR. 2010. Transplantation of adipose tissue and stem cells: role in metabolism and disease. Nature reviews Endocrinology 6(4):195-213.
Trayhurn P, Beattie JH. 2001. Physiological role of adipose tissue: white adipose tissue as an endocrine and secretory organ. The Proceedings of the Nutrition Society 60(3):329-339.
Trayhurn P WI. 2004. Adipokines: inflammation and the pleiotropic role of white adipose tissue. Br J Nutr 92:347.
Trayhurn P, Wood IS. 2004. Adipokines: inflammation and the pleiotropic role of white adipose tissue. The British journal of nutrition 92(3):347-355.
Trendelenburg G, Dirnagl U. 2005. Neuroprotective role of astrocytes in cerebral ischemia: focus on ischemic preconditioning. Glia 50(4):307-320.
Tretter L, Adam-Vizi V. 2004. Generation of reactive oxygen species in the reaction catalyzed by alpha-ketoglutarate dehydrogenase. The Journal of neuroscience : the official journal of the Society for Neuroscience 24(36):7771-7778.
Trogdon JG, Finkelstein EA, Feagan CW, Cohen JW. 2012. State- and payer-specific estimates of annual medical expenditures attributable to obesity. Obesity (Silver Spring, Md) 20(1):214-220.
Trujillo ME SP. 2005. Adiponectin-journey from an adipocyte secretory protein to biomarker of the metabolic syndrome. J Intern Med 257:167.

Tsao TS, Murrey HE, Hug C, Lee DH, Lodish HF. 2002. Oligomerization state-dependent activation of NF-kappa B signaling pathway by adipocyte complement-related protein of 30 kDa (Acrp30). The Journal of biological chemistry 277(33):29359-29362.

Turtzo LC, Lane MD. 2002. Completing the loop: neuron-adipocyte interactions and the control of energy homeostasis. Hormone and metabolic research = Hormon- und Stoffwechselforschung = Hormones et metabolisme 34(11-12):607-615.

Ujiie M, Dickstein DL, Carlow DA, Jefferies WA. 2003. Blood-brain barrier permeability precedes senile plaque formation in an Alzheimer disease model. Microcirculation 10(6):463-470.

Umemura K, Yamashita N, Yu X, Arima K, Asada T, Makifuchi T, Murayama S, Saito Y, Kanamaru K, Goto Y, Kohsaka S, Kanazawa I, Kimura H. 2006. Autotaxin expression is enhanced in frontal cortex of Alzheimer-type dementia patients. Neuroscience letters 400(1-2):97-100.

Umezu-Goto M, Kishi Y, Taira A, Hama K, Dohmae N, Takio K, Yamori T, Mills GB, Inoue K, Aoki J, Arai H. 2002. Autotaxin has lysophospholipase D activity leading to tumor cell growth and motility by lysophosphatidic acid production. J Cell Biol 158(2):227-233.

Une K, Takei YA, Tomita N, Asamura T, Ohrui T, Furukawa K, Arai H. 2011. Adiponectin in plasma and cerebrospinal fluid in MCI and Alzheimer's disease. European journal of neurology : the official journal of the European Federation of Neurological Societies 18(7):1006-1009.

Uwechue NM, Marx MC, Chevy Q, Billups B. 2012. Activation of glutamate transport evokes rapid glutamine release from perisynaptic astrocytes. The Journal of physiology 590(Pt 10):2317-2331.

Uysal KT, Wiesbrock SM, Marino MW, Hotamisligil GS. 1997. Protection from obesity-induced insulin resistance in mice lacking TNF-alpha function. Nature 389(6651):610-614.

Vaccarino FM, Fagel DM, Ganat Y, Maragnoli ME, Ment LR, Ohkubo Y, Schwartz ML, Silbereis J, Smith KM. 2007. Astroglial cells in development, regeneration, and repair. The Neuroscientist : a review journal bringing neurobiology, neurology and psychiatry 13(2):173-185.

Vachharajani V, Granger DN. 2009. Adipose tissue: a motor for the inflammation associated with obesity. IUBMB Life 61(4):424-430.

Valko M, Rhodes CJ, Moncol J, Izakovic M, Mazur M. 2006. Free radicals, metals and antioxidants in oxidative stress-induced cancer. Chemico-biological interactions 160(1):1-40.

van de Beek D, de Gans J, McIntyre P, Prasad K. 2003. Corticosteroids in acute bacterial meningitis. The Cochrane database of systematic reviews(3):Cd004405.

van der Heide LP, Ramakers GM, Smidt MP. 2006. Insulin signaling in the central nervous system: learning to survive. Progress in neurobiology 79(4):205-221.

van der Marck MA, Dicke HC, Uc EY, Kentin ZH, Borm GF, Bloem BR, Overeem S, Munneke M. 2012. Body mass index in Parkinson's disease: a meta-analysis. Parkinsonism & related disorders 18(3):263-267.

Van Gaal LF, Mertens IL, De Block CE. 2006. Mechanisms linking obesity with cardiovascular disease. Nature 444(7121):875-880.

van Meeteren LA, Brinkmann V, Saulnier-Blache JS, Lynch KR, Moolenaar WH. 2008. Anticancer activity of FTY720: phosphorylated FTY720 inhibits autotaxin, a metastasis-enhancing and angiogenic lysophospholipase D. Cancer letters 266(2):203-208.

van Meeteren LA, Ruurs P, Stortelers C, Bouwman P, van Rooijen MA, Pradere JP, Pettit TR, Wakelam MJ, Saulnier-Blache JS, Mummery CL, Moolenaar WH, Jonkers J. 2006.

Autotaxin, a secreted lysophospholipase D, is essential for blood vessel formation during development. Molecular and cellular biology 26(13):5015-5022.
Vaughan DE. 2005. PAI-1 and atherothrombosis. Journal of Thrombosis and Haemostasis 3(8):1879-1883.
Veerhuis R, Nielsen HM, Tenner AJ. 2011. Complement in the brain. Molecular immunology 48(14):1592-1603.
Vendrell J, Broch M, Vilarrasa N, Molina A, Gomez JM, Gutierrez C, Simon I, Soler J, Richart C. 2004. Resistin, adiponectin, ghrelin, leptin, and pro-inflammatory cytokines: relationships in obesity. Obesity research 12(6):962-971.
Venkatesh B, Hickman I, Nisbet J, Cohen J, Prins J. 2009. Changes in serum adiponectin concentrations in critical illness: a preliminary investigation. Critical care 13(4):R105.
Versini M, Jeandel PY, Rosenthal E, Shoenfeld Y. 2014. Obesity in autoimmune diseases: not a passive bystander. Autoimmunity reviews 13(9):981-1000.
Vincent HK, Vincent KR, Bourguignon C, Braith RW. 2005. Obesity and postexercise oxidative stress in older women. Med Sci Sports Exerc 37(2):213-219.
Vincent VA, Tilders FJ, Van Dam AM. 1997. Inhibition of endotoxin-induced nitric oxide synthase production in microglial cells by the presence of astroglial cells: a role for transforming growth factor beta. Glia 19(3):190-198.
Virchow R, Die cellularpathologie in ihrer begründung auf physiologische und pathologische gewebelehre. A. Hirschwald; Berlin: 1858. 1858. Die cellularpathologie in ihrer begründung auf physiologische und pathologische gewebelehre. A. Hirschwald; Berlin: .
Vives-Bauza C, Przedborski S. 2011. Mitophagy: the latest problem for Parkinson's disease. Trends in molecular medicine 17(3):158-165.
Vogel WHB, Andreas. 2009. Brief History of Vision and Ocular Medicine. Kugler Publications. p p. 97. ISBN 90-6299-6220-X.
Voloboueva LA, Suh SW, Swanson RA, Giffard RG. 2007. Inhibition of Mitochondrial Function in Astrocytes: Implications for Neuroprotection. Journal of neurochemistry 102(4):1383-1394.
Vong L, Ye C, Yang Z, Choi B, Chua S, Jr., Lowell BB. 2011. Leptin action on GABAergic neurons prevents obesity and reduces inhibitory tone to POMC neurons. Neuron 71(1):142-154.
Vos PE, Lamers KJ, Hendriks JC, van Haaren M, Beems T, Zimmerman C, van Geel W, de Reus H, Biert J, Verbeek MM. 2004. Glial and neuronal proteins in serum predict outcome after severe traumatic brain injury. Neurology 62(8):1303-1310.
Waites CL, Craig AM, Garner CC. 2005. Mechanisms of vertebrate synaptogenesis. Annual review of neuroscience 28:251-274.
Waki H, Tontonoz P. 2007a. Endocrine functions of adipose tissue. Annu Rev Pathol 2:31-56.
Waki H, Tontonoz P. 2007b. Endocrine Functions of Adipose Tissue. Annual Review of Pathology: Mechanisms of Disease 2(1):31-56.
Waki H, Yamauchi T, Kamon J, Ito Y, Uchida S, Kita S, Hara K, Hada Y, Vasseur F, Froguel P, Kimura S, Nagai R, Kadowaki T. 2003. Impaired multimerization of human adiponectin mutants associated with diabetes. Molecular structure and multimer formation of adiponectin. The Journal of biological chemistry 278(41):40352-40363.
Wakselman S, Bechade C, Roumier A, Bernard D, Triller A, Bessis A. 2008. Developmental neuronal death in hippocampus requires the microglial CD11b integrin and DAP12 immunoreceptor. The Journal of neuroscience : the official journal of the Society for Neuroscience 28(32):8138-8143.

Wallach D, Varfolomeev EE, Malinin NL, Goltsev YV, Kovalenko AV, Boldin MP. 1999. Tumor necrosis factor receptor and Fas signaling mechanisms. Annual review of immunology 17:331-367.

Walley AJ, Asher JE, Froguel P. 2009. The genetic contribution to non-syndromic human obesity. Nature reviews Genetics 10(7):431-442.

Wan Z, Mah D, Simtchouk S, Klegeris A, Little JP. 2014. Globular adiponectin induces a pro-inflammatory response in human astrocytic cells. Biochemical and biophysical research communications 446(1):37-42.

Wang H, Chu WS, Hemphill C, Elbein SC. 2002a. Human resistin gene: molecular scanning and evaluation of association with insulin sensitivity and type 2 diabetes in Caucasians. The Journal of clinical endocrinology and metabolism 87(6):2520-2524.

Wang Y, Lam KS, Chan L, Chan KW, Lam JB, Lam MC, Hoo RC, Mak WW, Cooper GJ, Xu A. 2006. Post-translational modifications of the four conserved lysine residues within the collagenous domain of adiponectin are required for the formation of its high molecular weight oligomeric complex. The Journal of biological chemistry 281(24):16391-16400.

Wang Y, Xu A, Knight C, Xu LY, Cooper GJ. 2002b. Hydroxylation and glycosylation of the four conserved lysine residues in the collagenous domain of adiponectin. Potential role in the modulation of its insulin-sensitizing activity. The Journal of biological chemistry 277(22):19521-19529.

Ward MA, Carlsson CM, Trivedi MA, Sager MA, Johnson SC. 2005. The effect of body mass index on global brain volume in middle-aged adults: a cross sectional study. BMC Neurol 5:23.

Warren HS, Fitting C, Hoff E, Adib-Conquy M, Beasley-Topliffe L, Tesini B, Liang X, Valentine C, Hellman J, Hayden D, Cavaillon JM. 2010. Resilience to bacterial infection: difference between species could be due to proteins in serum. J Infect Dis 201(2):223-232.

Weber B, Barros LF. 2015. The Astrocyte: Powerhouse and Recycling Center. Cold Spring Harbor perspectives in biology.

Weiner JA, Hecht JH, Chun J. 1998. Lysophosphatidic acid receptor gene vzg-1/lpA1/edg-2 is expressed by mature oligodendrocytes during myelination in the postnatal murine brain. The Journal of comparative neurology 398(4):587-598.

Weisberg SP, McCann D, Desai M, Rosenbaum M, Leibel RL, Ferrante AW, Jr. 2003. Obesity is associated with macrophage accumulation in adipose tissue. J Clin Invest 112(12):1796-1808.

Werner F, Jain MK, Feinberg MW, Sibinga NE, Pellacani A, Wiesel P, Chin MT, Topper JN, Perrella MA, Lee ME. 2000. Transforming growth factor-beta 1 inhibition of macrophage activation is mediated via Smad3. The Journal of biological chemistry 275(47):36653-36658.

Whitehead JP, Richards AA, Hickman IJ, Macdonald GA, Prins JB. 2006. Adiponectin--a key adipokine in the metabolic syndrome. Diabetes, obesity & metabolism 8(3):264-280.

Whitmer RA. 2007. Type 2 diabetes and risk of cognitive impairment and dementia. Current neurology and neuroscience reports 7(5):373-380.

Whitmer RA, Gunderson EP, Quesenberry CP, Jr., Zhou J, Yaffe K. 2007. Body mass index in midlife and risk of Alzheimer disease and vascular dementia. Current Alzheimer research 4(2):103-109.

Whitmer RA, Gustafson DR, Barrett-Connor E, Haan MN, Gunderson EP, Yaffe K. 2008. Central obesity and increased risk of dementia more than three decades later. Neurology 71(14):1057-1064.

Whitney NP, Eidem TM, Peng H, Huang Y, Zheng JC. 2009. Inflammation mediates varying effects in neurogenesis: relevance to the pathogenesis of brain injury and neurodegenerative disorders. Journal of neurochemistry 108(6):1343-1359.
Whitwell JL. 2010. Progression of atrophy in Alzheimer's disease and related disorders. Neurotoxicity research 18(3-4):339-346.
WHO. 2000. Obesity: preventing and managing the global epidemic. Report of a WHO consultation. World Health Organization technical report series 894:i-xii, 1-253.
WHO. 2011. Global data base on Body Mass Index-BMI classification. World Health Organization Report of a WHO Expert Consultation.
WHO. 2015a. Obesity and demographics. World Health Organization.
WHO. 2015b. Overweight. World Health Organization, Global Health Observatory (GHO) data Risk factors
Wiese S, Karus M, Faissner A. Astrocytes as a source for extracellular matrix molecules and cytokines. Front Pharmacol 3:120.
Wiesner G, Brown RE, Robertson GS, Imran SA, Ur E, Wilkinson M. 2006. Increased expression of the adipokine genes resistin and fasting-induced adipose factor in hypoxic/ischaemic mouse brain. Neuroreport 17(11):1195-1198.
Wikipedia O. 2009. Thermogenesis in brown adipocytes.
Wilkinson M, Brown R, Imran SA, Ur E. 2007. Adipokine gene expression in brain and pituitary gland. Neuroendocrinology 86(3):191-209.
Wilkinson M, Morash B, Ur E. 2000. The brain is a source of leptin. Frontiers of hormone research 26:106-125.
Williams K, Alvarez X, Lackner AA. 2001. Central nervous system perivascular cells are immunoregulatory cells that connect the CNS with the peripheral immune system. Glia 36(2):156-164.
Williams MA, Qiu C, Muy-Rivera M, Vadachkoria S, Song T, Luthy DA. 2004. Plasma adiponectin concentrations in early pregnancy and subsequent risk of gestational diabetes mellitus. The Journal of clinical endocrinology and metabolism 89(5):2306-2311.
Wilson CJ, Finch CE, Cohen HJ. 2002. Cytokines and cognition--the case for a head-to-toe inflammatory paradigm. Journal of the American Geriatrics Society 50(12):2041-2056.
Wilson JX. 1997. Antioxidant defense of the brain: a role for astrocytes. Canadian journal of physiology and pharmacology 75(10-11):1149-1163.
Wisse LE, Reijmer YD, Ter Telgte A, Kuijf HJ, Leemans A, Luijten PR, Koek HL, Geerlings MI, Biessels GJ. 2015. Hippocampal Disconnection in Early Alzheimer's Disease: A 7 Tesla MRI Study. Journal of Alzheimer's disease : JAD.
WL. A. 1893. The neuroglia elements of the brain. . BMJ 2:227–230.
Wolf AM, Wolf D, Rumpold H, Enrich B, Tilg H. 2004. Adiponectin induces the anti-inflammatory cytokines IL-10 and IL-1RA in human leukocytes. Biochemical and biophysical research communications 323(2):630-635.
Wood PM, Bunge RP. 1991. The origin of remyelinating cells in the adult central nervous system: the role of the mature oligodendrocyte. Glia 4(2):225-232.
World Health Organization W. 2014. Global status report on noncommunicable diseases 2014. WHO Global status report on noncommunicable diseases
Wronska A, Kmiec Z. 2012. Structural and biochemical characteristics of various white adipose tissue depots. Acta physiologica (Oxford, England) 205(2):194-208.
Xia MQ, Hyman BT. 1999. Chemokines/chemokine receptors in the central nervous system and Alzheimer's disease. Journal of neurovirology 5(1):32-41.

Xia MQ, Qin SX, Wu LJ, Mackay CR, Hyman BT. 1998. Immunohistochemical study of the beta-chemokine receptors CCR3 and CCR5 and their ligands in normal and Alzheimer's disease brains. The American journal of pathology 153(1):31-37.

Xu A, Wang Y, Keshaw H, Xu LY, Lam KS, Cooper GJ. 2003. The fat-derived hormone adiponectin alleviates alcoholic and nonalcoholic fatty liver diseases in mice. The Journal of clinical investigation 112(1):91-100.

Xu Y, Kim ER, Zhao R, Myers MG, Jr., Munzberg H, Tong Q. 2013. Glutamate release mediates leptin action on energy expenditure. Molecular metabolism 2(2):109-115.

Yamada K, Watanabe M. 2002. Cytodifferentiation of Bergmann glia and its relationship with Purkinje cells. Anatomical science international 77(2):94-108.

Yamagata T, Muroya K, Mukasa T, Igarashi H, Momoi M, Tsukahara T, Arahata K, Kumagai H, Momoi T. 1995. Hepatocyte growth factor specifically expressed in microglia activated Ras in the neurons, similar to the action of neurotrophic factors. Biochemical and biophysical research communications 210(1):231-237.

Yamauchi T, Kamon J, Ito Y, Tsuchida A, Yokomizo T, Kita S, Sugiyama T, Miyagishi M, Hara K, Tsunoda M, Murakami K, Ohteki T, Uchida S, Takekawa S, Waki H, Tsuno NH, Shibata Y, Terauchi Y, Froguel P, Tobe K, Koyasu S, Taira K, Kitamura T, Shimizu T, Nagai R, Kadowaki T. 2003a. Cloning of adiponectin receptors that mediate antidiabetic metabolic effects. Nature 423(6941):762-769.

Yamauchi T, Kamon J, Ito Y, Tsuchida A, Yokomizo T, Kita S, Sugiyama T, Miyagishi M, Hara K, Tsunoda M, Murakami K, Ohteki T, Uchida S, Takekawa S, Waki H, Tsuno NH, Shibata Y, Terauchi Y, Froguel P, Tobe K, Koyasu S, Taira K, Kitamura T, Shimizu T, Nagai R, Kadowaki T. 2003b. Cloning of adiponectin receptors that mediate antidiabetic metabolic effects. Nature 423(6941):762-769.

Yamauchi T, Kamon J, Waki H, Terauchi Y, Kubota N, Hara K, Mori Y, Ide T, Murakami K, Tsuboyama-Kasaoka N, Ezaki O, Akanuma Y, Gavrilova O, Vinson C, Reitman ML, Kagechika H, Shudo K, Yoda M, Nakano Y, Tobe K, Nagai R, Kimura S, Tomita M, Froguel P, Kadowaki T. 2001. The fat-derived hormone adiponectin reverses insulin resistance associated with both lipoatrophy and obesity. Nature medicine 7(8):941-946.

Yamauchi T, Nio Y, Maki T, Kobayashi M, Takazawa T, Iwabu M, Okada-Iwabu M, Kawamoto S, Kubota N, Kubota T, Ito Y, Kamon J, Tsuchida A, Kumagai K, Kozono H, Hada Y, Ogata H, Tokuyama K, Tsunoda M, Ide T, Murakami K, Awazawa M, Takamoto I, Froguel P, Hara K, Tobe K, Nagai R, Ueki K, Kadowaki T. 2007. Targeted disruption of AdipoR1 and AdipoR2 causes abrogation of adiponectin binding and metabolic actions. Nature medicine 13(3):332-339.

Yanagida K, Masago K, Nakanishi H, Kihara Y, Hamano F, Tajima Y, Taguchi R, Shimizu T, Ishii S. 2009. Identification and characterization of a novel lysophosphatidic acid receptor, p2y5/LPA6. The Journal of biological chemistry 284(26):17731-17741.

Yang SY, Lee J, Park CG, Kim S, Hong S, Chung HC, Min SK, Han JW, Lee HW, Lee HY. 2002. Expression of autotaxin (NPP-2) is closely linked to invasiveness of breast cancer cells. Clinical & experimental metastasis 19(7):603-608.

Yang Y, Mou L, Liu N, Tsao MS. 1999. Autotaxin expression in non-small-cell lung cancer. American journal of respiratory cell and molecular biology 21(2):216-222.

Yasuda T, Mochizuki H. 2010. Use of growth factors for the treatment of Parkinson's disease. Expert review of neurotherapeutics 10(6):915-924.

Yau SY, Li A, Hoo RLC, Ching YP, Christie BR, Lee TMC, Xu A, So K-F. 2014. Physical exercise-induced hippocampal neurogenesis and antidepressant effects are mediated by the adipocyte hormone adiponectin. Proceedings of the National Academy of Sciences of the United States of America 111(44):15810-15815.

Ye X, Chun J. 2010. Lysophosphatidic acid (LPA) signaling in vertebrate reproduction. Trends in endocrinology and metabolism: TEM 21(1):17-24.
Ye X, Hama K, Contos JJ, Anliker B, Inoue A, Skinner MK, Suzuki H, Amano T, Kennedy G, Arai H, Aoki J, Chun J. 2005. LPA3-mediated lysophosphatidic acid signalling in embryo implantation and spacing. Nature 435(7038):104-108.
Ye X, Skinner MK, Kennedy G, Chun J. 2008. Age-dependent loss of sperm production in mice via impaired lysophosphatidic acid signaling. Biology of reproduction 79(2):328-336.
Yi CX, Meyer CW, Jastroch M. 2013. Leptin action in the brain: How (and when) it makes fat burn. Molecular metabolism 2(2):63-64.
Yi CX, Tschop MH. 2012. Brain-gut-adipose-tissue communication pathways at a glance. Disease models & mechanisms 5(5):583-587.
Yokota T, Oritani K, Takahashi I, Ishikawa J, Matsuyama A, Ouchi N, Kihara S, Funahashi T, Tenner AJ, Tomiyama Y, Matsuzawa Y. 2000. Adiponectin, a new member of the family of soluble defense collagens, negatively regulates the growth of myelomonocytic progenitors and the functions of macrophages. Blood 96(5):1723-1732.
Yoo MS, Chun HS, Son JJ, DeGiorgio LA, Kim DJ, Peng C, Son JH. 2003. Oxidative stress regulated genes in nigral dopaminergic neuronal cells: correlation with the known pathology in Parkinson's disease. Brain research Molecular brain research 110(1):76-84.
Yoshiyama Y, Sato H, Seiki M, Shinagawa A, Takahashi M, Yamada T. 1998. Expression of the membrane-type 3 matrix metalloproteinase (MT3-MMP) in human brain tissues. Acta neuropathologica 96(4):347-350.
Yu EPK, Bennett MR. 2014. Mitochondrial DNA damage and atherosclerosis. Trends in Endocrinology & Metabolism 25(9):481-487.
Yung YC, Stoddard NC, Chun J. 2014. LPA receptor signaling: pharmacology, physiology, and pathophysiology. Journal of lipid research 55(7):1192-1214.
Yusuf S, Hawken S, Ounpuu S, Dans T, Avezum A, Lanas F, McQueen M, Budaj A, Pais P, Varigos J, Lisheng L. 2004. Effect of potentially modifiable risk factors associated with myocardial infarction in 52 countries (the INTERHEART study): case-control study. Lancet 364(9438):937-952.
Zehntner SP, Brisebois M, Tran E, Owens T, Fournier S. 2003. Constitutive expression of a costimulatory ligand on antigen-presenting cells in the nervous system drives demyelinating disease. FASEB journal : official publication of the Federation of American Societies for Experimental Biology 17(13):1910-1912.
Zerlin M, Levison SW, Goldman JE. 1995. Early patterns of migration, morphogenesis, and intermediate filament expression of subventricular zone cells in the postnatal rat forebrain. The Journal of neuroscience : the official journal of the Society for Neuroscience 15(11):7238-7249.
Zhang F, Jiang L. 2015. Neuroinflammation in Alzheimer's disease. Neuropsychiatric disease and treatment 11:243-256.
Zhang G, Zhao Z, Xu S, Ni L, Wang X. 1999. Expression of autotaxin mRNA in human hepatocellular carcinoma. Chinese medical journal 112(4):330-332.
Zhang P, Tian B. 2014. Metabolic syndrome: an important risk factor for Parkinson's disease. Oxid Med Cell Longev 2014:729194.
Zhang R, Wu J, Liu D, Shan H, Zhang J. 2013. Anti-inflammatory effect of full-length adiponectin and pro-inflammatory effect of globular adiponectin in esophageal adenocarcinoma cells. Oncology research 21(1):15-21.
Zhang W, Wang T, Pei Z, Miller DS, Wu X, Block ML, Wilson B, Zhang W, Zhou Y, Hong JS, Zhang J. 2005. Aggregated alpha-synuclein activates microglia: a process leading to

disease progression in Parkinson's disease. FASEB journal : official publication of the Federation of American Societies for Experimental Biology 19(6):533-542.
Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM. 1994. Positional cloning of the mouse obese gene and its human homologue. Nature 372(6505):425-432.
Zhang YW, Zhang JQ, Liu C, Wei P, Zhang X, Yuan QY, Yin XT, Wei LQ, Cui JG, Wang J. 2015. Memory Dysfunction in Type 2 Diabetes Mellitus Correlates with Reduced Hippocampal CA1 and Subiculum Volumes. Chinese medical journal 128(4):465-471.
Zhao Y, Natarajan V. 2013. Lysophosphatidic acid (LPA) and its receptors: role in airway inflammation and remodeling. Biochimica et biophysica acta 1831(1):86-92.
Zhao Z, Xu S, Zhang G. 1999. [A study on expression of autotaxin mRNA in hepatocellular carcinoma]. Zhonghua gan zang bing za zhi = Zhonghua ganzangbing zazhi = Chinese journal of hepatology 7(3):140-141.
Zheng M, Liu J, Ruan Z, Tian S, Ma Y, Zhu J, Li G. 2013. Intrahippocampal injection of Aβ1-42 inhibits neurogenesis and down-regulates IFN-γ and NF-κB expression in hippocampus of adult mouse brain. Amyloid 20(1):13-20.
Zheng Y, Voice JK, Kong Y, Goetzl EJ. 2000. Altered expression and functional profile of lysophosphatidic acid receptors in mitogen-activated human blood T lymphocytes. FASEB journal : official publication of the Federation of American Societies for Experimental Biology 14(15):2387-2389.
Zheng ZQ, Fang XJ, Qiao JT. 2004. Dual action of lysophosphatidic acid in cultured cortical neurons: survival and apoptogenic. Sheng li xue bao : [Acta physiologica Sinica] 56(2):163-171.
Zheng ZQ, Fang XJ, Zhang Y, Qiao JT. 2005. Neuroprotective effect of lysophosphatidic acid on AbetaP31-35-induced apoptosis in cultured cortical neurons. Sheng li xue bao : [Acta physiologica Sinica] 57(3):289-294.
Zhou H, Liu J, Ren L, Liu W, Xing Q, Men L, Song G, Du J. 2014. Relationship between [corrected] spatial memory in diabetic rats and protein kinase Cgamma, caveolin-1 in the hippocampus and neuroprotective effect of catalpol. Chinese medical journal 127(5):916-923.
Zlokovic BV, Jovanovic S, Miao W, Samara S, Verma S, Farrell CL. 2000. Differential regulation of leptin transport by the choroid plexus and blood-brain barrier and high affinity transport systems for entry into hypothalamus and across the blood-cerebrospinal fluid barrier. Endocrinology 141(4):1434-1441.

ANNEX

BOOK CHAPTER

Influence Of Obesity On Neurodegenerative Diseases

Chapter 16

Influence of Obesity on Neurodegenerative Diseases

Rana Awada , Avinash Parimisetty and
Christian Lefebvre d'Hellencourt

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/53671

1. Introduction

Obesity is one of the greatest public health challenges of the 21st century. Obesity prevalence has been increasing globally at an alarming rate, particularly among children. The progressively increased prevalence of obesity over the past decades among children, as well as adults, is not limited to the US and other industrialized nations but is also evident in developing countries [1].The World Health Organization (WHO) estimated the prevalence of obesity at more than 1 billion overweight adults, with at least 500 million reaching the level of obese. As this continues to increase, by 2015, WHO estimates the number of overweighed adults will balloon to 2.3 billion with more than 700 million obese. Worldwide, obesity is currently responsible for 2–8% of health care costs and approximately 10–13% of deaths [2].

Fundamental causes of the current obesity epidemic are associated with sedentary lifestyles, increased consumption of energy-dense foods high in saturated fats and sugars and reduced physical activity. All of which correlate with the profound changes occurring in behavioral patterns of communities across societies as a consequence of increased urbanization and industrialization and often the disappearance of traditional lifestyles [3]. However, it is now appreciated that the progression to obesity represents a complex interaction of genetics, metabolism, as well as diet and physical activity level.

Clinically, obesity is defined by measurements of body mass index [4] or waist circumference and waist to hip ratio [5]. Body mass index (BMI) is a simple index weight-to-height defined as a person's weight in kilograms divided by the square of his/her height in meters (kg/m^2). According to WHO guidelines, a BMI 25 kg/m^2 identifies overweight and a BMI of 30 kg/m^2 or higher identifies an individual as obese. Physiologically, obesity is an excessive accumulation of fat in adipose tissue in the form of triglycerides, which can negatively affect health. Obesity is associated with number of metabolic disorders, increased expression of

pro-inflammatory markers and elevated risk for various disease including type 2 diabetes, cardiovascular disease, gastrointestinal disorders, respiratory difficulties, and various types of cancer [6]. In a more general nature, it has been suggested that obesity may accelerate the normal process of aging [7] (figure 1).

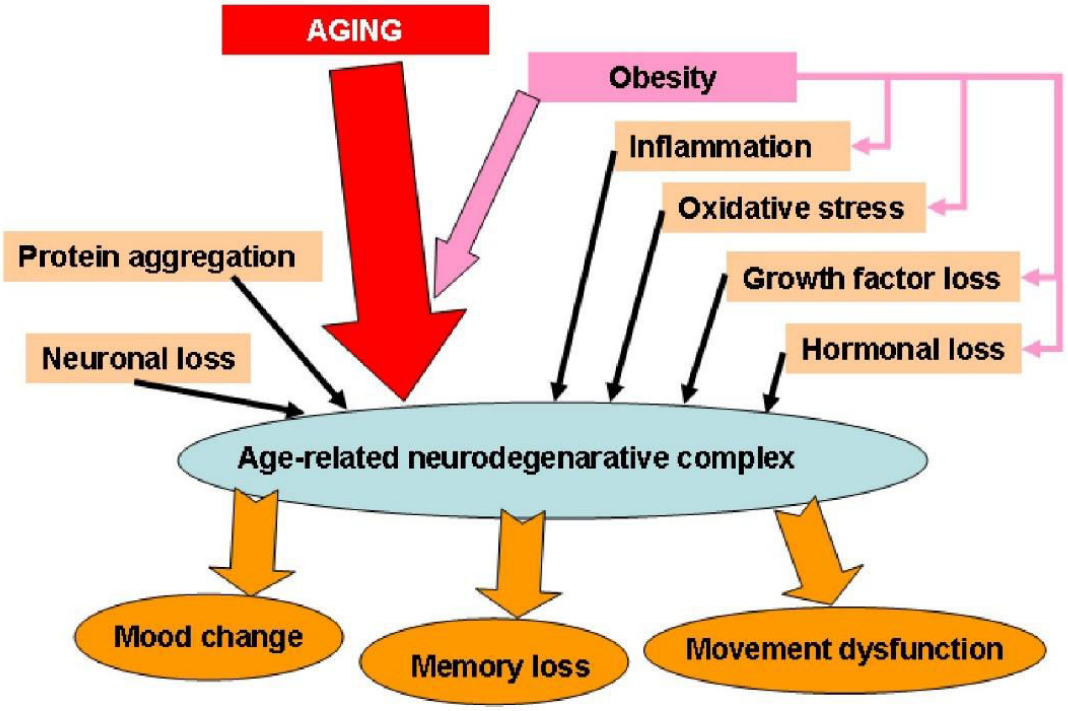


Figure 1. Obesity accelerates age and age related pathologies. Adapted from [8]

2. Obesity and inflammatory mediators

Adipose tissue has long been regarded as tissue storage of fat in the form of triglycerides; however, it is now recognized as an endocrine tissue producing a number of different factors, including inflammatory-related factors, acting at a physiological level [9].

Two forms of adipose tissues exist in mammals: the brown fat and the white fat (figure 2).

Brown adipose tissue is involved in the regulation of the body temperature. In humans, until recently, it was thought that brown fat was only present in the newborn and infant [12]. The extensive use of positron emission tomography (PET) in cancer medical imaging has changed this dogma. An evaluation of fluorodeoxyglucose PET (FDG PET) data from adult cancer patients indicated a high level of glucose consumption in specific body regions corresponding to brown fat [10], presumably in order to maintain normal body temperature

within an air conditioned room as this was not observed when patients were in a warm environment. White adipose tissue (WAT) is a source of energy involved in heat insulation and mechanical cushion. WAT represents around 15-20% of body weight and in obese individuals it increases up to 50%. WAT is composed of several different cell types, including preadipocytes, mature adipocytes, macrophages, endothelial cells which are involved in WAT homeostasis (Figure 3) [13, 14]. It is worth noting the presence of stem cells in the WAT, which are extensively studied for their potential in therapeutic reparation and even for the treatment of obesity and metabolic disorders [15-17].

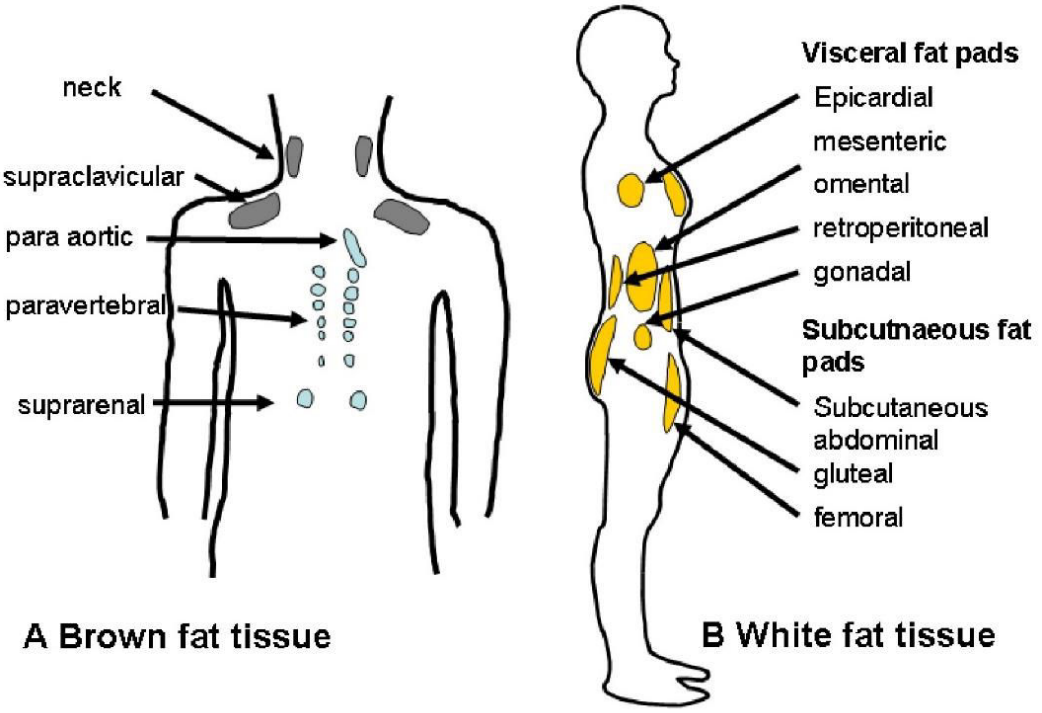


Figure 2. Brown fat (A) and white fat (B) tissue distribution in adult from [10, 11]

Different functional properties have been identified for WAT depending upon location in the subcutaneous or visceral areas. For example, a correlation exists between visceral obesity and increased risk of insulin resistance and cardiovascular diseases, while an increase of subcutaneous fat is associated with favorable plasma lipid profiles [11]. Adipose tissue was not usually thought of as an immune or inflammatory organ based upon studies demonstrating that loss of adipose tissue is associated with a decrease in markers of inflammation. It is now well accepted however, that adipose tissue is a key player in the development of inflammation [19]. Excess fat tissue in the obese environment contributes to a low-grade chronic inflammation [20] with elevated production of pro-inflammatory cytokines, such as tumor necrosis factor alpha (TNFα), interleukin -6 (IL-6) and IL-1 [21, 22]. The visceral adi-

pocytes significantly contribute in this role as they are metabolically active and produce a higher level of pro-inflammatory cytokines [11, 23-25].

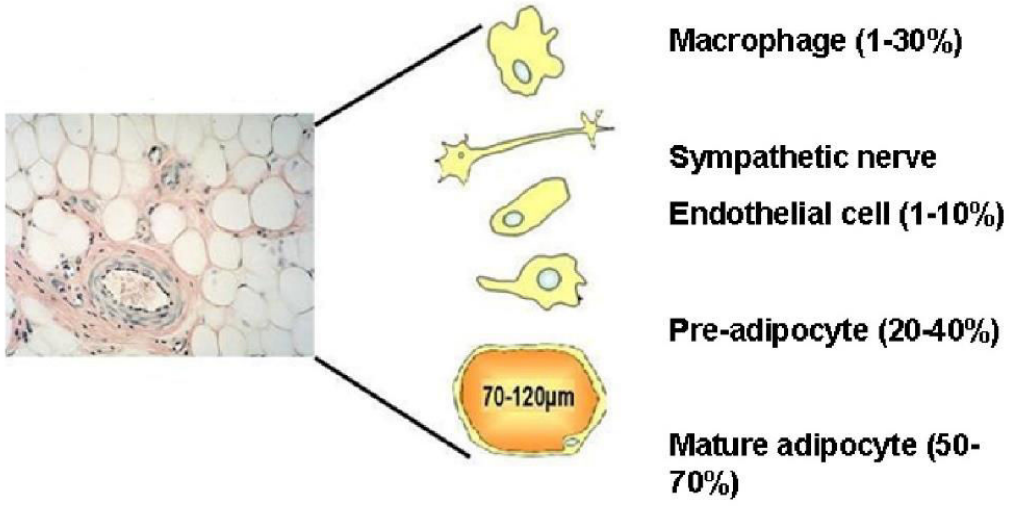


Figure 3. Cells present in the fat tissue adapted from [13, 18]

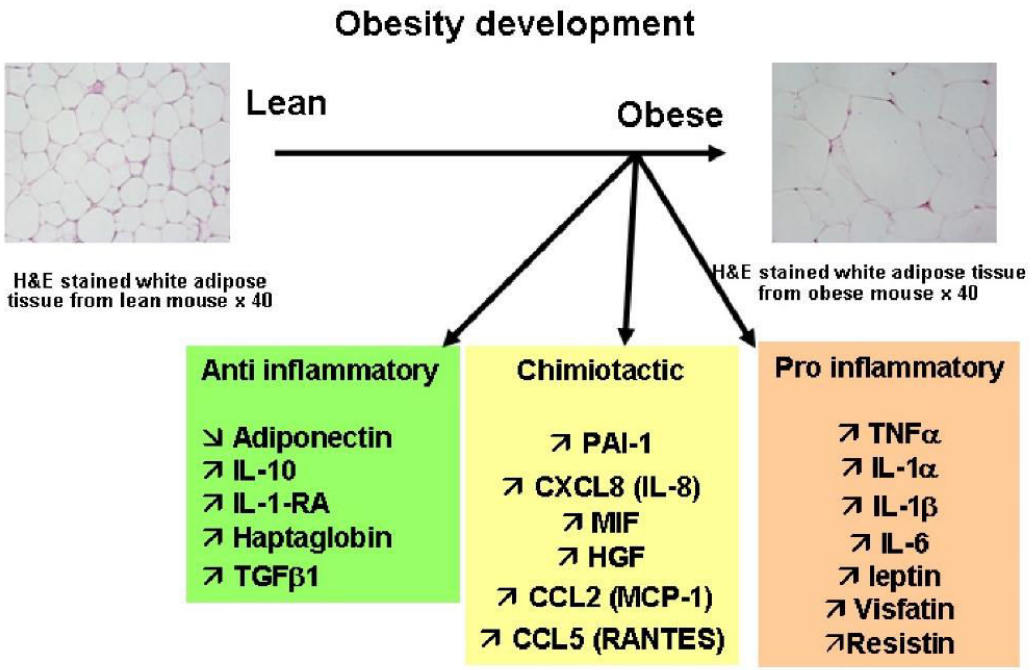


Figure 4. Inflammatory factors produced by WAT in obese situations. [26-35]

WAT is considered as an important organ in the regulation of many pathological processes by producing several inflammatory factors including, chemokines, cytokines and adipokines (also named adipocytokines). During the development of obesity expression of these factors is modified (figure 4).

Adipocytes secrete various chemo-attractants that recruit monocytes into the WAT. Obese adipose tissue exhibits an increased expression of Monocyte Chemoattractant Protein 1 (MCP-1) and of its receptor CCR2. The signaling of MCP1 / CCR2 has a direct impact on the development of obesity (for review see [36]). CCL-2, another chemokine with capability to recruit macrophages, has also a high level of expression in obese adipose tissue; however, it does not appear to be critical for adipose tissue macrophage recruitment [37]. Several other chemokines are also likely to play a role in the recruitment of monocytes/macrophages into the adipose tissue, such as MCP2, MCP4, migration inhibitory factor (MIF), macrophage inflammatory protein-1α (MIP-1α), MIP-1β, or MIP2-α [26, 38]. The adipocytes are not alone in the elevated inflammatory condition of obesity in that the number of macrophages is also higher in the obese environment thus, providing an additional cellular source of inflammatory factors [19].

Experimental animal studies have served a critical role in advancing our knowledge with regards to the biological relationship between adipose tissue, obesity, and inflammation. The first study to show a link between obesity and inflammation was the work of Hotamisligil and colleagues in 1993 in which they demonstrated that TNFα expression was up-regulated in adipose tissue of genetically obese mice [39]. Additional work reported that the number of bone-marrow derived macrophages present in white adipose tissue directly correlated with obesity [19]. In addition to macrophages, it has been demonstrated that pre-adipocytes and mature adipocytes also produce inflammatory factors. The mechanisms that initiate and trigger the inflammation are not yet totally elucidated, but different hypothesis have been proposed. A number of factors could trigger an inflammatory response and among them the saturated fatty acids may play a contributing role. For example, palmitate, an abundant nutritional fatty acid, could bind to the inflammation-related toll like receptors (TLR) leading to activation of a signalling cascade and the activation of the transcription factor NF-kB (nuclear factor kappa-light-chain-enhancer of activated B cells) [40]. NF-kB is involved in many cellular processes including immune and inflammatory responses. Upon activation and nuclear translocation, NF-kB can further induce the production of inflammatory cytokines, including TNF and IL-1. An alternative, but as relevant a process, is the recognition of a diverse range of stress and damage signals by inflammasomes. These are a group of protein complexes including the Nod-Like Receptor (NLR) proteins that can directly activate caspase-1 leading to the secretion of pro-inflammatory cytokines and pyroptotic cell death (for review see [41]).

Recently inflammasomes and their activation of down-stream events have been shown to play a major role in the development of obesity, insulin resistance, and diabetes [42, 43]. Another hypothesis linking inflammation and obesity is supported by Burcelin's group and involves the intestinal flora equilibrium. In this model, a high fat diet is proposed to

increase the gram-negative bacteria proportion in the intestine; this increases intestine permeability and the absorption of lipopolysacharide (LPS; the wall component of the gram-negative bacteria). Upon this increased absorption, TLR activation leads to an up-regulation of the inflammatory response [44, 45]. These two hypotheses are not mutually exclusive but rather it is likely that the two mechanisms coexist. While the classic localization of TLRs is on macrophages our laboratory and others have shown the presence of functional Toll-like receptors (TLRs) on human adipocytes including the expression of TLR type 2 (TLR2) and TLR type 4 (TLR4) [46, 47] providing evidence for the potential of an adipocyte receptor-mediated response.

Adipokines are defined as soluble mediators that are mainly, but not exclusively, produced by adipocytes and exert their biological function in an autocrine, paracrine or systemic manner. Over 50 adipokines have been identified, and they generally function as hormones to influence energy homeostasis and feeding [22, 48]. The following sections will focus on a review of two specific adipokines (leptin and adiponectin) and an additional factor (autotaxin) produced by WAT. Information is presented supporting that these factors and their activation may provide an important link between obesity and related inflammatory disorders.

Leptin was identified in 1994 as the 16 kDa protein product of the obese (ob) gene [49]. It displays immune-regulatory effects by increasing the production of pro-inflammatory cytokines by macrophages [50]. It is best known as an important regulator of energy balance through its actions in the brain to suppress appetite and increase energy expenditure [51]. Leptin in the blood enters the brain via a transport mechanism that can be saturated [52]. Upon entry it is believed to act primarily on the hypothalamic centers thus possibly providing a target for its effects upon appetite. In addition to the hypothalamus, leptin receptors (OBR) are widely expressed in numerous extra-hypothalamic regions of the brain, including the hippocampus, cerebellum, amygdala, and brain stem [53]. There are many splice variants of the receptor; those with short cytoplasmic domains are expressed in multiple tissues while the one with long cytoplasmic domains (OB-Rb) are expressed in specific brain regions. OB-Rb stimulates the JAK/STAT3 pathway and PI3K which are necessary for the leptin effects on food intake and hepatic glucose metabolism [54, 55].

Adiponectin, a prototypic adipocytokine is an anti-inflammatory adipokine secreted by adipocytes [56-58]. It plays a major role in regulation of insulin sensitivity and in obesity the levels of adiponectin are diminished due to a decreased release from WAT [59]. A deficiency in adiponectin is associated with exaggerated inflammatory response in patients with critical illness, including sepsis [32, 60] and with the development of a proinflammatory phenotype in in animal models of polymicrobial sepsis [61, 62]. Further studies demonstrated that adiponectin deficiency is associated with increased leukocyte and platelet adhesion as well as blood brain barrier dysfunction with cecal ligation and puncture induced sepsis in mice [63].

Autotaxin (ATX), also known as ectonucleotide pyrophosphatase phosphodiesterase-2 (ENPP2), is a secreted enzyme with lysophospholipase D (lysoPLD) activity involved in hydrolysis of lysophosphatidylcholine (LPC) into lysophosphatidic acid (LPA) [64]. LPA is bioactive phospholipid involved in numerous biological activities, including cell proliferation,

differentiation, and migration acting via specific G-protein coupled receptors [65]. The LPA strongly influences proliferation and differentiation of pre-adipocytes *via* the activation of LPA1 receptor [66, 67]. Anti-inflammatory properties for LPA have been suggested based upon the ability to inhibit, in mice, the LPS-induced inflammatory response of macrophages [68]. The expression of ATX is up-regulated during adipogenesis [69, 70] as well as in adipocytes from obese-diabetic db/db mice and in adipose tissue obtained from glucose-intolerant obese women subjects [69, 71]. The role of ATX in inflammation is less clear, but LPA seems to demonstrate some anti-inflammatory properties as it inhibits LPS-induced inflammation in cultured macrophages and in mice. Based upon these findings, it has been suggested that in addition to its role in cancer and LPA production, ATX may be involved in adipose tissue development and/or obesity-associated pathologies such as diabetes.

3. Influence of obesity on CNS

It is only relatively recently that the concept that obesity could have an effect on the brain has been emerging. Associations between obesity and various neurological disorders have been reported including sleep apnea, anxiety, manic depressive disorders, increased risk of developing cerebrovascular accident (CVA), and other neurological disorders [18, 72]. Additional consideration has been raised that obesity may be linked to various progressive and aging-related neurodegenerative diseases such as Parkinson's disease, Alzheimer's disease (AD), and autoimmune nervous system diseases like multiple sclerosis.

Over the last decade, a number of magnetic resonance imaging (MRI) and computed tomography (CT) studies have reported alterations in brain morphology of overweight/obese individuals. Initial studies demonstrated a higher BMI and/or waist-to-hip ratio in middle-aged individuals associated with a reduction in whole brain volume [73-75]. A similar association was observed with temporal lobe atrophy in elderly women [76] with additional evidence of hippocampal atrophy [77]. Debette et al. [78] reported a link between abdominal fat and reduced brain volume in otherwise healthy middle-aged adults. This study reported an inverse association between various obesity indicators (BMI, waist circumference, waist-to-hip ratio, and abdominal fat) and brain volume as determined by structural MRI of 733 participants. Independent of other obesity indicators, waist-to-hip ratio was found to be associated with increased temporal horn volume. Pannacciulli et al., 2006 reported gray matter reductions in the left postcentral gyrus, bilateral putamen, and right cerebellar regions in obese individuals as detected using voxel-based morphometry [79]. Gender differences have been suggested with a male-specific association between increasing BMI and smaller cerebellum, midbrain, frontal, termporal, and parietal cortex [74]. In a cohort of 95 obese women between the ages of 52 and 92, gray matter reductions were reported in the left orbitofrontal, right inferior frontal, right precentral gyri, and right cerebellar regions [80]. In contrast, increased volumes in white matter in the frontal, temporal, and parietal lobes were also reported [80]. In a cross-sectional study of normal elderly individuals showing no sign of

cognitive deficit, tensor-based morphometry unveiled atrophy in the white and gray matter of the frontal lobes, anterior cingulate gyrus, hippocampus, and thalamus in both male and female subjects with a high BMI (BMI > 30) as compared to individuals with a normal BMI (18.5–25) [81]. Upon further investigation, the brain volume reduction in gray and white matter was found to be associated with a common variant of the fat mass and obesity associated (*FTO*) gene [82]. Three-dimensional MRI brain maps of 206 healthy elderly participants showed an association between brain volume and the risk allele of the *FTO* gene known to be strongly associated with higher body-mass index. Participants who carried at least one copy of the allele had marked reductions in the volume of various brain structures compared to average volumes in non-carriers and in the general population. Carriers of the allele had, on average, an 8% deficit in the frontal lobe, 12% deficit in the occipital lobe (percentage units are expressed in terms of the average volumes seen in the general population of carriers and non-carriers). A reduction in temporal lobe volume was observed in participants with a higher BMI, but not in carriers of the risk allele. A pronounced effect of BMI was seen in carriers of the FTO allele showing volume deficits in all the other lobes of the brain, as well as in the brain stem and cerebellum. The authors proposed as a strong hypothesis that "BMI affects brain structure and that FTO exerts some additive detectable effect over and above whatever the BMI of the person happens to be" [82].

The possible relationship between neurodegeneration and obesity in animal models and in humans has been studied now for over a decade with a primary focus on the possibility that obesity and related metabolic disorders exacerbate neurodegeneration and thereby, promote cognitive decline and increase vulnerability to brain injury [73]. Based upon the identification of hereditary neurodegenerative disorders associated with obesity such as Alstrom, Bardet-Biedl or Prader-Willi syndromes, some studies have addressed the possibility that neurodegeneration in the brain may be a causal factor for obesity [83]. A more recent association between obesity and neurological function is based upon correlations with biological processes of oxidative stress and inflammation. While the causal nature of these processes to neurodegeneration has not been definitively established, it is widely accepted that neuroinflammation and oxidative stress responses occur with clinical manifestation of the disease. Given the recent reports of adipokines within the body fat and the elevation of these inflammatory factors with stimulation, a more direct linkage between obesity and various human diseases, including neurodegenerative disease, has been hypothesized. In the past decade, a linkage has been demonstrated between being overweight in middle age and increased risk for AD and other forms of dementia [84, 85]. However, as to date, the exact nature of the elevated risk has not been identified and characterized. There however, have been a number of hypotheses put forth, many including a role for inflammation. As previously stated, WAT can produce an array of inflammatory-related factors, for which expression levels may be modified in obesity. It has been proposed that an obesity-related chronic low-grade inflammation can serve to change the environment leading to a priming the brain for subsequent insults leading to a heightened inflammatory response and possibly exacerbation of the damage (figure 5).

http://dx.doi.org/10.5772/53671

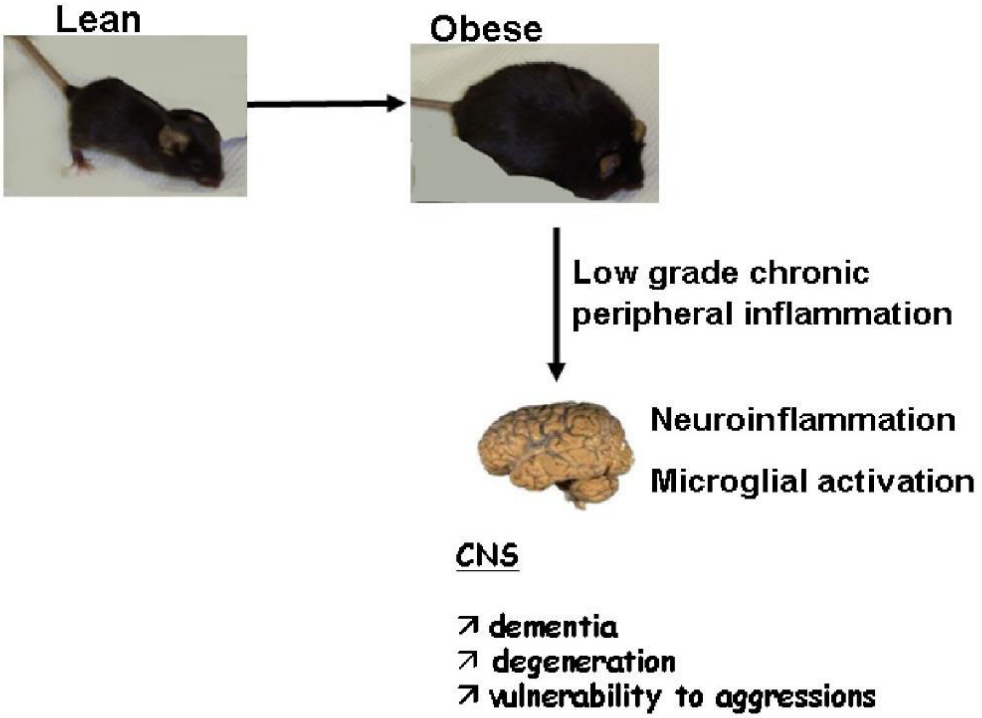


Figure 5. low grade chronic inflammation affect the response of the brain to later injury.

Obesity has a major negative impact on cognitive function due to vascular defects, impaired insulin metabolism and signaling pathway or a defect in glucose transport mechanisms in brain [86]. As shown in figure 4, leptin level is increased in obesity but there is also evidence that leptin signaling may become less effective in obesity, provoking a leptin-resistance status [87-89]. Thus, obesity, as it relates to leptin, may be due to a lack of leptin or of its receptor(s) but may also be a consequence of a signaling defect. Interestingly, leptin has protective effects in the brain both *in vitro* and *in vivo* and thus, has been suggested to be a good candidate as a link between obesity and neurodegeneration [90]. Similar to leptin, ATX is increased in obesity. LPA receptors are present in the CNS but the potential effect of ATX on oxidative stress or neuroinflammation was not known. In a recent study, Awada et al. [91] demonstrated that ATX synthesis and secretion by the brain immune cell, the microglia, have a protective effect by mitigating intracellular oxidation. These data suggests a novel anti-oxidant role for ATX in the brain. In contrast, adiponectin level is lowered with obesity [92]. In the CNS, adiponectin has been shown to improve cerebrovascular injury in mice [93, 94]. A deficiency in adiponectin in the mouse increases the severity of seizure activity [95] while presence of adiponectin provides a level of protection to hippocampal neurons against kainic acid-induced excitotoxicity [96]. It is likely that other factors produced by the WAT could have some effects on the CNS and further investigations are needed to decipher this complex network.

4. Susceptibility of the CNS to obesity in animal studies

Animal models of obesity have been very useful and important for understanding the regulation of food intake and imbalance in energy expenditure. The initial models examined spontaneous single gene mutations leading to the loss of the gene function [97]. The first of these models described is the agouti mouse [98, 99]. In addition to rats, other species have been used to study obesity related issues. These include, pigs, chicken, and even bats [97, 100, 101]. As several genes have been found to be involved in energy balance regulation, the advancement of methods for the overexpression or silencing of genes has allowed for a dramatic increase in the number of mouse models of obesity.

There is a growing body of evidence that nutrition could affect the inflammatory status of the brain [102, 103]. High dietary fat is a significant risk for cerebral oxidative stress development, neuronal inflammation, vascular dementia, AD, and Parkinson disease [104-108]. High fat diet induces a rapid (24 hours) temporary inflammation in the CNS, which can potentially progress to a chronic condition in obese mice as well as in human and leads to gliosis and mediobasal hypothalamus neuronal injury [109].

In genetic murine model of obesity, an increased susceptibility of CNS to trauma has been observed; obesity is an aggravating factor in chemical-induced neurodegeneration. In mice deficient for the leptin gene (ob/ob), the effects of two neurotoxicants are exacerbated, methamphetamine (METH), which affects dopaminergic neurons and kainic acid (KA), affecting the hippocampus [110]. The ob/ob mice are also more susceptible to seizure induced by the gamma-aminobutyric acid A receptor (GABAAR) antagonist, pentylenetetrazol (PTZ) [111].

It is now known that in distinct neurogenic sites of the brain the presence of stem/progenitor cells allows for the generation of new neurons over the full lifespan [112]. This process is influenced by a number of factors including cytokines, hormones, growth factors, and exercise [112-116]. The regulatory effects of growth factors demonstrate a level of specificity for brain regions with brain-derived neurotrophic factor (BDNF) showing prominent effects in the hippocampus while ciliary neurotrophic factor (CNTF) induces neurogenesis in the hypothalamus. In this case the neurogenesis occurs in the satiety centers inducing a persistent weight loss [117]. More importantly, with regards to inflammatory factors, injury to the brain such as ischemia [118], epilepsy [119], or chemically induced neurodegeneration [120] induce an increase in neurogenesis. This induction has been termed "injury-induced neurogenesis". A relationship between adult neurogenesis and obesity has been demonstrated in the decrease in the turnover of new neurons in the hypothalamic arcuate nucleus (region playing a key role in body weight regulation) in obese mice (high fat diet or ob/ob) [121]. While the research effort targeted toward this area of the effects of nutrition or obesity on adult neurogenesis is in its infancy, it is likely that a link similar to what has been found with neurodegeneration, may be found for molecules such as Omega 3 fatty acids, flavonoids, and polyphenols [122-124].

5. Conclusion

It is now well accepted that obesity is associated with several pathologies including neuropathies and the ability of the nervous system to repair following injury. While further research is needed in characterizing the nature of the effect of obesity on the nervous system there are current studies suggesting that such effects can be modified. For example, resveratrol or ursolic acid have been shown to attenuate obesity-associated nervous system inflammation resulting in an improvement of memory deficits in mice fed a high-fat diet. [125, 126]. Given the accelerated increase in obesity and neurodegenerative diseases as well as the influence of childhood health status and adult disease, there is a critical need to better understand the relationship between obesity and the nervous system. Identification of the critical factors underlying the various changes seen in the brain and its response to injury as a function of age, nutritional status, and body mass, i.e., obesity will lay the foundation for developing therapeutic interventions that will be applicable to the human population.

Acknowledgements

We would like to thanks Dr. G. Jean Harry (NTP/NIEHS/NIH) for her comments and edition. We would like to thank the 'Region La Reunion', Europe (CPER/FEDER) for its funding supports. AP is funded by fellowships from 'Conseil Régional de La Réunion'.

Author details

Rana Awada , Avinash Parimisetty and Christian Lefebvre d'Hellencourt*

*Address all correspondence to: Christian.Lefebvre-d-Hellencourt@univ-reunion.fr

Groupe d'Etude sur l'Inflammation Chronique et l'Obesité (GEICO), EA 4516, Plateforme CYROI, UFR Santé, Université de La Réunion, Ile de La Réunion, France

References

[1] Prentice AM. The emerging epidemic of obesity in developing countries. Int J Epidemiol 2006; 35(1) 93-9

[2] World Health Organisation (WHO). Obesity in Europe. http://www.euro.who.int/obesity. 2008

[3] Obesity: preventing and managing the global epidemic. Report of a WHO consultation. World Health Organ Tech Rep Ser 2000; 894(i-xii, 1-253

[4] Mei Z, Grummer-Strawn LM, Pietrobelli A, Goulding A, Goran MI and Dietz WH. Validity of body mass index compared with other body-composition screening indexes for the assessment of body fatness in children and adolescents. Am J Clin Nutr 2002; 75(6) 978-85

[5] Eckel RH, Grundy SM and Zimmet PZ. The metabolic syndrome. Lancet 2005; 365(9468) 1415-28

[6] Haslam DW and James WP. Obesity. Lancet 2005; 366(9492) 1197-209

[7] Tzanetakou IP, Katsilambros NL, Benetos A, Mikhailidis DP and Perrea DN. "Is obesity linked to aging?": adipose tissue and the role of telomeres. Ageing Res Rev 2012; 11(2) 220-9

[8] Granholm AC, Boger H and Emborg ME. Mood, memory and movement: an age-related neurodegenerative complex? Curr Aging Sci 2008; 1(2) 133-9

[9] Harwood HJ, Jr. The adipocyte as an endocrine organ in the regulation of metabolic homeostasis. Neuropharmacology 2012; 63(1) 57-75

[10] Nedergaard J, Bengtsson T and Cannon B. Unexpected evidence for active brown adipose tissue in adult humans. Am J Physiol Endocrinol Metab 2007; 293(2) E444-52

[11] Wronska A and Kmiec Z. Structural and biochemical characteristics of various white adipose tissue depots. Acta Physiol (Oxf) 2012; 205(2) 194-208

[12] Cannon B and Nedergaard J. Brown adipose tissue: function and physiological significance. Physiol Rev 2004; 84(1) 277-359

[13] Hauner H. Secretory factors from human adipose tissue and their functional role. Proc Nutr Soc 2005; 64(2) 163-9

[14] Hauner H. The new concept of adipose tissue function. Physiology & Behavior 2004; 83(4) 653-8

[15] Cawthorn WP, Scheller EL and MacDougald OA. Adipose tissue stem cells meet preadipocyte commitment: going back to the future. J Lipid Res 2011; 53(2) 227-46

[16] Tran TT and Kahn CR. Transplantation of adipose tissue and stem cells: role in metabolism and disease. Nat Rev Endocrinol 2010; 6(4) 195-213

[17] Roche R, Hoareau L, Mounet F and Festy F. Adult stem cells for cardiovascular diseases: the adipose tissue potential. Expert Opin Biol Ther 2007; 7(6) 791-8

[18] Palaniyandi R, Awada R, Harry GJ and Lefebvre d'Hellencourt C. White fat tissue, obesity and possible role in neurodegeneration in Harry GJ and Tilson HA (ed) White fat tissue, obesity and possible role in neurodegeneration. Book 2010

[19] Weisberg SP, McCann D, Desai M, Rosenbaum M, Leibel RL and Ferrante AW, Jr. Obesity is associated with macrophage accumulation in adipose tissue. J Clin Invest 2003; 112(12) 1796-808

[20] Greenberg AS and Obin MS. Obesity and the role of adipose tissue in inflammation and metabolism. Am J Clin Nutr 2006; 83(2) 461S-5S

[21] Moschen AR, Kaser A, Enrich B, Mosheimer B, Theurl M, Niederegger H and Tilg H. Visfatin, an adipocytokine with proinflammatory and immunomodulating properties. J Immunol 2007; 178(3) 1748-58

[22] Tilg H and Moschen AR. Adipocytokines: mediators linking adipose tissue, inflammation and immunity. Nat Rev Immunol 2006; 6(10) 772-83

[23] Van Harmelen V, Reynisdottir S, Eriksson P, Thorne A, Hoffstedt J, Lonnqvist F and Arner P. Leptin secretion from subcutaneous and visceral adipose tissue in women. Diabetes 1998; 47(6) 913-7

[24] Fain JN, Madan AK, Hiler ML, Cheema P and Bahouth SW. Comparison of the release of adipokines by adipose tissue, adipose tissue matrix, and adipocytes from visceral and subcutaneous abdominal adipose tissues of obese humans. Endocrinology 2004; 145(5) 2273-82

[25] Lafontan M and Langin D. Lipolysis and lipid mobilization in human adipose tissue. Prog Lipid Res 2009; 48(5) 275-97

[26] Tilg H and Moschen AR. Role of adiponectin and PBEF/visfatin as regulators of inflammation: involvement in obesity-associated diseases. Clin Sci (Lond) 2008; 114(4) 275-88

[27] Berndt J, Kloting N, Kralisch S, Kovacs P, Fasshauer M, Schon MR, Stumvoll M and Bluher M. Plasma visfatin concentrations and fat depot-specific mRNA expression in humans. Diabetes 2005; 54(10) 2911-6

[28] Azuma K, Katsukawa F, Oguchi S, Murata M, Yamazaki H, Shimada A and Saruta T. Correlation between serum resistin level and adiposity in obese individuals. Obes Res 2003; 11(8) 997-1001

[29] Sartipy P and Loskutoff DJ. Monocyte chemoattractant protein 1 in obesity and insulin resistance. Proc Natl Acad Sci U S A 2003; 100(12) 7265-70

[30] Madani R, Karastergiou K, Ogston NC, Miheisi N, Bhome R, Haloob N, Tan GD, Karpe F, Malone-Lee J, Hashemi M, Jahangiri M and Mohamed-Ali V. RANTES release by human adipose tissue in vivo and evidence for depot-specific differences. Am J Physiol Endocrinol Metab 2009; 296(6) E1262-8

[31] Chiellini C, Santini F, Marsili A, Berti P, Bertacca A, Pelosini C, Scartabelli G, Pardini E, Lopez-Soriano J, Centoni R, Ciccarone AM, Benzi L, Vitti P, Del Prato S, Pinchera A and Maffei M. Serum haptoglobin: a novel marker of adiposity in humans. J Clin Endocrinol Metab 2004; 89(6) 2678-83

[32] Hillenbrand A, Knippschild U, Weiss M, Schrezenmeier H, Henne-Bruns D, Huber-Lang M and Wolf AM. Sepsis induced changes of adipokines and cytokines - septic patients compared to morbidly obese patients. BMC Surg 2010; 10(26

[33] Bell LN, Ward JL, Degawa-Yamauchi M, Bovenkerk JE, Jones R, Cacucci BM, Gupta CE, Sheridan C, Sheridan K, Shankar SS, Steinberg HO, March KL and Considine RV. Adipose tissue production of hepatocyte growth factor contributes to elevated serum HGF in obesity. Am J Physiol Endocrinol Metab 2006; 291(4) E843-8

[34] Fain JN. Release of inflammatory mediators by human adipose tissue is enhanced in obesity and primarily by the nonfat cells: a review. Mediators Inflamm 2010; 2010(513948

[35] Meier CA, Bobbioni E, Gabay C, Assimacopoulos-Jeannet F, Golay A and Dayer JM. IL-1 receptor antagonist serum levels are increased in human obesity: a possible link to the resistance to leptin? J Clin Endocrinol Metab 2002; 87(3) 1184-8

[36] Panee J. Monocyte Chemoattractant Protein 1 (MCP-1) in obesity and diabetes. Cytokine 2012;

[37] Clement S, Juge-Aubry C, Sgroi A, Conzelmann S, Pazienza V, Pittet-Cuenod B, Meier CA and Negro F. Monocyte chemoattractant protein-1 secreted by adipose tissue induces direct lipid accumulation in hepatocytes. Hepatology 2008; 48(3) 799-807

[38] Gonzalez-Castejon M and Rodriguez-Casado A. Dietary phytochemicals and their potential effects on obesity: a review. Pharmacol Res 2011; 64(5) 438-55

[39] Hotamisligil GS, Shargill NS and Spiegelman BM. Adipose expression of tumor necrosis factor-alpha: direct role in obesity-linked insulin resistance. Science 1993; 259(5091) 87-91

[40] Shi H, Kokoeva MV, Inouye K, Tzameli I, Yin H and Flier JS. TLR4 links innate immunity and fatty acid-induced insulin resistance. J Clin Invest 2006; 116(11) 3015-25

[41] Strowig T, Henao-Mejia J, Elinav E and Flavell R. Inflammasomes in health and disease. Nature 481(7381) 278-86

[42] Stienstra R, Tack CJ, Kanneganti TD, Joosten LA and Netea MG. The inflammasome puts obesity in the danger zone. Cell Metab 2012; 15(1) 10-8

[43] Stienstra R, van Diepen JA, Tack CJ, Zaki MH, van de Veerdonk FL, Perera D, Neale GA, Hooiveld GJ, Hijmans A, Vroegrijk I, van den Berg S, Romijn J, Rensen PC, Joosten LA, Netea MG and Kanneganti TD. Inflammasome is a central player in the induction of obesity and insulin resistance. Proc Natl Acad Sci U S A 2011; 108(37) 15324-9

[44] Cani PD, Bibiloni R, Knauf C, Waget A, Neyrinck AM, Delzenne NM and Burcelin R. Changes in gut microbiota control metabolic endotoxemia-induced inflammation in high-fat diet-induced obesity and diabetes in mice. Diabetes 2008; 57(6) 1470-81

[45] Nicholson JK, Holmes E, Kinross J, Burcelin R, Gibson G, Jia W and Pettersson S. Host-gut microbiota metabolic interactions. Science 2012; 336(6086) 1262-7

[46] Bes-Houtmann S, Roche R, Hoareau L, Gonthier MP, Festy F, Caillens H, Gasque P, Lefebvre d'Hellencourt C and Cesari M. Presence of functional TLR2 and TLR4 on human adipocytes. Histochem Cell Biol 2007; 127(2) 131-7

[47] Meijer K, de Vries M, Al-Lahham S, Bruinenberg M, Weening D, Dijkstra M, Kloosterhuis N, van der Leij RJ, van der Want H, Kroesen BJ, Vonk R and Rezaee F. Human primary adipocytes exhibit immune cell function: adipocytes prime inflammation independent of macrophages. PLoS ONE 2011; 6(3) e17154

[48] Ahima RS and Osei SY. Adipokines in obesity. Front Horm Res 2008; 36(182-97

[49] Zhang Y, Proenca R, Maffei M, Barone M, Leopold L and Friedman JM. Positional cloning of the mouse obese gene and its human homologue. Nature 1994; 372(6505) 425-32

[50] Loffreda S, Yang SQ, Lin HZ, Karp CL, Brengman ML, Wang DJ, Klein AS, Bulkley GB, Bao C, Noble PW, Lane MD and Diehl AM. Leptin regulates proinflammatory immune responses. Faseb J 1998; 12(1) 57-65

[51] Friedman JM and Halaas JL. Leptin and the regulation of body weight in mammals. Nature 1998; 395(6704) 763-70

[52] Banks WA, Kastin AJ, Huang W, Jaspan JB and Maness LM. Leptin enters the brain by a saturable system independent of insulin. Peptides 1996; 17(2) 305-11

[53] Elmquist JK, Bjorbaek C, Ahima RS, Flier JS and Saper CB. Distributions of leptin receptor mRNA isoforms in the rat brain. J Comp Neurol 1998; 395(4) 535-47

[54] Buettner C, Pocai A, Muse ED, Etgen AM, Myers MG, Jr. and Rossetti L. Critical role of STAT3 in leptin's metabolic actions. Cell Metab 2006; 4(1) 49-60

[55] Gautron L and Elmquist JK. Sixteen years and counting: an update on leptin in energy balance. J Clin Invest 2011; 121(6) 2087-93

[56] Ohashi K, Ouchi N and Matsuzawa Y. Anti-inflammatory and anti-atherogenic properties of adiponectin. Biochimie 2012;

[57] Ouchi N and Walsh K. Adiponectin as an anti-inflammatory factor. Clin Chim Acta 2007; 380(1-2) 24-30

[58] Robinson K, Prins J and Venkatesh B. Clinical review: adiponectin biology and its role in inflammation and critical illness. Crit Care 2011; 15(2) 221

[59] Arita Y, Kihara S, Ouchi N, Takahashi M, Maeda K, Miyagawa J, Hotta K, Shimomura I, Nakamura T, Miyaoka K, Kuriyama H, Nishida M, Yamashita S, Okubo K, Matsubara K, Muraguchi M, Ohmoto Y, Funahashi T and Matsuzawa Y. Paradoxical

decrease of an adipose-specific protein, adiponectin, in obesity. Biochem Biophys Res Commun 1999; 257(1) 79-83

[60] Venkatesh B, Hickman I, Nisbet J, Cohen J and Prins J. Changes in serum adiponectin concentrations in critical illness: a preliminary investigation. Crit Care 2009; 13(4) R105

[61] Uji Y, Yamamoto H, Tsuchihashi H, Maeda K, Funahashi T, Shimomura I, Shimizu T, Endo Y and Tani T. Adiponectin deficiency is associated with severe polymicrobial sepsis, high inflammatory cytokine levels, and high mortality. Surgery 2009; 145(5) 550-7

[62] Teoh H, Quan A, Bang KW, Wang G, Lovren F, Vu V, Haitsma JJ, Szmitko PE, Al-Omran M, Wang CH, Gupta M, Peterson MD, Zhang H, Chan L, Freedman J, Sweeney G and Verma S. Adiponectin deficiency promotes endothelial activation and profoundly exacerbates sepsis-related mortality. Am J Physiol Endocrinol Metab 2008; 295(3) E658-64

[63] Vachharajani V, Cunningham C, Yoza B, Carson J, Jr., Vachharajani TJ and McCall C. Adiponectin-deficiency exaggerates sepsis-induced microvascular dysfunction in the mouse brain. Obesity (Silver Spring) 2012; 20(3) 498-504

[64] Umezu-Goto M, Kishi Y, Taira A, Hama K, Dohmae N, Takio K, Yamori T, Mills GB, Inoue K, Aoki J and Arai H. Autotaxin has lysophospholipase D activity leading to tumor cell growth and motility by lysophosphatidic acid production. J Cell Biol 2002; 158(2) 227-33

[65] Ishii I, Fukushima N, Ye X and Chun J. Lysophospholipid receptors: signaling and biology. Annu Rev Biochem 2004; 73(321-54

[66] Simon MF, Daviaud D, Pradere JP, Gres S, Guigne C, Wabitsch M, Chun J, Valet P and Saulnier-Blache JS. Lysophosphatidic acid inhibits adipocyte differentiation via lysophosphatidic acid 1 receptor-dependent down-regulation of peroxisome proliferator-activated receptor gamma2. J Biol Chem 2005; 280(15) 14656-62

[67] Valet P, Pages C, Jeanneton O, Daviaud D, Barbe P, Record M, Saulnier-Blache JS and Lafontan M. Alpha2-adrenergic receptor-mediated release of lysophosphatidic acid by adipocytes. A paracrine signal for preadipocyte growth. J Clin Invest 1998; 101(7) 1431-8

[68] Fan H, Zingarelli B, Harris V, Tempel GE, Halushka PV and Cook JA. Lysophosphatidic acid inhibits bacterial endotoxin-induced pro-inflammatory response: potential anti-inflammatory signaling pathways. Mol Med 2008; 14(7-8) 422-8

[69] Ferry G, Tellier E, Try A, Gres S, Naime I, Simon MF, Rodriguez M, Boucher J, Tack I, Gesta S, Chomarat P, Dieu M, Raes M, Galizzi JP, Valet P, Boutin JA and Saulnier-Blache JS. Autotaxin is released from adipocytes, catalyzes lysophosphatidic acid

synthesis, and activates preadipocyte proliferation. Up-regulated expression with adipocyte differentiation and obesity. J Biol Chem 2003; 278(20) 18162-9

[70] Gesta S, Simon MF, Rey A, Sibrac D, Girard A, Lafontan M, Valet P and Saulnier-Blache JS. Secretion of a lysophospholipase D activity by adipocytes: involvement in lysophosphatidic acid synthesis. J Lipid Res 2002; 43(6) 904-10

[71] Boucher J, Quilliot D, Praderes JP, Simon MF, Gres S, Guigne C, Prevot D, Ferry G, Boutin JA, Carpene C, Valet P and Saulnier-Blache JS. Potential involvement of adipocyte insulin resistance in obesity-associated up-regulation of adipocyte lysophospholipase D/autotaxin expression. Diabetologia 2005; 48(3) 569-77

[72] Whitmer RA. The epidemiology of adiposity and dementia. Curr Alzheimer Res 2007; 4(2) 117-22

[73] Bruce-Keller AJ, Keller JN and Morrison CD. Obesity and vulnerability of the CNS. Biochim Biophys Acta 2009; 1792(5) 395-400

[74] Taki Y, Kinomura S, Sato K, Inoue K, Goto R, Okada K, Uchida S, Kawashima R and Fukuda H. Relationship between body mass index and gray matter volume in 1,428 healthy individuals. Obesity (Silver Spring) 2008; 16(1) 119-24

[75] Ward MA, Carlsson CM, Trivedi MA, Sager MA and Johnson SC. The effect of body mass index on global brain volume in middle-aged adults: a cross sectional study. BMC Neurol 2005; 5(23

[76] Gustafson D, Lissner L, Bengtsson C, Bjorkelund C and Skoog I. A 24-year follow-up of body mass index and cerebral atrophy. Neurology 2004; 63(10) 1876-81

[77] Jagust W, Harvey D, Mungas D and Haan M. Central obesity and the aging brain. Arch Neurol 2005; 62(10) 1545-8

[78] Debette S, Beiser A, Hoffmann U, Decarli C, O'Donnell CJ, Massaro JM, Au R, Himali JJ, Wolf PA, Fox CS and Seshadri S. Visceral fat is associated with lower brain volume in healthy middle-aged adults. Ann Neurol 2010; 68(2) 136-44

[79] Pannacciulli N, Del Parigi A, Chen K, Le DS, Reiman EM and Tataranni PA. Brain abnormalities in human obesity: a voxel-based morphometric study. Neuroimage 2006; 31(4) 1419-25

[80] Walther K, Birdsill AC, Glisky EL and Ryan L. Structural brain differences and cognitive functioning related to body mass index in older females. Hum Brain Mapp 2010; 31(7) 1052-64

[81] Raji CA, Ho AJ, Parikshak NN, Becker JT, Lopez OL, Kuller LH, Hua X, Leow AD, Toga AW and Thompson PM. Brain structure and obesity. Hum Brain Mapp 2010; 31(3) 353-64

[82] Ho AJ, Stein JL, Hua X, Lee S, Hibar DP, Leow AD, Dinov ID, Toga AW, Saykin AJ, Shen L, Foroud T, Pankratz N, Huentelman MJ, Craig DW, Gerber JD, Allen AN,

Corneveaux JJ, Stephan DA, DeCarli CS, DeChairo BM, Potkin SG, Jack CR, Jr., Weiner MW, Raji CA, Lopez OL, Becker JT, Carmichael OT and Thompson PM. A commonly carried allele of the obesity-related FTO gene is associated with reduced brain volume in the healthy elderly. Proc Natl Acad Sci U S A 2010; 107(18) 8404-9

[83] Ristow M. Neurodegenerative disorders associated with diabetes mellitus. J Mol Med 2004; 82(8) 510-29

[84] Gustafson D. Adiposity indices and dementia. Lancet Neurol 2006; 5(8) 713-20

[85] Gustafson D, Rothenberg E, Blennow K, Steen B and Skoog I. An 18-year follow-up of overweight and risk of Alzheimer disease. Arch Intern Med 2003; 163(13) 1524-8

[86] Naderali EK, Ratcliffe SH and Dale MC. Obesity and Alzheimer's disease: a link between body weight and cognitive function in old age. Am J Alzheimers Dis Other Demen 2009; 24(6) 445-9

[87] Myers MG, Jr., Heymsfield SB, Haft C, Kahn BB, Laughlin M, Leibel RL, Tschop MH and Yanovski JA. Challenges and opportunities of defining clinical leptin resistance. Cell Metab 2012; 15(2) 150-6

[88] St-Pierre J and Tremblay ML. Modulation of leptin resistance by protein tyrosine phosphatases. Cell Metab 2012; 15(3) 292-7

[89] Shimizu H, Oh IS, Okada S and Mori M. Leptin resistance and obesity. Endocr J 2007; 54(1) 17-26

[90] Doherty GH. Obesity and the ageing brain: could leptin play a role in neurodegeneration? Curr Gerontol Geriatr Res 2011; 2011(708154

[91] Awada R, Rondeau P, Gres S, Saulnier-Blache JS, Lefebvre d'Hellencourt C and Bourdon E. Autotaxin protects microglial cells against oxidative stress. Free Radic Biol Med 2012; 52(2) 516-26

[92] Hu E, Liang P and Spiegelman BM. AdipoQ is a novel adipose-specific gene dysregulated in obesity. J Biol Chem 1996; 271(18) 10697-703

[93] Chen B, Liao WQ, Xu N, Xu H, Wen JY, Yu CA, Liu XY, Li CL, Zhao SM and Campbell W. Adiponectin protects against cerebral ischemia-reperfusion injury through anti-inflammatory action. Brain Res 2009; 1273(129-37

[94] Nishimura M, Izumiya Y, Higuchi A, Shibata R, Qiu J, Kudo C, Shin HK, Moskowitz MA and Ouchi N. Adiponectin prevents cerebral ischemic injury through endothelial nitric oxide synthase dependent mechanisms. Circulation 2008; 117(2) 216-23

[95] Lee EB, Warmann G, Dhir R and Ahima RS. Metabolic dysfunction associated with adiponectin deficiency enhances kainic acid-induced seizure severity. J Neurosci 2012; 31(40) 14361-6

[96] Jeon BT, Shin HJ, Kim JB, Kim YK, Lee DH, Kim KH, Kim HJ, Kang SS, Cho GJ, Choi WS and Roh GS. Adiponectin protects hippocampal neurons against kainic acid-induced excitotoxicity. Brain Res Rev 2009; 61(2) 81-8

[97] Speakman J, Hambly C, Mitchell S and Krol E. Animal models of obesity. Obes Rev 2007; 8 Suppl 1(55-61

[98] Dickies MM. A new viable yellow mutation in the house mouse. J Hered 1962; 53(84-6

[99] Yen TT, Gill AM, Frigeri LG, Barsh GS and Wolff GL. Obesity, diabetes, and neoplasia in yellow A(vy)/- mice: ectopic expression of the agouti gene. Faseb J 1994; 8(8) 479-88

[100] Dietrich HM. Housing, breeding and selecting chickens of the Obese strain (OS) with spontaneous autoimmune thyroiditis. Lab Anim 1989; 23(4) 345-52

[101] Hen G, Yosefi S, Simchaev V, Shinder D, Hruby VJ and Friedman-Einat M. The melanocortin circuit in obese and lean strains of chicks. J Endocrinol 2006; 190(2) 527-35

[102] Keller JN. Special issue: Reciprocal interactions between diet, metabolism, and the nervous system. Foreword. Biochim Biophys Acta 2009; 1792(5) 393-4

[103] Zhang L, Bruce-Keller AJ, Dasuri K, Nguyen AT, Liu Y and Keller JN. Diet-induced metabolic disturbances as modulators of brain homeostasis. Biochim Biophys Acta 2009; 1792(5) 417-22

[104] Kalmijn S. Fatty acid intake and the risk of dementia and cognitive decline: a review of clinical and epidemiological studies. J Nutr Health Aging 2000; 4(4) 202-7

[105] Hida K, Wada J, Eguchi J, Zhang H, Baba M, Seida A, Hashimoto I, Okada T, Yasuhara A, Nakatsuka A, Shikata K, Hourai S, Futami J, Watanabe E, Matsuki Y, Hiramatsu R, Akagi S, Makino H and Kanwar YS. Visceral adipose tissue-derived serine protease inhibitor: a unique insulin-sensitizing adipocytokine in obesity. Proc Natl Acad Sci U S A 2005; 102(30) 10610-5

[106] Bousquet M, St-Amour I, Vandal M, Julien P, Cicchetti F and Calon F. High-fat diet exacerbates MPTP-induced dopaminergic degeneration in mice. Neurobiol Dis 2012; 45(1) 529-38

[107] Choi JY, Jang EH, Park CS and Kang JH. Enhanced susceptibility to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine neurotoxicity in high-fat diet-induced obesity. Free Radic Biol Med 2005; 38(6) 806-16

[108] Morris JK, Bomhoff GL, Stanford JA and Geiger PC. Neurodegeneration in an animal model of Parkinson's disease is exacerbated by a high-fat diet. Am J Physiol Regul Integr Comp Physiol 299(4) R1082-90

[109] Thaler JP, Yi CX, Schur EA, Guyenet SJ, Hwang BH, Dietrich MO, Zhao X, Sarruf DA, Izgur V, Maravilla KR, Nguyen HT, Fischer JD, Matsen ME, Wisse BE, Morton

GJ, Horvath TL, Baskin DG, Tschop MH and Schwartz MW. Obesity is associated with hypothalamic injury in rodents and humans. J Clin Invest 2012; 122(1) 153-62

[110] Sriram K, Benkovic SA, Miller DB and O'Callaghan JP. Obesity exacerbates chemically induced neurodegeneration. Neuroscience 2002; 115(4) 1335-46

[111] Erbayat-Altay E, Yamada KA, Wong M and Thio LL. Increased severity of pentylenetetrazol induced seizures in leptin deficient ob/ob mice. Neurosci Lett 2008; 433(2) 82-6

[112] Ming GL and Song H. Adult neurogenesis in the mammalian brain: significant answers and significant questions. Neuron 2011; 70(4) 687-702

[113] Anderson MF, Aberg MA, Nilsson M and Eriksson PS. Insulin-like growth factor-I and neurogenesis in the adult mammalian brain. Brain Res Dev Brain Res 2002; 134(1-2) 115-22

[114] Ciaroni S, Cuppini R, Cecchini T, Ferri P, Ambrogini P, Cuppini C and Del Grande P. Neurogenesis in the adult rat dentate gyrus is enhanced by vitamin E deficiency. J Comp Neurol 1999; 411(3) 495-502

[115] Valente T, Hidalgo J, Bolea I, Ramirez B, Angles N, Reguant J, Morello JR, Gutierrez C, Boada M and Unzeta M. A diet enriched in polyphenols and polyunsaturated fatty acids, LMN diet, induces neurogenesis in the subventricular zone and hippocampus of adult mouse brain. J Alzheimers Dis 2009; 18(4) 849-65

[116] van Praag H, Christie BR, Sejnowski TJ and Gage FH. Running enhances neurogenesis, learning, and long-term potentiation in mice. Proc Natl Acad Sci U S A 1999; 96(23) 13427-31

[117] Kokoeva MV, Yin H and Flier JS. Neurogenesis in the hypothalamus of adult mice: potential role in energy balance. Science 2005; 310(5748) 679-83

[118] Bachner D, Ahrens M, Betat N, Schroder D and Gross G. Developmental expression analysis of murine autotaxin (ATX). Mech Dev 1999; 84(1-2) 121-5

[119] Bengzon J, Kokaia Z, Elmer E, Nanobashvili A, Kokaia M and Lindvall O. Apoptosis and proliferation of dentate gyrus neurons after single and intermittent limbic seizures. Proc Natl Acad Sci U S A 1997; 94(19) 10432-7

[120] Harry GJ, McPherson CA, Wine RN, Atkinson K and Lefebvre d'Hellencourt C. Trimethyltin-induced neurogenesis in the murine hippocampus. Neurotox Res 2004; 5(8) 623-7

[121] McNay DE, Briancon N, Kokoeva MV, Maratos-Flier E and Flier JS. Remodeling of the arcuate nucleus energy-balance circuit is inhibited in obese mice. J Clin Invest 2012; 122(1) 142-52

[122] Dias GP, Cavegn N, Nix A, do Nascimento Bevilaqua MC, Stangl D, Zainuddin MS, Nardi AE, Gardino PF and Thuret S. The role of dietary polyphenols on adult hippo-

campal neurogenesis: molecular mechanisms and behavioural effects on depression and anxiety. Oxid Med Cell Longev 2012; 2012(541971

[123] Stangl D and Thuret S. Impact of diet on adult hippocampal neurogenesis. Genes Nutr 2009; 4(4) 271-82

[124] Zainuddin MS and Thuret S. Nutrition, adult hippocampal neurogenesis and mental health. Br Med Bull 2012;

[125] Jeon BT, Jeong EA, Shin HJ, Lee Y, Lee DH, Kim HJ, Kang SS, Cho GJ, Choi WS and Roh GS. Resveratrol attenuates obesity-associated peripheral and central inflammation and improves memory deficit in mice fed a high-fat diet. Diabetes 2012; 61(6) 1444-54

[126] Lu J, Wu DM, Zheng YL, Hu B, Cheng W, Zhang ZF and Shan Q. Ursolic acid improves high fat diet-induced cognitive impairments by blocking endoplasmic reticulum stress and IkappaB kinase beta/nuclear factor-kappaB-mediated inflammatory pathways in mice. Brain Behav Immun 2011; 25(8) 1658-67

REVIEW ARTICLE-1

A Molecular Web: Endoplasmic Reticulum Stress, Inflammation, And Oxidative Stress

frontiers in
CELLULAR NEUROSCIENCE

REVIEW ARTICLE
published: 29 July 2014
doi: 10.3389/fncel.2014.00213

A molecular web: endoplasmic reticulum stress, inflammation, and oxidative stress

Namrata Chaudhari[1], Priti Talwar[1], Avinash Parimisetty[2], Christian Lefebvre d'Hellencourt[2] and Palaniyandi Ravanan[1]*

[1] Apoptosis and Cell Death Research Lab, School of Biosciences and Technology, Vellore Institute of Technology University, Vellore, India
[2] Groupe d'Etude sur l'Inflammation Chronique et l'Obésité, EA 41516, Plateforme CYROI, Université de La Réunion, Saint Denis de La Réunion, France

Edited by:
Lawrence Rajendran, University of Zurich, Switzerland

Reviewed by:
Thomas Launey, RIKEN, Japan
Julian Grosskreutz, University Hospital Jena, Germany

***Correspondence:**
Palaniyandi Ravanan, Apoptosis and Cell Death Research Lab, School of Biosciences and Technology, Vellore Institute of Technology University, Vellore 632014, Tamil Nadu, India
e-mail: ravanan.p@vit.ac.in

Execution of fundamental cellular functions demands regulated protein folding homeostasis. Endoplasmic reticulum (ER) is an active organelle existing to implement this function by folding and modifying secretory and membrane proteins. Loss of protein folding homeostasis is central to various diseases and budding evidences suggest ER stress as being a major contributor in the development or pathology of a diseased state besides other cellular stresses. The trigger for diseases may be diverse but, inflammation and/or ER stress may be basic mechanisms increasing the severity or complicating the condition of the disease. Chronic ER stress and activation of the unfolded-protein response (UPR) through endogenous or exogenous insults may result in impaired calcium and redox homeostasis, oxidative stress via protein overload thereby also influencing vital mitochondrial functions. Calcium released from the ER augments the production of mitochondrial Reactive Oxygen Species (ROS). Toxic accumulation of ROS within ER and mitochondria disturbs fundamental organelle functions. Sustained ER stress is known to potentially elicit inflammatory responses via UPR pathways. Additionally, ROS generated through inflammation or mitochondrial dysfunction could accelerate ER malfunction. Dysfunctional UPR pathways have been associated with a wide range of diseases including several neurodegenerative diseases, stroke, metabolic disorders, cancer, inflammatory disease, diabetes mellitus, cardiovascular disease, and others. In this review, we have discussed the UPR signaling pathways, and networking between ER stress-induced inflammatory pathways, oxidative stress, and mitochondrial signaling events, which further induce or exacerbate ER stress.

Keywords: endoplasmic reticulum stress, inflammation, oxidative stress, NF-κB, IRE1α, calcium

INTRODUCTION

Endoplasmic reticulum (ER) is a cardinal membrane-bound organelle comprising of interconnected highly branched tubules, vesicles, and cisternae. ER structure can be categorized as domains like nuclear envelope domain (array of proteins that are synthesized on rough ER and concentrated within the inner membrane), the rough and smooth ER domain (due to presence and absence of bound ribosomes respectively), and the regions that contact other organelles like plasma membrane, Golgi, vacuoles, mitochondria, peroxisomes, late endosomes, and lysosomes (for ease of direct transfer of lipids to their membranes and efficient calcium signaling) (Voeltz et al., 2002). ER functions majorly in translocating and integrating proteins (secretory and membrane proteins respectively), assisting their folding and transport (extracellular or to cell membrane), lipid biosynthesis, and maintaining calcium homeostasis. It is also a site for post translational modification (*N*-linked glycosylation) of proteins and is considered as a signaling organelle (Berridge, 2002; Fagone and Jackowski, 2009; Sammels et al., 2010; Braakman and Bulleid, 2011). Ribosomes embedded on rough endoplasmic reticulum (RER) are sites for protein synthesis and secretion. Smooth endoplasmic reticulum (SER) lacks bound ribosomes, therefore, inefficient in protein synthesis but is important for fatty acid and phospholipids synthesis, carbohydrate metabolism, lipid bilayer assembly, and regulation of calcium homeostasis. Cell type, cell function, and cell needs determine the role of ER in the cell. For example, liver cells high in SER aid in drug detoxification; while plasma cells, beta cells, and other secretory cells are rich in RER to meet their secretory protein demand; sarcoplasmic reticulum, a specialized form of the ER is more in muscle cells functioning in muscle contractions and relaxation (Alberts et al., 2002).

The excursion of secretory or membrane protein begins at ER. A protein destined to be modified in ER is marked with an N-terminal ER signal sequence. During co-translational modification, a signal recognition particle (SRP) recognizes and binds to signal sequence on nascent protein, ribosome, and SRP receptor on ER membrane, after which ribosome-nascent polypeptide chain complex is rapidly transferred to a membrane protein translocator, the SEC61 translocon. Signal peptide is cleaved by signal peptidase on ER membrane and finally the nascent chain enters the ER lumen via the translocon. In post translational modification, interaction of SEC61 translocon and completely synthesized protein is sufficient for its ER targeting (Lodish et al., 2000).

The proteins in ER are folded and modified under the vigilance of ER resident molecular chaperones and folding enzymes that

accelerate rate limiting reactions in protein folding mechanisms and thus assist them in attaining appropriate conformation and maturation. The glucose-regulated proteins (GRP) system including chaperones of Hsp70 family and co-chaperones of HSP40 family (Kampinga and Craig, 2010), ER lectin-like chaperone system including calnexin (CNX) and/or calreticulin (CRT) along with glucosidases and transferases (Rutkevich and Williams, 2011), and finally the protein disulfide isomerase (PDI) family of disulfide bond oxidase, reductase, and isomerase enzymes (Appenzeller-Herzog and Ellgaard, 2008) are the pillars of Endoplasmic Reticulum Quality Control system (ERQC). ERQC system has its particular vital role to play in converting a protein from nascent to native state (Nishikawa et al., 2005; Bukau et al., 2006; Hebert and Molinari, 2007; Araki and Nagata, 2011). The aptly folded proteins are transported to Golgi via vesicular carriers and finally escorted to their destinations (plasma membrane or lysosomal membrane or loaded into granules for secretion) (Szul and Sztul, 2011). Selective chaperones of ERQC system and specific mannose lectins like ER degradation-enhancing α-mannosidase-like protein (EDEM) are capable of tagging unassembled, misfolded, or unfolded proteins, which facilitates their identification and retrotranslocation to cytosol via SEC61 translocon where they are steered to ubiquitin proteasome degradation system or are eliminated by autophagic degradation (Smith et al., 2011).

Here, we review the signaling events occurring within ER under stressed conditions, its counter effect on other organelles, and give crisp information on interaction between ER stress pathways, oxidative stress, and inflammation.

UNFOLDED-PROTEIN RESPONSE

Accumulation of unfolded/misfolded/mutated proteins (Hetz and Soto, 2006; Viana et al., 2012), disturbances in cellular redox regulation and endogenous reactive oxygen species (ROS) production (Fedoroff, 2006), hypoxia (Feldman et al., 2005; Sawada et al., 2008), hyperglycemia, and hyperlipidemia (Fonseca et al., 2011; Back et al., 2012), aberrations in calcium regulation (Gorlach et al., 2006), viral infections (He, 2006; Zhang and Wang, 2012) act as stress signals and alter ER homeostasis making it dysfunctional. In response to such diverse signals, ER elicits a protective or adaptive response called unfolded-protein response (UPR) with an aim to restore ER homeostasis; however, if the stress signal is severe and/or prolonged, ER triggers cell death pathways (Szegezdi et al., 2006; Kim et al., 2008a; Cheng and Yang, 2011; Benbrook and Long, 2012).

Stress signals culminate in overloading ER with proteins and exhausting the ER machinery. ER stress is thought to be and in certain cases proved to play a key role in diseases like Alzheimer's disease (Salminen et al., 2009; Viana et al., 2012), Parkinson's disease (Wang and Takahashi, 2007; Cali et al., 2011), amyotrophic lateral sclerosis (ALS) (Lautenschlaeger et al., 2012; Tadic et al., 2014), poly glutamine diseases (Vidal et al., 2011), ischemia (Doroudgar et al., 2009), atherosclerosis (Zhou and Tabas, 2013), bipolar disorder (Hayashi et al., 2009), prion diseases (Xu and Zhu, 2012), cancer (Tsai and Weissman, 2010), diabetes (Papa, 2012), auto immune disorders (Zhong et al., 2012), and cardiovascular disorders (Minamino et al., 2010). Interestingly, there are reports demonstrating that ER stress inhibition could protect against neuronal injury (Qi et al., 2004; Sokka et al., 2007), ischemia (Nakka et al., 2010), cardiovascular diseases (Teng et al., 2011), respiratory disorders (Hoffman et al., 2013), atherosclerosis (Zhou et al., 2013), and sleep apnea (Zhu et al., 2008), in *in vivo* murine models.

THE MACHINERY OF UPR

The adaptive UPR comprises of signal transduction pathways initiated by ER proximal UPR transmembrane proteins: inositol-requiring kinase 1 (IRE1α), activating transcription factor 6 (ATF6), and double-stranded RNA-activated protein kinase (PKR)-like endoplasmic reticulum kinase (PERK) in an attempt to restore homeostasis and normal ER functions (Schroder and Kaufman, 2005). These UPR transducer proteins are negatively

Abbreviations: ADNF, activity-dependent neurotrophic factor; AIP1, ASK1-interacting protein-1; ALS, amyotrophic lateral sclerosis; AMPA, α-amino-3-hydroxy-5-methylisoxazole-4-propionate acid; AP-1, activator protein-1; APR, acute phase response; ARD, ankyrin repeat domain; ASK1, apoptosis signal-regulating kinase; ATF4, activating transcription factor 4; ATF6, activating transcription factor 6; ATP, adenosine 5-triphosphate; Bad, Bcl-2-associated death promoter; BAFF, B cell activating factor; BAPTA-AM, 1,2-Bis(2-aminophenoxy)ethane-*N*,*N*,*N*′,*N*′-tetraacetic acid tetrakis(acetoxymethyl)ester; Bcl2, B-cell lymphoma 2; Bcl xl, B-cell lymphoma-extra large; BHA, butylated hydroxyanisole; Bim, B-cell lymphoma 2 interacting mediator of cell death; BIP, immunoglobulin heavy chain binding protein; CDDO, 2-cyano-3,12-dioxoolean-1,9-dien-28-oic acid; CDDO Me, methyl 2-cyano-3,12-dioxooleana-1,9(11)dien-28-oate, Bardoxolone methyl; CEMB, cyano enone of methyl boswellates; CHOP, CCAAT/enhancer-binding protein homologous protein; CNX, Calnexin; CREB, cAMP response-element-binding; CRP, C-reactive protein; CRT, Calreticulin; DTT, dithiothreitol; EDEM, ER degradation-enhancing α-mannosidase-like protein; eIF2α, eukaryotic translation initiation factor; ER, endoplasmic reticulum; ERAD, ER-associated degradation; ERKs, extracellular signal-regulated kinases; ERO, ER membrane associated oxidoreductin; ERSE, ER stress response element; ERQC, Endoplasmic reticulum quality control system; FAD, Flavin adenine dinucleotide; FoxO1, fork head box O1; GLS, Golgi-localization signal; GRP, glucose-regulated protein; GSH, glutathione; GSSH, glutathione to oxidized glutathione; IBD, inflammatory bowel disease; IEC, intestinal epithelial cells; IKK, IκB kinase; IAPs, inhibitor of apoptotic proteins; IL-1R, interleukin 1-receptor; InsP3, inositol 1,4,5-triphosphate RyRs, ryanodine receptors; IRAK2, interleukin-1 receptor-associated kinase-2; IRE1α, inositol-requiring kinase 1; IRES, internal ribosome entry site; JAB1, Jun activation domain-binding protein-1; JIK, c-Jun-N-terminal inhibitory kinase; LPS, lipopolysaccharides; MAM, mitochondrial associated membranes; MAPK, mitogen activated protein kinase; MAPKKK, mitogen activated protein kinase kinase kinase; MCP1, monocyte chemoattractant protein-1; MM, multiple myeloma; NAC, *N*-acetyl-l-cysteine; nACh, nicotinic acetylcholine; NDGA, nordihydroguaiaretic acid; NEMO, NF-kappa-B essential modulator; NES, nuclear export sequence; NF-κB, nuclear factor κB; NGF, nerve growth factor; NIK, NF-κB-inducing kinase; NLS, nuclear localization signal; NMDA, *N*-methyl-d-Aspartate; NO, nitric oxide; NOD, non-obese diabetic; Nrf2, nuclear erythroid 2 p45-related factor 2; Oasis, old astrocyte specifically induced substance; PBA, 4-phenylbutyrate; PDI, protein disulfide isomerase; PDTC, pyrrolidine dithiocarbamate; PERK, double-stranded RNA-activated protein kinase (PKR)-like endoplasmic reticulum kinase; RER, rough endoplasmic reticulum; RHR, Rel homology region; RIP, regulated intramembrane proteolysis; RNS, reactive nitrogen species; ROI, reactive oxygen intermediates; ROS, reactive oxygen species; S1P, site-1 protease; S2P, site-2 protease; SAP, serum amyloid P-component; sAPP, secreted ß-amyloid precursor protein; SER, smooth endoplasmic reticulum; SERCA, Sarco/ER calcium-ATPase; SOD, superoxide dismutase; SRP, signal recognition particle; sXBP1, spliced XBP1; TAD, transcription activation domain; TCA, tricarboxylic acid; TLR, Toll-like receptor 4; TMB-8, 3,4,5-trimethoxybenzoic acid 8-(diethylamino)octyl ester; TNFR, tumor necrosis factor receptor; TRAF2, tumor necrosis factor receptor-associated factor 2; TUDCA, taurine-conjugated ursodeoxycholic acid; UPR, unfolded-protein response; XBP1, X-box-binding protein 1.

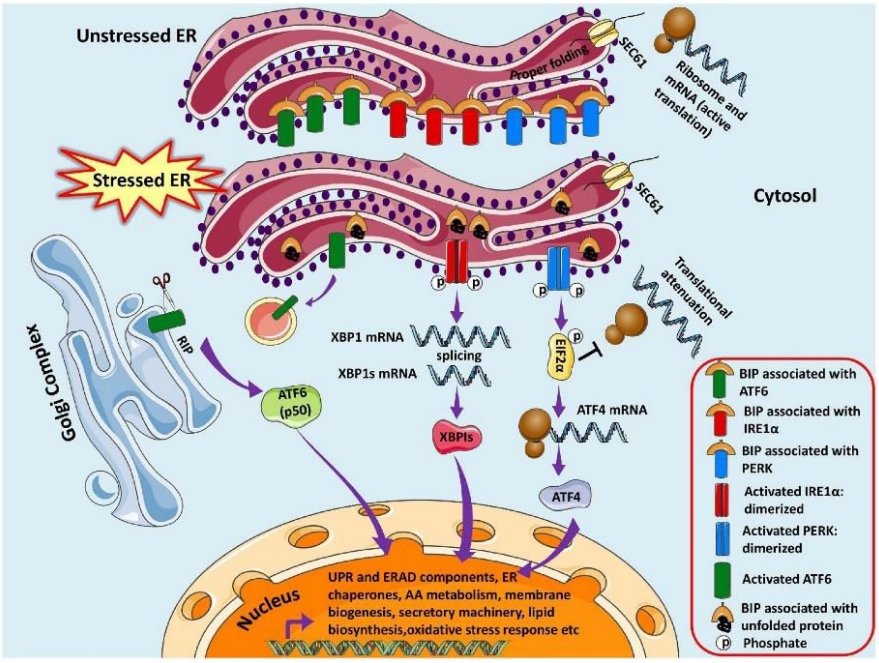


FIGURE 1 | Unfolded protein response pathways are shown. The newly synthesized protein destined for modification in ER enters through SEC61 channel and undergoes folding and maturation. Under basal/unstressed conditions, the BIP (immunoglobulin heavy chain binding protein) binds to ATF6 (activating transcription factor 6), IRE1α (inositol-requiring kinase 1), and PERK [double-stranded RNA-activated protein kinase (PKR)-like endoplasmic reticulum kinase] thereby inhibiting them. During ER stress, BIP dissociates from the three UPR sensors and binds to unfolded/misfolded proteins, thus initiating adaptive signaling events to help ER recover from the stress. Dissociation of BIP from ATF6 unmasks a Golgi-localization signal (GLS, not shown here), which facilitates its translocation to Golgi where it undergoes regulated intramembrane proteolysis (RIP) by resident proteases, Site-1 protease (S1P) and Site-2 protease (S2P). The released ATF6 acts as a transcription factor, which travels to the nucleus and binds to ER-stress response elements (ERSE) and induces transcription of several genes, including BIP, CHOP (CCAAT/enhancer-binding protein homologous protein), and X-box-binding protein 1 (XBP1). Similarly IRE1α is activated and undergoes homodimerization and autophosphorylation, thereby activating the endoribonuclease activity, which splices XBP1 mRNA to spliced XBP1 mRNA, which codes for a transcription factor XBP1s that translocates to nucleus and regulates genes involved in UPR and ER-associated degradation (ERAD). Finally, the release of BIP activates PERK pathway, which initiates a global translational arrest by phosphorylating the translation initiation factor 2α (EIF2α), thus decreasing ER protein load. ATF4 (Activating Transcription Factor 4) mRNA escapes translational suppression exclusively as it possesses internal ribosome entry site (IRES) sequences in the 5′-untranslated regions. ATF4 enters nucleus and regulates expression of UPR target genes.

regulated by the chaperone GRP78/BIP (immunoglobulin heavy chain binding protein) in unstressed or healthy ER at their luminal ends (amino terminal), however, increase in unfolded proteins causes dissociation of Grp78/BIP thereby releasing the inhibition and thus eliciting the response (Bertolotti et al., 2000; Pfaffenbach and Lee, 2011) (**Figure 1**).

THE IRE1α PATHWAY

Homo-oligomerization of activated IRE1α opens the Ser/Thr kinase domain at the cytosolic carboxyl terminal, aligning it for trans-autophosphorylation thereby activating the endoribonuclease domain (Shamu and Walter, 1996; Sidrauski and Walter, 1997; Liu et al., 2003). X-box-binding protein 1 (XBP1) mRNA is spliced unconventionally by this RNAase domain of IRE1α; cleaving a 26 nucleotide intron to produce a spliced mRNA that codes for bZIP-family transcription factor sXBP1 (spliced XBP1). Once it is translocated to the nucleus, it can dimerize or act in tandem with other co-regulators and regulate several genes involved in UPR and ER-associated degradation (ERAD) by binding to ER stress response element (ERSE) promoter (Yoshida et al., 1998, 2001a; Lee et al., 2003; Van Huizen et al., 2003). The dimerized and activated IRE1α collaborates with modulators and adaptors on the cytosolic end to initiate signaling events in response to the intensity and duration of stress. Adaptor-like tumor necrosis factor receptor (TNFR)-associated factor 2 (TRAF2), an E3 ubiquitin ligase, recruits apoptosis signal-regulating kinase (ASK1), a mitogen-activated protein kinase kinase kinase (MAPKKK) that has been shown to relay various stress signals to the downstream MAPKs consequently activating Jun N-terminal kinase (JNK) and p38 MAP kinase (Derijard et al., 1995; Urano et al., 2000; Nishitoh et al., 2002). IRE1α also triggers activation of other kinases such as extracellular signal-regulated kinases (ERKs) as well as nuclear

factor κB (NF-κB) pathways (Kaneko et al., 2003; Nguyen et al., 2004; Hu et al., 2006).

THE PERK PATHWAY

Double-stranded RNA-activated protein kinase (PKR)-like endoplasmic reticulum kinase, another UPR mediator, also undergoes similar dimerization and autophosphorylation (Ma et al., 2002a) to initiate a transient cellular translational arrest by phosphorylating at serine residue 51 and inactivating eukaryotic translation initiation factor (eIF2α) (Harding et al., 1999), the most studied and well-known substrate of PERK, thereby reducing protein load on ER and causing cell-cycle arrest (Brewer and Diehl, 2000). However, the phosphorylated eIF2 α can translate genes with internal ribosome entry site (IRES) sequences in the 5′-untranslated regions sidestepping the eIF2α translational block (Vattem and Wek, 2004; Schroder and Kaufman, 2005); one such gene being *ATF4*, which encodes ATF4 (Activating Transcription Factor 4), a member of the cAMP response-element-binding (CREB) family of transcription factors (Lu et al., 2004) that induces expression of several genes central in cell survival and UPR (Harding et al., 2000a,b, 2003; Scheuner et al., 2001; Ma et al., 2002b; Raven et al., 2008). This PERK-mediated translational block is also essential for activation of NF-κB (Jiang et al., 2003). One of the downstream substrate of PERK is Keap1, which sequesters the nuclear erythroid 2 p45-related factor 2 (Nrf2) in cytoplasm. PERK phosphorylates Keap1, thereby freeing Nrf2 of inhibition and facilitating its nuclear import for expression of antioxidant and detoxification enzymes (Cullinan et al., 2003; Cullinan and Diehl, 2006).

THE ATF6 PATHWAY

Release of GRP78, unmasks the Golgi-localization signal (GLS), forms ER membrane tethered ATF6 proteins expediting their translocation to the Golgi (Chen et al., 2002); where they undergo regulated intramembrane proteolysis (RIP) by resident proteases Site-1 protease (S1P) and Site-2 protease (S2P) (Ye et al., 2000) cleaving at a juxtamembrane site, producing and releasing active ATF6 transcription factors into the cytosol (Haze et al., 1999). They will then migrate into the nucleus and homodimerize or heterodimerize with other transcription factors to regulate gene expression of proteins involved in UPR (Yoshida et al., 1998, 2001b; Yamamoto et al., 2007; Adachi et al., 2008). Not a long ago, new membrane-bound bZIP transcription factors were identified with similar structural and proteolysis pattern as ATF6. These include Luman (CREB3), Oasis (old astrocyte specifically induced substance, CREB3L1), BBF2H7 (CREB3L2), CREBH (CREB3L3), and Tisp40 (CREB4, CREB3L4) (Bailey and O'Hare, 2007). Regardless of structural similarity with ATF6, these factors execute distinctive functions in regulating UPR based on activating stimuli/stress, cell type, and response element binding (Asada et al., 2011). CREBH, a liver-specific transcription factor does not serve as a UPR transactivator for expression of classic ER chaperone genes regulated under ERSE; however, it modulates ER stress response genes that contain ERSE in their promoter regions. CREBH is cleaved upon ER stress thereby activating expression of acute phase response (APR) genes like those encoding serum amyloid P-component (SAP) and C-reactive protein (CRP) providing a correlation between ER stress and acute inflammatory responses (Zhang et al., 2006).

Thus, all the three UPR sensors are regulated by one master regulator, i.e., GRP78/BIP (Hendershot, 2004). The mission of ER under stress is to thus increase the expression of transcription factors, which orchestrates and initiates production of ER chaperones and genes involved in ERAD, to provide tolerance to the stress and restore homeostasis. Thus, ER mandatorily has to be dynamic to meet its changing needs, which are managed by integrated signaling pathways that constantly monitor the levels of ER machinery. If the UPR is incompetent to abolish the stress, ER prompts a cell death program, which can be apoptotic (Szegezdi et al., 2006; Tabas and Ron, 2011; Gorman et al., 2012), non-apoptotic (Ullman et al., 2008), or autophagic (Ogata et al., 2006; Yorimitsu et al., 2006; Ullman et al., 2008; Cheng and Yang, 2011) therefore resulting in cellular demise (Heath-Engel et al., 2008; Benbrook and Long, 2012; Logue et al., 2013).

INTEGRATION OF ER STRESS AND OTHER PATHWAYS IN NEURODEGENERATION

The major cellular communicating compartments include nucleus, ER, mitochondria, and Golgi that initiate signaling pathways to help the cell respond to various intracellular and extracellular signals/stresses. Besides UPR, other pathways emanating from ER collaborate with mitochondria and nucleus to regulate the cellular responses (**Figure 2**). The interaction among reactive species formation, disturbed calcium homeostasis, mitochondrial collapse, and inflammation is a common phenomenon existing in various disorders and its connection with ER stress is recently being explored and demands more research. The complications underlying neurodegenerative disorders are multifactorial and may include genetic predisposition (Bertram and Tanzi, 2005; Lill and Bertram, 2011), environmental factors (Cannon and Greenamyre, 2011), cellular stressors such as oxidative stress and free radical production (Gandhi and Abramov, 2012), excitotoxicity (Dong et al., 2009), neuroinflammation (Glass et al., 2010), disruption of calcium-regulating systems (Bezprozvanny, 2009), mitochondrial dysfunction (Lezi and Swerdlow, 2012), and misfolded protein accumulation (Matus et al., 2011).

Central nervous system being most aerobic includes neurons, which are differentiated and are highly mitochondrial dependent. They require massive amount of adenosine 5-triphosphate (ATP); to satisfy their tremendous metabolic demands, for maintaining ionic gradients across the cell membranes and for neurotransmission, thereby being very sensitive to mitochondrial stress (Ames, 2000; Nicholls and Budd, 2000). Mitochondria are known to play a central role in many vital cell functions including generation of cellular energy in the form of ATP, maintaining intracellular calcium homeostasis, ROS formation, apoptosis, fatty acid oxidation, and steroid synthesis. Mitochondrial stress characterized by abnormal mitochondrial morphology, biochemical dysfunction, and disturbed redox homeostasis is a common manifestation in brain aging and several chronic neurodegenerative diseases like Alzheimer's disease, Parkinson's disease, ALS, and Huntington's disease (Beal, 2005; Lezi and Swerdlow, 2012). Likewise, mitochondrial dysfunction is also predominant in pathogenesis of cancer, cardiovascular diseases, and several metabolic diseases like

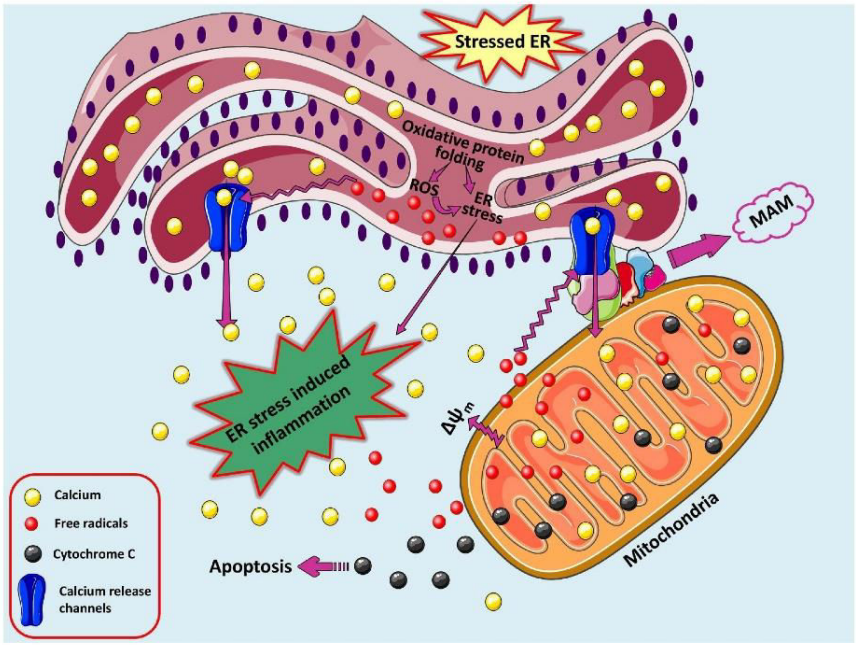


FIGURE 2 | A loop of oxidative stress, ER stress leading to inflammation. During protein overload, ROS are generated in the ER as a part of an oxidative folding process during electron transfer between protein disulfide isomerase (PDI) and endoplasmic reticulum oxidoreductin-1 (ERO-1). ROS can target ER resident proteins, enzymes, and chaperones (not shown) and ER based calcium (Ca^{2+}) channels, leading to the release of calcium from the ER into the cytosol and ER-stress signaling. Increased cytosolic calcium and calcium entry in mitochondria from ER via MAM-associated channels can stimulate mitochondria metabolism to produce more ROS. Increased mitochondrial calcium concentration causes cytochrome *c* release, altered membrane potential that eventually triggers cellular death programs. The increased protein folding demand, calcium and ROS signaling integrates with UPR pathways and can potentially lead to inflammatory responses.

obesity, insulin resistance, and diabetes (Lesnefsky et al., 2001; Wallace, 2005; Kim et al., 2008b; Mantena et al., 2008; Hu and Liu, 2011).

Mitochondria have a noteworthy role in neurodegenerative diseases, where various disease-specific proteins (misfolded/mutated) interact with mitochondria and/or affect mitochondrial function (Johri and Beal, 2012). There are emerging evidences suggesting that triggering of UPR pathways by cellular and/or extracellular accumulation of misfolded/aberrant proteins in several human neurodegenerative conditions could cause severe loss in neuronal functions and viability (Doyle et al., 2011; Bernales et al., 2012). In addition, imbalance in Ca^{2+} homeostasis, which is significant in neurodegenerative diseases affects mitochondria and ER as they are physically and functionally tightly interconnected via mitochondrial-associated membranes (MAM), which participates in Ca^{2+} signaling, lipid transport, energy metabolism, and cell survival. Thus, apart from coordinating pathways involved in cell life and death, their physical association is vital and any loss of communication in them is known to complicate and worsen the prevailing conditions in neurodegenerative diseases (Malhotra and Kaufman, 2011; Vannuvel et al., 2013).

CALCIUM LINKS ER AND MITOCHONDRIA

In the brain, calcium participates in various intracellular and extracellular processes and its fluctuations across the plasma membrane and between intracellular compartments play crucial roles in integral functions of neurons like neuronal excitability, synaptic transmission and plasticity, memory formation, neurotransmitter release, activation of specific calcium-dependent signal transduction pathways, and gene transcription (Berridge, 1998). The free cytosolic calcium levels in neurons are maintained around 0.1–0.5 μM, while the extracellular levels being ~1 mM. On encountering stimulatory signals, extracellular calcium influx or calcium release from intracellular stores is triggered. Calcium can enter the neuronal cell by voltage-operated channels or by receptor-operated channels, on the plasma membrane controlled by neurotransmitters. Later includes glutamate receptors like *N*-methyl-D-aspartate (NMDA), α-amino-3-hydroxy-5-methylisoxazole-4-propionate acid (AMPA), kainic acid, nicotinic acetylcholine (nACh), serotonin (5-HT3) and adenosine 5-triphosphate (ATP) P2X receptors. Na-K-ATPase and Ca^{2+}-ATPases maintain ionic gradient along the neuronal membrane at the expense of considerable amounts of ATP. The main intracellular calcium store is the

ER (10–100 mM), acting as a major buffering system that functions as a sink for Ca^{2+} storage, from where calcium can be released into the cytosol via activation of the inositol 1,4,5-triphosphate receptors [InsP3Rs, sensitive to inositol 1,4,5-triphosphate (InsP3)] or ryanodine receptors (RyRs, sensitive to cyclic ADP ribose). Both the InsP3Rs and the RYRs are also sensitive to Ca^{2+}, and this process of calcium-induced calcium release can set up propagated calcium waves. Resting cytosolic calcium levels are partly maintained by calcium-binding and calcium-buffering proteins (e.g., calmodulin, calcineurin, calretinin, calbindin, or parvalbumin) or by active uptake into internal stores by the Sarco/ER calcium-ATPase (SERCA) at the ER membrane or by the mitochondrial uniporter. Minor transient alterations in calcium levels occurring during physiological processes are salient to neuronal functioning; however, pathological conditions accompanied by prolonged increase in calcium levels can overwhelm the calcium regulatory network, making the cell difficult to recuperate. Rampant fluctuations in this highly regulated calcium homeostasis not only disturbs normal brain physiology but also hampers neuronal integrity and viability (Kawamoto et al., 2012). An accumulation of misfolded proteins in the ER lumen can cause leaky calcium efflux from the ER, possibly via inositol-triphosphate receptors (Deniaud et al., 2008). Additionally during Ca^{2+} overload, calcium influx increases in mitochondria and ER, thereby causing changes in mitochondrial pH and ROS production accompanied by altered mitochondrial membrane potential and opening of permeability transition pore with subsequent release of cytochrome *c*, cardiolipin peroxidation, and activation of several calcium-dependent proteins and kinases (Smaili et al., 2009). Thus, calcium-induced ROS increase and ROS-mediated calcium vulnerability create a self amplifying loop (Peng and Jou, 2010).

In the adaptive phase of UPR, ER-to-mitochondria Ca^{2+} transfer under regulation by MAM proteome maintains mitochondrial metabolism and ATP production that safeguards cellular functions. However, severe ER stress induces mitochondrial Ca^{2+} overload, ROS accumulation, and ATP depletion and thus activates the mitochondria-dependent apoptosis (Raturi and Simmen, 2013). Prolonged stimulation of neuronal glutamate receptors results in excitotoxicity due to massive calcium influx activating several enzymes and proteases eventually causing neuronal loss in neurodegenerative diseases. This is accompanied by excessive nitric oxide (NO) production by neuronal NO synthase (Calabrese et al., 2000; Dong et al., 2009). Significant loss in PDI function due to NO-mediated S-nitrosylation causes dysregulated protein folding and accumulation of polyubiquitinated proteins leading to neuronal death via ER stress. This was backed up by the fact that *S*-nitrosylated PDI was found in neurodegenerative diseased brains suggesting ER dysfunction as critical factor that relates NO-induced cellular stress to neurodegeneration (Uehara et al., 2006). Chen et al. (2013) identified NO-mediated S-nitrosylation of PDI as one of the liable factors for accumulation of mutant superoxide dismutase (SOD) 1 aggregates in ALS.

OXIDATIVE STRESS IN ER AND MITOCHONDRIA

Free radicals are generated from two sources: the UPR-regulated oxidative folding machinery in the ER (Bhandary et al., 2012) and mitochondria (Cadenas and Davies, 2000). ROS and reactive nitrogen species (RNS) are byproducts of normal cellular metabolism and are also produced in response to multiple stresses in various compartments of cell by enzymatic or non-enzymatic processes. ROS and RNS are known to play dichotomous role as being both harmful at high concentrations while beneficial at moderate/low concentrations and in harmony with cellular antioxidant defense mechanisms that they maintain cellular redox homeostasis (Calabrese et al., 2000; Finkel, 2011; Dasuri et al., 2013). Oxidative stress is a condition in which the production of reactive species is alarmingly high and the available antioxidant defenses is limited; resulting in damage to DNA, proteins, sugars, and lipids by the excessive free radicals (Valko et al., 2007). Superoxide anion (triplet stage molecular oxygen) produced majorly in mitochondria is a primary ROS and is a precursor for many secondary ROS, including hydrogen peroxide, hydroxyl radical, hypochlorous acid, and hydroperoxyl radical. Similarly nitric oxide is produced during arginine metabolism and can be converted to various other RNS such as nitrosonium cation, nitrite and nitrate radical, nitroxyl anion, or peroxynitrite. Complexes I and III of mitochondrial respiratory chain and α-ketoglutarate dehydrogenase in the tricarboxylic acid (TCA) cycle contribute to ROS production. There also exist mitochondrial enzymes that detoxify/scavenge free radicals (Stamler et al., 1992; Turrens, 2003).

Endoplasmic reticulum provides an exclusive oxidizing-folding environment to the proteins to facilitate disulfide bond formation and this process is believed to contribute to 25% of ROS generated by cell (Tu and Weissman, 2004; Malhotra and Kaufman, 2007). PDI, a member of the thioredoxin superfamily catalyzes disulfide bond formation through thiol-disulfide oxidation, reduction, and isomerization. A flavin adenine dinucleotide (FAD)-dependent reaction is carried out by ERO-1 (ER membrane associated oxidoreductin) involving transfer of electrons from reduced PDI to molecular oxygen (O_2), resulting in ER protein folding-induced oxidative stress. Erroneous disulfide bonds are reduced by glutathione (GSH) thereby decreasing the reduced glutathione to oxidized glutathione (GSSH) ratio and altering the redox environment in the ER. The ER ratio of GSH/GSSH ranges from 1:1 to 3:1, while the cellular ratio ranges from 30:1 to 100:1. Lastly, ER transmembrane protein NADPH oxidase complex, Nox4 is also involved in producing superoxide anion and hydrogen peroxide (Hwang et al., 1992; Santos et al., 2009).

ER STRESS AND INFLAMMATION

Endoplasmic reticulum stress and inflammation are related in a way that under acute trigger, they safeguard the cellular viability and functions and when chronically induced they are destructive and go beyond physiological control. Recent research reveal connections at multiple levels between UPR and inflammation and therefore focuses are now deviated to understand involvement of ER stress in specialized cells and tissues concerning inflammatory and immune responses. ER stress-induced inflammation primarily serves to limit the tissue damage and facilitate tissue repair; however, it largely depends on the target cell type, the disease stage, and the type of ER stressor. Several inflammatory chronic diseases including diabetes, obesity, neurodegenerative diseases, atherosclerosis, arthritis, respiratory diseases, irritable bowel syndrome, cardiovascular diseases, cancer, and many metabolic diseases have

ER stress as factor involved in progression of the disease (Pahl and Baeuerle, 1995; Zhang and Kaufman, 2008; Garg et al., 2012; Verfaillie et al., 2013).

NF-κB – A KEY PLAYER IN MEDIATING INFLAMMATORY RESPONSE

NF-κB plays an essential role of transcriptional regulator in mediating inflammatory responses, immune and stress responses, and regulates apoptosis, proliferation, differentiation, and development. Under various stimulatory conditions by inflammatory cytokines, viral and bacterial infections, or physical trigger by UV irradiation or cellular physiological stresses, NF-κB is activated and NF-κB target genes are expressed. NF-κB is a dimeric protein comprising of different combinations of five Rel family members [p65 (RelA), RelB, c-Rel, p105/p50 (NF-κB1) and p100/52 (NF-κB2)] therefore forming homo or heterodimers. They contain a conserved ~300 amino acid region called Rel Homology Region (RHR), which is composed of DNA-binding domain at its N-terminal, followed by a short flexible linker, C terminal dimerization domain, and finally a nuclear localization signal (NLS) that completes the RHR. p65/RelA, c-Rel, and RelB, contain a transcription activation domain (TAD) beyond the RHR at extreme carboxy terminal ends and are responsible for transcribing NF-κB target genes. Consequently, NF-κB dimers having minimum one of them (p65/RelA, c-Rel, and RelB) are active transcription factors. NF-κB dimers solely made of p50 and p52 subunits repress transcription since they lack TAD, inspite of being capable of nuclear localization and DNA binding. Unless required, NF-κB is sequestered in cytoplasm in its inactive form by constitutively expressing inhibitors of NF-κB (IκB: IκBα, IκBβ, IκBε) and is activated by signal-induced phosphorylation and consequential proteosome degradation of inhibitor by IκB kinase (IKK). IκBα, IκBβ, and IκBε contain a central ankyrin repeat domain (ARD), which has conserved serine residues for phosphorylation by IKK and also conserved lysine amino acids for poly-ubiquitination at its amino terminal. IκBα exclusively has a functional nuclear export sequence (NES), which is not masked on binding to NF-κB therefore restrains the complex NF-κB:IκBα to cytoplasm. NF-κB:IκBβ complexes can reside stably in either nucleus or cytoplasm. IκBε and IκBα are found to function as negative feedback regulators for NF-κB escorting it back to cytoplasm (Arenzana-Seisdedos et al., 1995; Kearns et al., 2006). All the three inhibitors of NF-κB at their carboxy terminal ends have a short sequence rich in the amino acids proline, glutamic acid, serine, and threonine. NF-κB precursor's p100 and p105 are also inhibitors of NF-κB in an entire different fashion involving multiprotein assemblies. Dissociation of IκB from NF-κB unmasks NLS, which facilitates NF-κB's translocation in nucleus thereby transcribing NF-κB target genes involved in inflammatory responses. The IKK is composed of three subunits: IKKα and IKKβ are legitimate kinases while IKKγ (NEMO, NF-kappa-B essential modulator which interacts with IKKβ) is known to play a regulatory role instead. The canonical pathway involves activation of cytokine receptors [(TNFR), interleukin 1 (IL-1) receptor (IL-1R)], antigen receptors and pattern-recognition receptors, Toll-like receptor 4 (TLR4), and subsequent phosphorylation of IκBα by IKKβ and NEMO thereby freeing mostly p65-containing heterodimers for nuclear translocation. The alternative or non-canonical pathways are triggered by activation of specific members of the TNF cytokine family, such as CD40 ligand, B cell activating factor (BAFF) and lymphotoxin-β following IKKα-mediated processing of precursor protein p100 to p52 forming active p52–RelB complexes. NF-κB-inducing kinase (NIK) is known to activate IKKα, whereas IKKβ can be activated by multiple kinases (Dixit and Mak, 2002; Hayden and Ghosh, 2008; Huxford and Ghosh, 2009; Lawrence, 2009; Oeckinghaus et al., 2011).

THE ER STRESS AS A TRIGGER FOR INFLAMMATION

Pahl and Baeuerle in 1995 demonstrated that NF-κB as a mixture of p5O/p65 and p5O/c-rel heterodimers, functions in a novel ER-nucleus signal transduction pathway upon gradual accumulation of proteins in ER caused by over expression of the immunoglobulin μ heavy chains. Similarly, tunicamycin, 2-deoxyglucose, brefeldin A, and thapsigargin are capable of eliciting UPR (grp induction) and activating NF-κB. However, castanospermine (glucosidase inhibitor) and dithiothreitol (DTT) potentially activate the UPR but not the NF-κB pathway; and tumor necrosis factor α (TNFα) and p65 over expression activate NF-κB but not the UPR. On the other hand, synthetic triterpenoids such as 2-cyano-3,12-dioxoolean-1,9-dien-28-oic acid (CDDO), Methyl 2-cyano-3,12-dioxooleana-1,9(11)dien-28-oate, Bardoxolone methyl (CDDO-Me) and cyano enone of methyl boswellates (CEMB) have been known to induce UPR and block the NF-κB signaling (Zou et al., 2008; Ravanan et al., 2011a,b). Another interesting finding was that preincubation with the antioxidant pyrrolidinedithiocarbamate (PDTC) inhibited the NF-κB activation induced by tunicamycin, 2-deoxyglucose, and brefeldin A implicating oxidative stress as a trigger for NF-κB activation (Pahl and Baeuerle, 1995). In their further research, they found that ER stress-mediated NF-κB activation depends on ER calcium efflux (by thapsigargin or cyclopiazonic acid), followed by formation of reactive oxygen intermediates (ROI). Preincubation with calcium chelators like 1,2-bis(2-aminophenoxy)ethane-*N*,*N*,*N*′,*N*′-tetraacetic acid tetrakis(acetoxymethyl)ester (BAPTA-AM) and 3,4,5-trimethoxybenzoic acid 8-(diethylamino)octyl ester (TMB-8); and antioxidants like *N*-acetyl-L-cysteine (NAC), butylated hydroxyanisole (BHA), rotenone, nordihydroguaiaretic acid (NDGA), DTT, and pyrrolidine dithiocarbamate (PDTC, to less extent) blocked NF-κB activation. In addition, calcium chelators inhibited ROI formation in response to thapsigargin and cyclopiazonic acid, implying that calcium release precedes ROI formation ultimately mediating ER overload-dependent NF-κB activation. Use of tepoxalin showed that ROI production was a result of cyclooxygenases or lipoxygenases in response to thapsigargin (Pahl and Baeuerle, 1996, 1997).

The close link between ER stress and inflammation is a likely contributor to the integration of ER function and metabolic homeostasis, considering the significant role of inflammation in metabolic diseases (Hotamisligil, 2006). For instance, leptin (an important adipokine acts on receptors in the hypothalamus of the brain where it inhibits appetite) resistance has been known to cause obesity and interestingly ER stress has been found to induce leptin resistance (Hosoi et al., 2008). Non-obese diabetic (NOD) mice exhibited dysfunction of the islet β-cell and glaring ER stress prior to onset of hyperglycemia in type 1 diabetes

conditions. Several fold increase in NF-κB target genes indicated clear crosstalk between ER stress, cytokine signaling, and inflammation defining the loss of β-cells and their functions in NOD mice (Tersey et al., 2012). Pre-existing ER stress though mild, sensitizes the pancreatic cells to weak inflammatory stimulus causing an aggravated inflammatory response and accelerating the development of type 1 diabetes. Miani et al. (2012) showed involvement of ER stress specifically IRE1α-XBP1 pathway in NF-κB activation and expression of its target genes via modulation of fork head box O1 (FoxO1) protein concluding that β-cell-ER stress triggers exacerbated local inflammation. ER-stressed smooth muscle cells locally boost leukocyte adhesion by forming a hyaluronan rich extracellular matrix promoting inflammatory responses (Majors et al., 2003). Paneth and Goblet cells of intestinal epithelium are XBP1-dependent for survival and to execute their secretory functions. Intestinal inflammation in inflammatory bowel disease (IBD) emerges from dysfunction in these cells due to excessive ER stress, which may be an after effect of broad range of factors like primary genetic (abnormalities in UPR, XBP1) and secondary (inflammation, environmental) (Kaser and Blumberg, 2009; Kaser et al., 2011). There exist some complex connections between ER stress and inflammatory responses, which are cell type or condition specific and vary with different metabolic conditions causing vivid increase in inflammatory mediators. All the three sensors of UPR pathway are capable of NF-κB activation (Hotamisligil, 2010) (**Figure 3**).

IRE1α–NF-κB

ER stress-induced activation of IRE1α kinase activity recruits adaptor protein TNF receptor-associated factor 2 (TRAF2), c-Jun-N-terminal inhibitory kinase (JIK), Jun activation domain-binding protein-1 (JAB1), and ASK1-interacting protein 1 (AIP1) to initiate downstream signaling pathways. IRE1α decides the cellular fate, i.e., survival via UPR or death via apoptosis; regulatory mechanisms of which are not yet entirely understood. JAB1 was suggested to be a decisive molecule: interacting with IRE1α under weak ER stress stimulation to suppress UPR and dissociating to allow IRE1α activation and the UPR under strong ER

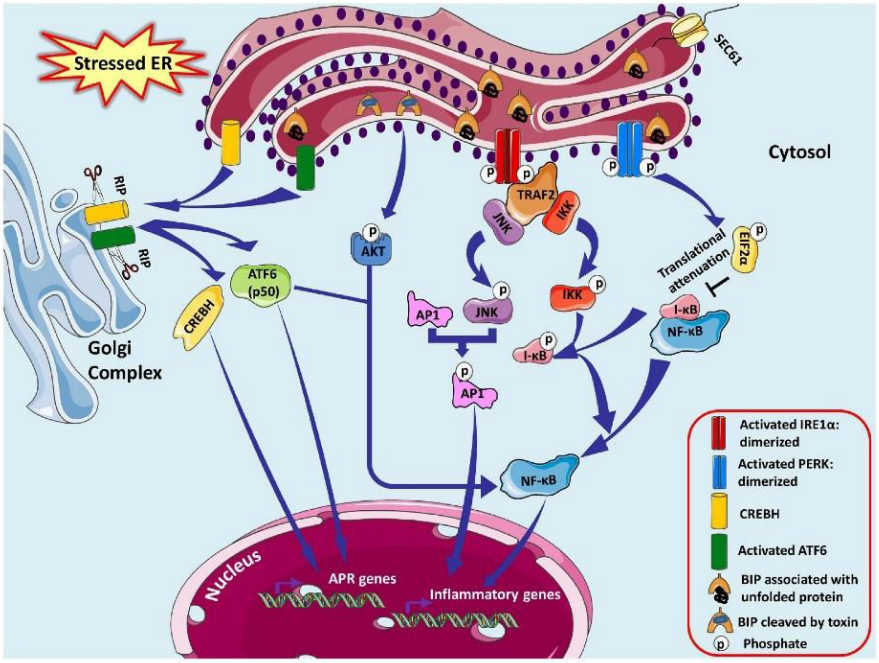


FIGURE 3 | Endoplasmic reticulum stress-associated NFκB activation is shown. ER stress-induced activation of IRE1α kinase activity recruits adaptor protein tumor-necrosis factor-α (TNF-α) receptor-associated factor 2 (TRAF2), which further recruits c-Jun N-terminal kinase (JNK) activating several transcription factors and many apoptosis signaling proteins. JNK induces the expression of inflammatory genes by phosphorylating the transcription factor activator protein 1 (AP-1), which is a dimer of monomers from various protein families forming different combinations of AP-1. They induce inflammation via promoting transcription of genes for cytokines, chemokines, and other proinflammatory molecules. TRAF2 associates with IκB kinase (IKK) activating NF-κB by promoting degradation of IκBα resulting in NF-κB nuclear translocation. PERK branch of UPR also can potentially induce NF-κB not by IκBα phosphorylation or degradation but essentially by its eIF2α-mediated attenuation of translation; successively inhibiting the synthesis of IκBα. Subtilase cytotoxin from Shiga strain of *E. coli* makes a specific single site cleavage in GRP78, therefore capable of eliciting ER stress response pathways. CREBH and ATF6 may dimerize and synergistically activate transcription of major APR genes inducing a systemic inflammatory response in specific cells.

stressed conditions (Oono et al., 2004). Activated IRE1α recruits adaptor protein TRAF2, and c-JNK activation is followed. Dominant negative TRAF2 with a truncated N-terminal RING effector domain inhibited JNK activation via IRE1α (Urano et al., 2000). In response to ER stress-inducing agents like thapsigargin and tunicamycin, IRE1α–TRAF2 pathway is known to induce NF-κB activation, which is inhibited by both dominant negative IRE1α and TRAF2 (Leonardi et al., 2002; Kaneko et al., 2003). Hu et al. (2006) reported that the ER stress inducers induce IRE1α to form a complex with IKK essentially through TRAF2, thereby activating NF-κB by promoting degradation of IκBα resulting in NF-κB nuclear translocation. This was accompanied by upregulation of TNFα (but not FasL and TRAIL) in an IRE1α- and NF-κB-dependent manner (not via TNFR1 signaling) and downregulation of TRAF2, which weaken TNFα-induced activation of NF-κB and JNK, and triggered TNFα-induced apoptosis. JIK binds to both TRAF2 and IRE1α, causing increase in JNK activation under ER stress, thus modulating IRE1α–TRAF2 signaling events. TRAF2 also interacts with procaspase-12 under ER stress thereby promoting clustering and cleavage to its active caspase-12 form and initiating apoptotic signaling pathways (Yoneda et al., 2001). Luo et al. (2008) using AIP1 knockout cells concluded that AIP1 is involved in ER stress-activated IRE1α–JNK/XBP1 pathway and that IRE1α–AIP1 complex is essential for subsequent recruitment of TRAF2 to IRE1α. JNK, member of the mitogen activated protein kinase (MAPK) superfamily, phosphorylates and activates several transcription factors such as c-Jun, ATF2, p53, c-Myc, and many apoptosis signaling proteins like Bcl-2, Bcl-xL, Bad, and Bim (Bogoyevitch and Kobe, 2006). Activated JNK induces the expression of inflammatory genes by phosphorylating the transcription factor activator protein 1 (AP1), which is a dimer of monomers from various protein families like JUN, FOS, ATF, and MAF which interact to form different combinations of AP-1 (Davis, 2000). The various genes that are regulated by AP-1 are combination specific and execute different biological functions. They induce inflammation via promoting transcription of genes for cytokines, chemokines, and other proinflammatory molecules (Angel et al., 2001; Shaulian and Karin, 2001; Eferl and Wagner, 2003).

Knockdown experiments unveiled that loss of either IRE1α or PERK curtailed NF-κB signaling in ER stress pathways, signifying combinatorial and synergistic effect of both sensors for complete NF-κB activation. IRE1 (kinase domain)-TRAF2–IKK signaling, not via JNK, leads to ER stress-induced NF-κB activation (Tam et al., 2012). Spliced XBP1 and ATF4, induce production of the inflammatory cytokines/chemokines IL-8, IL-6, and monocyte chemoattractant protein 1 (MCP1) in human aortic endothelial cells (Gargalovic et al., 2006). Malfunctioning of Paneth cells and heightened proinflammatory response in XBP1-deficient intestinal epithelial cells (IEC) provided evidence of XBP1 anomalies in IEC as being exclusively responsible for intestinal inflammation (Kaser et al., 2008).

PERK–NF-κB

PERK branch of UPR can potentially induce NF-κB essentially by its eIF2α-mediated attenuation of translation; successively inhibiting the synthesis of IκBα. It was also found that half life of this inhibitor is less compared to NF-κB culminating in increased ratio of NF-κB to IκB, which facilitates NF-κB's nuclear translocation to activate its target genes in response to ER stress (Deng et al., 2004). Comparable results were also obtained by Wu et al. (2004), which demonstrated that ultraviolet light inhibits new IκBα synthesis that can be reversed by expression of an eIF2α (S51A) mutant.

ATF6–NF-κB

Subtilase cytotoxin from Shiga strain of *E. coli* is a serine protease, which enters the cell and makes a specific single site cleavage in GRP78 (Paton et al., 2006). This causes the GRP78 to dissociate from the ER stress sensors and activate an early ER stress response qualitatively similar to that observed with the certified UPR-inducing chemical agents (Wolfson et al., 2008). Yamazaki et al. (2009) made a significant contribution by dissecting the UPR pathways involved in subtilase triggered NF-κB activation. The initial breakdown of GRP78 provoked the UPR triggering transient Akt phosphorylation and subsequent NF-κB activation exclusively via ATF6 signaling; as dominant negative inhibition of IRE1α, XBP1, or PERK did not attenuate activation of NF-κB. Zhang et al. (2006) also claimed that under ER stress, CREBH and ATF6 may dimerize and synergistically activate transcription of major APR genes inducing a systemic inflammatory response.

NF-κB ACTIVATION IN NEURODEGENERATION

In the central nervous system, NF-κB complexes are expressed by neurons, glial cells, and oligodendrocytes. Constitutive NF-κB activity is fundamental to physiological processes of brain development, synaptic signaling that govern learning and memory, and neuroprotection. Nonetheless, inducible NF-κB activity is observed in pathological conditions like trauma, ischemia, neurodegenerative diseases, memory dysfunction, and many more. The existence of diverse NF-κB complexes (Rel/NF-κB homodimers and heterodimers), their specificities for certain cell types, activating stimuli, responsive genes, explains the contradictory function of NF-κB in cell death and survival (Bhakar et al., 2002; Pizzi and Spano, 2006). Stimulatory signals for NF-κB activation in neurons can be stress- or injury-related or on exposure to cytokines or other signaling molecules including TNFα, glutamate, NGF, ADNF, and sAPP. These are capable of triggering downstream kinase cascades like calcium/calmodulin-dependent kinase II, Akt, protein kinase C, which may terminate in high constitutive neuronal NF-κB activity leading to signal transduction pathways and expression of inflammatory cytokines, chemokines, immune receptors, and cell surface adhesion molecules (Pahl, 1999; Li and Stark, 2002). NF-κB induces genes supporting cell survival like those of inhibitors of apoptosis proteins (IAPs), BCL-2s, TRAF1/TRAF2, and SOD. Glucose deprivation induces injury and excitotoxicity stress elevates intracellular calcium concentrations in embryonic neurons to toxic levels, which on TNFα administration are decreased by regulating the expression of proteins involved in calcium homeostasis thereby promoting neuronal survival. TNFα also protects neurons by increasing expression of anti-apoptotic proteins Bcl-2 and Bcl-xL in hypoxic or nitric oxide-induced injury (Cheng et al., 1994; Mattson et al., 1997; Tamatani et al., 1999). Downregulation of NF-κB in the hippocampus is associated Aβ1–42-mediated decline of neurogenesis (Zheng et al., 2013).

In glial cells (microglia and astrocytes), inducible NF-kB activity may indirectly promote neuronal death by producing large amounts of NO, proinflammatory cytokines interleukin-1b (IL-1b), interleukin-6 (IL-6) and TNF-α, ROS, and excitotoxins (John et al., 2003; Kim and de Vellis, 2005; Hsiao et al., 2013). Thus activation of NF-κB in neurons could promote their survival, whereas activation of NF-κB in glial cells may induce the production of neurotoxins depending on the context, i.e., cell type, stimulus, duration, and threshold levels of effectors (Mattson and Meffert, 2006). Nonaka et al. (1999) demonstrated remarkable prolonged activation of NF-κB persistent up to a year in glia following brain trauma in experimental rats suggesting NF-κB's role in long-term inflammatory processes.

THERAPEUTIC IMPLICATIONS

Researchers have concentrated their focus on identifying small molecules acting as chaperones to help stabilize misfolded proteins facilitating protein folding and alleviating ER stress. 4-Phenyl butyric acid (4-PBA) and taurine-conjugated ursodeoxycholic acid (TUDCA) are being explored in this regard for various diseases like colitis, atherosclerosis, and type 2 diabetes (Ozcan et al., 2006; Erbay et al., 2009; Cao et al., 2013; Vang et al., 2014). It is also known that Lipopolysaccharides (LPS) can induce various ER stress markers such as GRP78, CHOP in the lung tissues of LPS-treated mice while an ER stress inhibitor 4-phenylbutyrate (PBA), can reduce the LPS-induced lung inflammation in mice model (Kim et al., 2013). A novel strategy of inhibiting IRE1α endonuclease activity, without affecting its kinase activity has come to light identifying small molecules like MK3946 and STF-083010, as therapeutic options for multiple myeloma (MM) therapy. MK3946, an IRE1α endoribonuclease domain inhibitor, blocked XBP1 splicing causing death of MM cells and/or enhanced sensitivity of MM cells to other ER stress-inducing drugs bortezomib and 17-AAG, thereby proving to be of efficient lead for MM treatment (Mimura et al., 2012). Dinaciclib (SCH727965), a potent inhibitor of cyclin-dependent kinase (CDKs) 1/2/5/9, at extremely low (e.g., nmol/L) concentrations downregulated thapsigargin and tunicamycin-induced XBP1s and Grp78 expression in human leukemia and myeloma cells in association with evident cell death. It also markedly reduced myeloma cell growth in *in vivo* models (Nguyen and Grant, 2014). Similarly, STF-083010 exhibited cytostatic and cytotoxic activity in various MM cell lines and also effectively reduced tumor grown as xenografts in NSG mice models. It showed significant *ex vivo* toxicity to $CD138^+$ cells (marker for plasma and MM cells) isolated from MM patients (Papandreou et al., 2011). Another approach in targeting UPR for therapeutic applications found GSK2656157, an ATP-competitive inhibitor of PERK enzyme, which inhibited multiple human tumor xenografts growth in mice (Atkins et al., 2013). In diet-induced obesity (DIO), C57BL/6J mice, atorvastatin treatment effectively improved pancreatic β cell function through improved proliferation, sensitivity to glucose, and attenuated ER stress (Chen et al., 2014). Gemigliptin, a dipeptidyl peptidase-IV inhibitor efficiently inhibited ER-stress-mediated apoptosis and inflammation in cardiomyocytes via Akt/PERK/CHOP and IRE1α/JNK-p38 pathways proposing to have direct beneficial effects to prevent the progression of cardiovascular diseases (Hwang et al., 2014). In Sprague-Dawley rats, post traumatic brain surgery administration of docosahexaenoic reduced ER stress marker proteins, ubiquitinated proteins, amyloid precursor protein/p-Tau proteins, and neurological deficits demanding further research (Begum et al., 2014). Wang et al. (2014) showed that Propofol, a clinically used anesthetic agent up regulates BiP, XBP1s, and cleaved ATF6, which may be involved in the adaptive ER stress and attenuates ER stress-induced phosphorylation of PERK and eIF2a, and inhibits the up-regulation of ATF4 and CHOP as well. Synthetic compound libraries targeting specific components of ER stress pathways are being evaluated in *in vitro* and *in vivo* conditions. For instance chromenone-based inhibitors of IRE1 RNase activity and small-molecule PERK inhibitors have been tested in cell lines (Pytel et al., 2014; Ranatunga et al., 2014). Interleukin-1 receptor-associated kinase-2 (IRAK2) is recently being identified as amplifier of IRE pathway in addition to its known functions in innate immunity. Therapeutic targeting of this molecule shall shed some light in understanding of pathogenesis of ER stress-related and inflammatory diseases (Benosman et al., 2013).

CONCLUSION

Endoplasmic reticulum is a dynamic organelle orchestrating several crucial pathways that decide cellular fate. It senses alterations in ER homeostasis and triggers UPR pathways with an aim to initially restore homeostasis by activating genes involved in protein folding and degrading machinery transcribed by factors like ATF6, XBP1, and ATF4. If unresolved, it initiates cell death pathways. Under such situations, UPR pathways triggers, affects, and integrates with mitochondrial cellular signaling pathways. Neurodegenerative diseases, inflammatory diseases, cardiovascular diseases, diabetes mellitus, cancer, and several metabolic diseases have perturbed ER functions, which contribute to their pathogenesis to some extent considering the existence of other complications like inflammation or oxidative stress. Recent findings have revealed interconnections between ER stress, inflammation, and oxidative stress pathways under pathological conditions. Thus, further understanding of the molecular mechanisms in these interconnecting pathways occurring in numerous diseases may lead to discovery of novel therapeutic targets.

However, comprehensive research is demanded to aim UPR in diseases, wherein side effects, efficacy, and safety are major concerns. Nonetheless, there still exists a dilemma regarding ER stress being a cause or consequence for a particular diseased condition. Mitigating ER stress will certainly be of therapeutic significance keeping in mind that components involved in ER stress-induced inflammation are targeted specifically with minimal side effects and only desired cells are acted upon sparing the healthy cells. Focused research and in-depth investigations in this direction are needed for a new therapeutic strategy.

ACKNOWLEDGMENTS

We apologize to all those investigators whose eminent work that we were unable to cite either due to space constraints or oversight. Palaniyandi Ravanan greatly acknowledges the financial support from the young investigators grant from SERB, Department of Science and Technology. Christian Lefebvre d'Hellencourt thanks the support from European Union and the Federative Structure for

Environment, Biodiversity, and Health from the University of La Réunion. The authors wish to greatly acknowledge the editor and reviewers for the suggestions and critical evaluation of the manuscript. The images are created using Servier Medical Art and we are thankful to www.servier.com for providing free online images.

REFERENCES

Adachi, Y., Yamamoto, K., Okada, T., Yoshida, H., Harada, A., and Mori, K. (2008). ATF6 is a transcription factor specializing in the regulation of quality control proteins in the endoplasmic reticulum. *Cell Struct. Funct.* 33, 75–89. doi:10.1247/csf.07044

Alberts, B., Johnson, A., Lewis, J., Raff, M., Roberts, K., and Walter, P. (2002). *Molecular Biology of the Cell.* New York: Garland Science.

Ames, A. III (2000). CNS energy metabolism as related to function. *Brain Res. Brain Res. Rev.* 34, 42–68. doi:10.1016/S0165-0173(00)00038-2

Angel, P., Szabowski, A., and Schorpp-Kistner, M. (2001). Function and regulation of AP-1 subunits in skin physiology and pathology. *Oncogene* 20, 2413–2423. doi:10.1038/sj.onc.1204380

Appenzeller-Herzog, C., and Ellgaard, L. (2008). The human PDI family: versatility packed into a single fold. *Biochim. Biophys. Acta* 1783, 535–548. doi:10.1016/j.bbamcr.2007.11.010

Araki, K., and Nagata, K. (2011). Protein folding and quality control in the ER. *Cold Spring Harb. Perspect. Biol.* 3, a007526. doi:10.1101/cshperspect.a007526

Arenzana-Seisdedos, F., Thompson, J., Rodriguez, M. S., Bachelerie, F., Thomas, D., and Hay, R. T. (1995). Inducible nuclear expression of newly synthesized I kappa B alpha negatively regulates DNA-binding and transcriptional activities of NF-kappa B. *Mol. Cell. Biol.* 15, 2689–2696.

Asada, R., Kanemoto, S., Kondo, S., Saito, A., and Imaizumi, K. (2011). The signalling from endoplasmic reticulum-resident bZIP transcription factors involved in diverse cellular physiology. *J. Biochem.* 149, 507–518. doi:10.1093/jb/mvr041

Atkins, C., Liu, Q., Minthorn, E., Zhang, S. Y., Figueroa, D. J., Moss, K., et al. (2013). Characterization of a novel PERK kinase inhibitor with antitumor and antiangiogenic activity. *Cancer Res.* 73, 1993–2002. doi:10.1158/0008-5472.CAN-12-3109

Back, S. H., Kang, S. W., Han, J., and Chung, H. T. (2012). Endoplasmic reticulum stress in the beta-cell pathogenesis of type 2 diabetes. *Exp. Diabetes Res.* 2012, 618396. doi:10.1155/2012/618396

Bailey, D., and O'Hare, P. (2007). Transmembrane bZIP transcription factors in ER stress signaling and the unfolded protein response. *Antioxid. Redox Signal.* 9, 2305–2321. doi:10.1089/ars.2007.1796

Beal, M. F. (2005). Mitochondria take center stage in aging and neurodegeneration. *Ann. Neurol.* 58, 495–505. doi:10.1002/ana.20624

Begum, G., Yan, H. Q., Li, L., Singh, A., Dixon, C. E., and Sun, D. (2014). Docosahexaenoic acid reduces ER stress and abnormal protein accumulation and improves neuronal function following traumatic brain injury. *J. Neurosci.* 34, 3743–3755. doi:10.1523/JNEUROSCI.2872-13.2014

Benbrook, D. M., and Long, A. (2012). Integration of autophagy, proteasomal degradation, unfolded protein response and apoptosis. *Exp. Oncol.* 34, 286–297.

Benosman, S., Ravanan, P., Correa, R. G., Hou, Y. C., Yu, M., Gulen, M. F., et al. (2013). Interleukin-1 receptor-associated kinase-2 (IRAK2) is a critical mediator of endoplasmic reticulum (ER) stress signaling. *PLoS ONE* 8:e64256. doi:10.1371/journal.pone.0064256

Bernales, S., Soto, M. M., and McCullagh, E. (2012). Unfolded protein stress in the endoplasmic reticulum and mitochondria: a role in neurodegeneration. *Front. Aging Neurosci.* 4:5. doi:10.3389/fnagi.2012.00005

Berridge, M. J. (1998). Neuronal calcium signaling. *Neuron* 21, 13–26. doi:10.1016/S0896-6273(00)80510-3

Berridge, M. J. (2002). The endoplasmic reticulum: a multifunctional signaling organelle. *Cell Calcium* 32, 235–249. doi:10.1016/S0143416002001823

Bertolotti, A., Zhang, Y., Hendershot, L. M., Harding, H. P., and Ron, D. (2000). Dynamic interaction of BiP and ER stress transducers in the unfolded-protein response. *Nat. Cell Biol.* 2, 326–332. doi:10.1038/35014014

Bertram, L., and Tanzi, R. E. (2005). The genetic epidemiology of neurodegenerative disease. *J. Clin. Invest.* 115, 1449–1457. doi:10.1172/JCI24761

Bezprozvanny, I. (2009). Calcium signaling and neurodegenerative diseases. *Trends Mol. Med.* 15, 89–100. doi:10.1016/j.molmed.2009.01.001

Bhakar, A. L., Tannis, L. L., Zeindler, C., Russo, M. P., Jobin, C., Park, D. S., et al. (2002). Constitutive nuclear factor-kappa B activity is required for central neuron survival. *J. Neurosci.* 22, 8466–8475.

Bhandary, B., Marahatta, A., Kim, H. R., and Chae, H. J. (2012). An involvement of oxidative stress in endoplasmic reticulum stress and its associated diseases. *Int. J. Mol. Sci.* 14, 434–456. doi:10.3390/ijms14010434

Bogoyevitch, M. A., and Kobe, B. (2006). Uses for JNK: the many and varied substrates of the c-Jun N-terminal kinases. *Microbiol. Mol. Biol. Rev.* 70, 1061–1095. doi:10.1128/MMBR.00025-06

Braakman, I., and Bulleid, N. J. (2011). Protein folding and modification in the mammalian endoplasmic reticulum. *Annu. Rev. Biochem.* 80, 71–99. doi:10.1146/annurev-biochem-062209-093836

Brewer, J. W., and Diehl, J. A. (2000). PERK mediates cell-cycle exit during the mammalian unfolded protein response. *Proc. Natl. Acad. Sci. U.S.A.* 97, 12625–12630. doi:10.1073/pnas.220247197

Bukau, B., Weissman, J., and Horwich, A. (2006). Molecular chaperones and protein quality control. *Cell* 125, 443–451. doi:10.1016/j.cell.2006.04.014

Cadenas, E., and Davies, K. J. (2000). Mitochondrial free radical generation, oxidative stress, and aging. *Free Radic. Biol. Med.* 29, 222–230. doi:10.1016/S0891-5849(00)00317-8

Calabrese, V., Bates, T. E., and Stella, A. M. (2000). NO synthase and NO-dependent signal pathways in brain aging and neurodegenerative disorders: the role of oxidant/antioxidant balance. *Neurochem. Res.* 25, 1315–1341. doi:10.1023/A:1007604414773

Cali, T., Ottolini, D., and Brini, M. (2011). Mitochondria, calcium, and endoplasmic reticulum stress in Parkinson's disease. *Biofactors* 37, 228–240. doi:10.1002/biof.159

Cannon, J. R., and Greenamyre, J. T. (2011). The role of environmental exposures in neurodegeneration and neurodegenerative diseases. *Toxicol. Sci.* 124, 225–250. doi:10.1093/toxsci/kfr239

Cao, S. S., Zimmermann, E. M., Chuang, B. M., Song, B., Nwokoye, A., Wilkinson, J. E., et al. (2013). The unfolded protein response and chemical chaperones reduce protein misfolding and colitis in mice. *Gastroenterology* 144, e1006. doi:10.1053/j.gastro.2013.01.023

Chen, X., Shen, J., and Prywes, R. (2002). The luminal domain of ATF6 senses endoplasmic reticulum (ER) stress and causes translocation of ATF6 from the ER to the Golgi. *J. Biol. Chem.* 277, 13045–13052. doi:10.1074/jbc.M110636200

Chen, X., Zhang, X., Li, C., Guan, T., Shang, H., Cui, L., et al. (2013). S-nitrosylated protein disulfide isomerase contributes to mutant SOD1 aggregates in amyotrophic lateral sclerosis. *J. Neurochem.* 124, 45–58. doi:10.1111/jnc.12046

Chen, Z. Y., Liu, S. N., Li, C. N., Sun, S. J., Liu, Q., Lei, L., et al. (2014). Atorvastatin helps preserve pancreatic beta cell function in obese C57BL/6 J mice and the effect is related to increased pancreas proliferation and amelioration of endoplasmic-reticulum stress. *Lipids Health Dis.* 13, 98. doi:10.1186/1476-511X-13-98

Cheng, B., Christakos, S., and Mattson, M. P. (1994). Tumor necrosis factors protect neurons against metabolic-excitotoxic insults and promote maintenance of calcium homeostasis. *Neuron* 12, 139–153. doi:10.1016/0896-6273(94)90159-7

Cheng, Y., and Yang, J. M. (2011). Survival and death of endoplasmic-reticulum-stressed cells: role of autophagy. *World J. Biol. Chem.* 2, 226–231. doi:10.4331/wjbc.v2.i10.226

Cullinan, S. B., and Diehl, J. A. (2006). Coordination of ER and oxidative stress signaling: the PERK/Nrf2 signaling pathway. *Int. J. Biochem. Cell Biol.* 38, 317–332. doi:10.1016/j.biocel.2005.09.018

Cullinan, S. B., Zhang, D., Hannink, M., Arvisais, E., Kaufman, R. J., and Diehl, J. A. (2003). Nrf2 is a direct PERK substrate and effector of PERK-dependent cell survival. *Mol. Cell. Biol.* 23, 7198–7209. doi:10.1128/MCB.23.20.7198-7209.2003

Dasuri, K., Zhang, L., and Keller, J. N. (2013). Oxidative stress, neurodegeneration, and the balance of protein degradation and protein synthesis. *Free Radic. Biol. Med.* 62, 170–185. doi:10.1016/j.freeradbiomed.2012.09.016

Davis, R. J. (2000). Signal transduction by the JNK group of MAP kinases. *Cell* 103, 239–252. doi:10.1016/S0092-8674(00)00116-1

Deng, J., Lu, P. D., Zhang, Y., Scheuner, D., Kaufman, R. J., Sonenberg, N., et al. (2004). Translational repression mediates activation of nuclear factor kappa B by phosphorylated translation initiation factor 2. *Mol. Cell. Biol.* 24, 10161–10168. doi:10.1128/MCB.24.23.10161-10168.2004

Deniaud, A., Sharaf El Dein, O., Maillier, E., Poncet, D., Kroemer, G., Lemaire, C., et al. (2008). Endoplasmic reticulum stress induces calcium-dependent permeability transition, mitochondrial outer membrane permeabilization and apoptosis. *Oncogene* 27, 285–299. doi:10.1038/sj.onc.1210638

Derijard, B., Raingeaud, J., Barrett, T., Wu, I. H., Han, J., Ulevitch, R. J., et al. (1995). Independent human MAP-kinase signal transduction pathways defined by MEK and MKK isoforms. *Science* 267, 682–685. doi:10.1126/science.7839144

Dixit, V., and Mak, T. W. (2002). NF-kappaB signaling. Many roads lead to Madrid. *Cell* 111, 615–619. doi:10.1016/S0092-8674(02)01166-2

Dong, X. X., Wang, Y., and Qin, Z. H. (2009). Molecular mechanisms of excitotoxicity and their relevance to pathogenesis of neurodegenerative diseases. *Acta Pharmacol. Sin.* 30, 379–387. doi:10.1038/aps.2009.24

Doroudgar, S., Thuerauf, D. J., Marcinko, M. C., Belmont, P. J., and Glembotski, C. C. (2009). Ischemia activates the ATF6 branch of the endoplasmic reticulum stress response. *J. Biol. Chem.* 284, 29735–29745. doi:10.1074/jbc.M109.018036

Doyle, K. M., Kennedy, D., Gorman, A. M., Gupta, S., Healy, S. J., and Samali, A. (2011). Unfolded proteins and endoplasmic reticulum stress in neurodegenerative disorders. *J. Cell. Mol. Med.* 15, 2025–2039. doi:10.1111/j.1582-4934.2011.01374.x

Eferl, R., and Wagner, E. F. (2003). AP-1: a double-edged sword in tumorigenesis. *Nat. Rev. Cancer* 3, 859–868. doi:10.1038/nrc1209

Erbay, E., Babaev, V. R., Mayers, J. R., Makowski, L., Charles, K. N., Snitow, M. E., et al. (2009). Reducing endoplasmic reticulum stress through a macrophage lipid chaperone alleviates atherosclerosis. *Nat. Med.* 15, 1383–1391. doi:10.1038/nm.2067

Fagone, P., and Jackowski, S. (2009). Membrane phospholipid synthesis and endoplasmic reticulum function. *J. Lipid Res.* 50(Suppl.), S311–S316. doi:10.1194/jlr.R800049-JLR200

Fedoroff, N. (2006). Redox regulatory mechanisms in cellular stress responses. *Ann. Bot.* 98, 289–300. doi:10.1093/aob/mcl128

Feldman, D. E., Chauhan, V., and Koong, A. C. (2005). The unfolded protein response: a novel component of the hypoxic stress response in tumors. *Mol. Cancer Res.* 3, 597–605. doi:10.1158/1541-7786.MCR-05-0221

Finkel, T. (2011). Signal transduction by reactive oxygen species. *J. Cell Biol.* 194, 7–15. doi:10.1083/jcb.201102095

Fonseca, S. G., Gromada, J., and Urano, F. (2011). Endoplasmic reticulum stress and pancreatic beta-cell death. *Trends Endocrinol. Metab.* 22, 266–274. doi:10.1016/j.tem.2011.02.008

Gandhi, S., and Abramov, A. Y. (2012). Mechanism of oxidative stress in neurodegeneration. *Oxid. Med. Cell Longev.* 2012, 428010. doi:10.1155/2012/428010

Garg, A. D., Kaczmarek, A., Krysko, O., Vandenabeele, P., Krysko, D. V., and Agostinis, P. (2012). ER stress-induced inflammation: does it aid or impede disease progression? *Trends Mol. Med.* 18, 589–598. doi:10.1016/j.molmed.2012.06.010

Gargalovic, P. S., Gharavi, N. M., Clark, M. J., Pagnon, J., Yang, W. P., He, A., et al. (2006). The unfolded protein response is an important regulator of inflammatory genes in endothelial cells. *Arterioscler. Thromb. Vasc. Biol.* 26, 2490–2496. doi:10.1161/01.ATV.0000242903.41158.a1

Glass, C. K., Saijo, K., Winner, B., Marchetto, M. C., and Gage, F. H. (2010). Mechanisms underlying inflammation in neurodegeneration. *Cell* 140, 918–934. doi:10.1016/j.cell.2010.02.016

Gorlach, A., Klappa, P., and Kietzmann, T. (2006). The endoplasmic reticulum: folding, calcium homeostasis, signaling, and redox control. *Antioxid. Redox Signal.* 8, 1391–1418. doi:10.1089/ars.2006.8.1391

Gorman, A. M., Healy, S. J., Jager, R., and Samali, A. (2012). Stress management at the ER: regulators of ER stress-induced apoptosis. *Pharmacol. Ther.* 134, 306–316. doi:10.1016/j.pharmthera.2012.02.003

Harding, H. P., Novoa, I., Zhang, Y., Zeng, H., Wek, R., Schapira, M., et al. (2000a). Regulated translation initiation controls stress-induced gene expression in mammalian cells. *Mol. Cell* 6, 1099–1108. doi:10.1016/S1097-2765(00)00108-8

Harding, H. P., Zhang, Y., Bertolotti, A., Zeng, H., and Ron, D. (2000b). Perk is essential for translational regulation and cell survival during the unfolded protein response. *Mol. Cell* 5, 897–904. doi:10.1016/S1097-2765(00)80330-5

Harding, H. P., Zhang, Y., and Ron, D. (1999). Protein translation and folding are coupled by an endoplasmic-reticulum-resident kinase. *Nature* 397, 271–274. doi:10.1038/16729

Harding, H. P., Zhang, Y., Zeng, H., Novoa, I., Lu, P. D., Calfon, M., et al. (2003). An integrated stress response regulates amino acid metabolism and resistance to oxidative stress. *Mol. Cell* 11, 619–633. doi:10.1016/S1097-2765(03)00105-9

Hayashi, A., Kasahara, T., Kametani, M., Toyota, T., Yoshikawa, T., and Kato, T. (2009). Aberrant endoplasmic reticulum stress response in lymphoblastoid cells from patients with bipolar disorder. *Int. J. Neuropsychopharmacol.* 12, 33–43. doi:10.1017/S1461145708009358

Hayden, M. S., and Ghosh, S. (2008). Shared principles in NF-kappaB signaling. *Cell* 132, 344–362. doi:10.1016/j.cell.2008.01.020

Haze, K., Yoshida, H., Yanagi, H., Yura, T., and Mori, K. (1999). Mammalian transcription factor ATF6 is synthesized as a transmembrane protein and activated by proteolysis in response to endoplasmic reticulum stress. *Mol. Biol. Cell* 10, 3787–3799. doi:10.1091/mbc.10.11.3787

He, B. (2006). Viruses, endoplasmic reticulum stress, and interferon responses. *Cell Death Differ.* 13, 393–403. doi:10.1038/sj.cdd.4401833

Heath-Engel, H. M., Chang, N. C., and Shore, G. C. (2008). The endoplasmic reticulum in apoptosis and autophagy: role of the BCL-2 protein family. *Oncogene* 27, 6419–6433. doi:10.1038/onc.2008.309

Hebert, D. N., and Molinari, M. (2007). In and out of the ER: protein folding, quality control, degradation, and related human diseases. *Physiol. Rev.* 87, 1377–1408. doi:10.1152/physrev.00050.2006

Hendershot, L. M. (2004). The ER function BiP is a master regulator of ER function. *Mt. Sinai J. Med.* 71, 289–297.

Hetz, C. A., and Soto, C. (2006). Stressing out the ER: a role of the unfolded protein response in prion-related disorders. *Curr. Mol. Med.* 6, 37–43.

Hoffman, S. M., Tully, J. E., Nolin, J. D., Lahue, K. G., Goldman, D. H., Daphtary, N., et al. (2013). Endoplasmic reticulum stress mediates house dust mite-induced airway epithelial apoptosis and fibrosis. *Respir. Res.* 14, 141. doi:10.1186/1465-9921-14-141

Hosoi, T., Sasaki, M., Miyahara, T., Hashimoto, C., Matsuo, S., Yoshii, M., et al. (2008). Endoplasmic reticulum stress induces leptin resistance. *Mol. Pharmacol.* 74, 1610–1619. doi:10.1124/mol.108.050070

Hotamisligil, G. S. (2006). Inflammation and metabolic disorders. *Nature* 444, 860–867. doi:10.1038/nature05485

Hotamisligil, G. S. (2010). Endoplasmic reticulum stress and the inflammatory basis of metabolic disease. *Cell* 140, 900–917. doi:10.1016/j.cell.2010.02.034

Hsiao, H. Y., Chen, Y. C., Chen, H. M., Tu, P. H., and Chern, Y. (2013). A critical role of astrocyte-mediated nuclear factor-kappaB-dependent inflammation in Huntington's disease. *Hum. Mol. Genet.* 22, 1826–1842. doi:10.1093/hmg/ddt036

Hu, F., and Liu, F. (2011). Mitochondrial stress: a bridge between mitochondrial dysfunction and metabolic diseases? *Cell. Signal.* 23, 1528–1533. doi:10.1016/j.cellsig.2011.05.008

Hu, P., Han, Z., Couvillon, A. D., Kaufman, R. J., and Exton, J. H. (2006). Autocrine tumor necrosis factor alpha links endoplasmic reticulum stress to the membrane death receptor pathway through IRE1alpha-mediated NF-kappaB activation and down-regulation of TRAF2 expression. *Mol. Cell. Biol.* 26, 3071–3084. doi:10.1128/MCB.26.8.3071-3084.2006

Huxford, T., and Ghosh, G. (2009). A structural guide to proteins of the NF-kappaB signaling module. *Cold Spring Harb. Perspect. Biol.* 1, a000075. doi:10.1101/cshperspect.a000075

Hwang, C., Sinskey, A. J., and Lodish, H. F. (1992). Oxidized redox state of glutathione in the endoplasmic reticulum. *Science* 257, 1496–1502.

Hwang, H. J., Jung, T. W., Ryu, J. Y., Hong, H. C., Choi, H. Y., Seo, J. A., et al. (2014). Dipeptidyl petidase-IV inhibitor (gemigliptin) inhibits tunicamycin-induced endoplasmic reticulum stress, apoptosis and inflammation in H9c2 cardiomyocytes. *Mol. Cell. Endocrinol.* 392, 1–7. doi:10.1016/j.mce.2014.04.017

Jiang, H. Y., Wek, S. A., McGrath, B. C., Scheuner, D., Kaufman, R. J., Cavener, D. R., et al. (2003). Phosphorylation of the alpha subunit of eukaryotic initiation factor 2 is required for activation of NF-kappaB in response to diverse cellular stresses. *Mol. Cell. Biol.* 23, 5651–5663. doi:10.1128/MCB.23.16.5651-5663.2003

John, G. R., Lee, S. C., and Brosnan, C. F. (2003). Cytokines: powerful regulators of glial cell activation. *Neuroscientist.* 9, 10–22. doi:10.1177/1073858402239587

Johri, A., and Beal, M. F. (2012). Mitochondrial dysfunction in neurodegenerative diseases. *J. Pharmacol. Exp. Ther.* 342, 619–630. doi:10.1124/jpet.112.192138

Kampinga, H. H., and Craig, E. A. (2010). The HSP70 chaperone machinery: J proteins as drivers of functional specificity. *Nat. Rev. Mol. Cell Biol.* 11, 579–592. doi:10.1038/nrm2941

Kaneko, M., Niinuma, Y., and Nomura, Y. (2003). Activation signal of nuclear factor-kappa B in response to endoplasmic reticulum stress is transduced via IRE1 and tumor necrosis factor receptor-associated factor 2. *Biol. Pharm. Bull.* 26, 931–935. doi:10.1248/bpb.26.931

Kaser, A., and Blumberg, R. S. (2009). Endoplasmic reticulum stress in the intestinal epithelium and inflammatory bowel disease. *Semin. Immunol.* 21, 156–163. doi:10.1016/j.smim.2009.01.001

Kaser, A., Flak, M. B., Tomczak, M. F., and Blumberg, R. S. (2011). The unfolded protein response and its role in intestinal homeostasis and inflammation. *Exp. Cell Res.* 317, 2772–2779. doi:10.1016/j.yexcr.2011.07.008

Kaser, A., Lee, A. H., Franke, A., Glickman, J. N., Zeissig, S., Tilg, H., et al. (2008). XBP1 links ER stress to intestinal inflammation and confers genetic risk for

human inflammatory bowel disease. *Cell* 134, 743–756. doi:10.1016/j.cell.2008.07.021

Kawamoto, E. M., Vivar, C., and Camandola, S. (2012). Physiology and pathology of calcium signaling in the brain. *Front. Pharmacol.* 3:61. doi:10.3389/fphar.2012.00061

Kearns, J. D., Basak, S., Werner, S. L., Huang, C. S., and Hoffmann, A. (2006). IkappaBepsilon provides negative feedback to control NF-kappaB oscillations, signaling dynamics, and inflammatory gene expression. *J. Cell Biol.* 173, 659–664. doi:10.1083/jcb.200510155

Kim, H. J., Jeong, J. S., Kim, S. R., Park, S. Y., Chae, H. J., and Lee, Y. C. (2013). Inhibition of endoplasmic reticulum stress alleviates lipopolysaccharide-induced lung inflammation through modulation of NF-kappaB/HIF-1alpha signaling pathway. *Sci. Rep.* 3, 1142. doi:10.1038/srep01142

Kim, I., Xu, W., and Reed, J. C. (2008a). Cell death and endoplasmic reticulum stress: disease relevance and therapeutic opportunities. *Nat. Rev. Drug Discov.* 7, 1013–1030. doi:10.1038/nrd2755

Kim, J. A., Wei, Y., and Sowers, J. R. (2008b). Role of mitochondrial dysfunction in insulin resistance. *Circ. Res.* 102, 401–414. doi:10.1161/CIRCRESAHA.107.165472

Kim, S. U., and De Vellis, J. (2005). Microglia in health and disease. *J. Neurosci. Res.* 81, 302–313. doi:10.1002/jnr.20562

Lautenschlaeger, J., Prell, T., and Grosskreutz, J. (2012). Endoplasmic reticulum stress and the ER mitochondrial calcium cycle in amyotrophic lateral sclerosis. *Amyotroph. Lateral Scler.* 13, 166–177. doi:10.3109/17482968.2011.641569

Lawrence, T. (2009). The nuclear factor NF-kappaB pathway in inflammation. *Cold Spring Harb. Perspect. Biol.* 1, a001651. doi:10.1101/cshperspect.a001651

Lee, A. H., Iwakoshi, N. N., and Glimcher, L. H. (2003). XBP-1 regulates a subset of endoplasmic reticulum resident chaperone genes in the unfolded protein response. *Mol. Cell. Biol.* 23, 7448–7459. doi:10.1128/MCB.23.21.7448-7459.2003

Leonardi, A., Vito, P., Mauro, C., Pacifico, F., Ulianich, L., Consiglio, E., et al. (2002). Endoplasmic reticulum stress causes thyroglobulin retention in this organelle and triggers activation of nuclear factor-kappa B via tumor necrosis factor receptor-associated factor 2. *Endocrinology* 143, 2169–2177. doi:10.1210/endo.143.6.8825

Lesnefsky, E. J., Moghaddas, S., Tandler, B., Kerner, J., and Hoppel, C. L. (2001). Mitochondrial dysfunction in cardiac disease: ischemia–reperfusion, aging, and heart failure. *J. Mol. Cell. Cardiol.* 33, 1065–1089. doi:10.1006/jmcc.2001.1378

Lezi, E., and Swerdlow, R. H. (2012). Mitochondria in neurodegeneration. *Adv. Exp. Med. Biol.* 942, 269–286. doi:10.1007/978-94-007-2869-1_12

Li, X., and Stark, G. R. (2002). NFkappaB-dependent signaling pathways. *Exp. Hematol.* 30, 285–296. doi:10.1016/S0301-472X(02)00777-4

Lill, C. M., and Bertram, L. (2011). Towards unveiling the genetics of neurodegenerative diseases. *Semin. Neurol.* 31, 531–541. doi:10.1055/s-0031-1299791

Liu, C. Y., Xu, Z., and Kaufman, R. J. (2003). Structure and intermolecular interactions of the luminal dimerization domain of human IRE1alpha. *J. Biol. Chem.* 278, 17680–17687. doi:10.1074/jbc.M300418200

Lodish, H., Berk, A., Zipursky, S. L., Matsudaira, P., Baltimore, D., and Darnell, J. (2000). *Molecular Cell Biology*. New York: W. H. Freeman.

Logue, S. E., Cleary, P., Saveljeva, S., and Samali, A. (2013). New directions in ER stress-induced cell death. *Apoptosis* 18, 537–546. doi:10.1007/s10495-013-0818-6

Lu, P. D., Harding, H. P., and Ron, D. (2004). Translation reinitiation at alternative open reading frames regulates gene expression in an integrated stress response. *J. Cell Biol.* 167, 27–33. doi:10.1083/jcb.200408003

Luo, D., He, Y., Zhang, H., Yu, L., Chen, H., Xu, Z., et al. (2008). AIP1 is critical in transducing IRE1-mediated endoplasmic reticulum stress response. *J. Biol. Chem.* 283, 11905–11912. doi:10.1074/jbc.M710557200

Ma, K., Vattem, K. M., and Wek, R. C. (2002a). Dimerization and release of molecular chaperone inhibition facilitate activation of eukaryotic initiation factor-2 kinase in response to endoplasmic reticulum stress. *J. Biol. Chem.* 277, 18728–18735. doi:10.1074/jbc.M200903200

Ma, Y., Brewer, J. W., Diehl, J. A., and Hendershot, L. M. (2002b). Two distinct stress signaling pathways converge upon the CHOP promoter during the mammalian unfolded protein response. *J. Mol. Biol.* 318, 1351–1365. doi:10.1016/S0022-2836(02)00234-6

Majors, A. K., Austin, R. C., De La Motte, C. A., Pyeritz, R. E., Hascall, V. C., Kessler, S. P., et al. (2003). Endoplasmic reticulum stress induces hyaluronan deposition and leukocyte adhesion. *J. Biol. Chem.* 278, 47223–47231. doi:10.1074/jbc.M304871200

Malhotra, J. D., and Kaufman, R. J. (2007). Endoplasmic reticulum stress and oxidative stress: a vicious cycle or a double-edged sword? *Antioxid. Redox Signal.* 9, 2277–2293. doi:10.1089/ars.2007.1782

Malhotra, J. D., and Kaufman, R. J. (2011). ER stress and its functional link to mitochondria: role in cell survival and death. *Cold Spring Harb. Perspect. Biol.* 3, a004424. doi:10.1101/cshperspect.a004424

Mantena, S. K., King, A. L., Andringa, K. K., Eccleston, H. B., and Bailey, S. M. (2008). Mitochondrial dysfunction and oxidative stress in the pathogenesis of alcohol- and obesity-induced fatty liver diseases. *Free Radic. Biol. Med.* 44, 1259–1272. doi:10.1016/j.freeradbiomed.2007.12.029

Mattson, M. P., Goodman, Y., Luo, H., Fu, W., and Furukawa, K. (1997). Activation of NF-kappaB protects hippocampal neurons against oxidative stress-induced apoptosis: evidence for induction of manganese superoxide dismutase and suppression of peroxynitrite production and protein tyrosine nitration. *J. Neurosci. Res.* 49, 681–697. doi:10.1002/(SICI)1097-4547(19970915)49:6<681::AID-JNR3>3.0.CO;2-3

Mattson, M. P., and Meffert, M. K. (2006). Roles for NF-kappaB in nerve cell survival, plasticity, and disease. *Cell Death Differ.* 13, 852–860. doi:10.1038/sj.cdd.4401837

Matus, S., Glimcher, L. H., and Hetz, C. (2011). Protein folding stress in neurodegenerative diseases: a glimpse into the ER. *Curr. Opin. Cell Biol.* 23, 239–252. doi:10.1016/j.ceb.2011.01.003

Miani, M., Colli, M. L., Ladriere, L., Cnop, M., and Eizirik, D. L. (2012). Mild endoplasmic reticulum stress augments the proinflammatory effect of IL-1beta in pancreatic rat beta-cells via the IRE1alpha/XBP1s pathway. *Endocrinology* 153, 3017–3028. doi:10.1210/en.2011-2090

Mimura, N., Fulciniti, M., Gorgun, G., Tai, Y. T., Cirstea, D., Santo, L., et al. (2012). Blockade of XBP1 splicing by inhibition of IRE1alpha is a promising therapeutic option in multiple myeloma. *Blood* 119, 5772–5781. doi:10.1182/blood-2011-07-366633

Minamino, T., Komuro, I., and Kitakaze, M. (2010). Endoplasmic reticulum stress as a therapeutic target in cardiovascular disease. *Circ. Res.* 107, 1071–1082. doi:10.1161/CIRCRESAHA.110.227819

Nakka, V. P., Gusain, A., and Raghubir, R. (2010). Endoplasmic reticulum stress plays critical role in brain damage after cerebral ischemia/reperfusion in rats. *Neurotox. Res.* 17, 189–202. doi:10.1007/s12640-009-9110-5

Nguyen, D. T., Kebache, S., Fazel, A., Wong, H. N., Jenna, S., Emadali, A., et al. (2004). Nck-dependent activation of extracellular signal-regulated kinase-1 and regulation of cell survival during endoplasmic reticulum stress. *Mol. Biol. Cell* 15, 4248–4260. doi:10.1091/mbc.E03-11-0851

Nguyen, T. K., and Grant, S. (2014). Dinaciclib (SCH727965) inhibits the unfolded protein response through a CDK1- and 5-dependent mechanism. *Mol. Cancer Ther.* 13, 662–674. doi:10.1158/1535-7163.MCT-13-0714

Nicholls, D. G., and Budd, S. L. (2000). Mitochondria and neuronal survival. *Physiol. Rev.* 80, 315–360.

Nishikawa, S., Brodsky, J. L., and Nakatsukasa, K. (2005). Roles of molecular chaperones in endoplasmic reticulum (ER) quality control and ER-associated degradation (ERAD). *J. Biochem.* 137, 551–555. doi:10.1093/jb/mvi068

Nishitoh, H., Matsuzawa, A., Tobiume, K., Saegusa, K., Takeda, K., Inoue, K., et al. (2002). ASK1 is essential for endoplasmic reticulum stress-induced neuronal cell death triggered by expanded polyglutamine repeats. *Genes Dev.* 16, 1345–1355. doi:10.1101/gad.992302

Nonaka, M., Chen, X. H., Pierce, J. E., Leoni, M. J., McIntosh, T. K., Wolf, J. A., et al. (1999). Prolonged activation of NF-kappaB following traumatic brain injury in rats. *J. Neurotrauma* 16, 1023–1034.

Oeckinghaus, A., Hayden, M. S., and Ghosh, S. (2011). Crosstalk in NF-kappaB signaling pathways. *Nat. Immunol.* 12, 695–708. doi:10.1038/ni.2065

Ogata, M., Hino, S., Saito, A., Morikawa, K., Kondo, S., Kanemoto, S., et al. (2006). Autophagy is activated for cell survival after endoplasmic reticulum stress. *Mol. Cell. Biol.* 26, 9220–9231. doi:10.1128/MCB.01453-06

Oono, K., Yoneda, T., Manabe, T., Yamagishi, S., Matsuda, S., Hitomi, J., et al. (2004). JAB1 participates in unfolded protein responses by association and dissociation with IRE1. *Neurochem. Int.* 45, 765–772. doi:10.1016/j.neuint.2004.01.003

Ozcan, U., Yilmaz, E., Ozcan, L., Furuhashi, M., Vaillancourt, E., Smith, R. O., et al. (2006). Chemical chaperones reduce ER stress and restore glucose homeostasis in a mouse model of type 2 diabetes. *Science* 313, 1137–1140. doi:10.1126/science.1128294

Pahl, H. L. (1999). Activators and target genes of Rel/NF-kappaB transcription factors. *Oncogene* 18, 6853–6866. doi:10.1038/sj.onc.1203239

Pahl, H. L., and Baeuerle, P. A. (1995). A novel signal transduction pathway from the endoplasmic reticulum to the nucleus is mediated by transcription factor NF-kappa B. *EMBO J.* 14, 2580–2588.

Pahl, H. L., and Baeuerle, P. A. (1996). Activation of NF-kappa B by ER stress requires both Ca2+ and reactive oxygen intermediates as messengers. *FEBS Lett.* 392, 129–136.

Pahl, H. L., and Baeuerle, P. A. (1997). The ER-overload response: activation of NF-kappa B. *Trends Biochem. Sci.* 22, 63–67. doi:10.1016/S0968-0004(96)10073-6

Papa, F. R. (2012). Endoplasmic reticulum stress, pancreatic beta-cell degeneration, and diabetes. *Cold Spring Harb. Perspect. Med.* 2, a007666. doi:10.1101/cshperspect.a007666

Papandreou, I., Denko, N. C., Olson, M., Van Melckebeke, H., Lust, S., Tam, A., et al. (2011). Identification of an Ire1alpha endonuclease specific inhibitor with cytotoxic activity against human multiple myeloma. *Blood* 117, 1311–1314. doi:10.1182/blood-2010-08-303099

Paton, A. W., Beddoe, T., Thorpe, C. M., Whisstock, J. C., Wilce, M. C., Rossjohn, J., et al. (2006). AB5 subtilase cytotoxin inactivates the endoplasmic reticulum chaperone BiP. *Nature* 443, 548–552. doi:10.1038/nature05124

Peng, T. I., and Jou, M. J. (2010). Oxidative stress caused by mitochondrial calcium overload. *Ann. N. Y. Acad. Sci.* 1201, 183–188. doi:10.1111/j.1749-6632.2010.05634.x

Pfaffenbach, K. T., and Lee, A. S. (2011). The critical role of GRP78 in physiologic and pathologic stress. *Curr. Opin. Cell Biol.* 23, 150–156. doi:10.1016/j.ceb.2010.09.007

Pizzi, M., and Spano, P. (2006). Distinct roles of diverse nuclear factor-kappaB complexes in neuropathological mechanisms. *Eur. J. Pharmacol.* 545, 22–28. doi:10.1016/j.ejphar.2006.06.027

Pytel, D., Seyb, K., Liu, M., Ray, S. S., Concannon, J., Huang, M., et al. (2014). Enzymatic characterization of ER stress-dependent kinase, PERK, and development of a high-throughput assay for identification of PERK inhibitors. *J. Biomol. Screen* 19, 1024–1034. doi:10.1177/1087057114525853

Qi, X., Hosoi, T., Okuma, Y., Kaneko, M., and Nomura, Y. (2004). Sodium 4-phenylbutyrate protects against cerebral ischemic injury. *Mol. Pharmacol.* 66, 899–908. doi:10.1124/mol.104.001339

Ranatunga, S., Tang, C. H., Kang, C. W., Kriss, C. L., Kloppenburg, B. J., Hu, C. C., et al. (2014). Synthesis of novel tricyclic chromenone-based inhibitors of IRE-1 RNase activity. *J. Med. Chem.* 57, 4289–4301. doi:10.1021/jm5002452

Raturi, A., and Simmen, T. (2013). Where the endoplasmic reticulum and the mitochondrion tie the knot: the mitochondria-associated membrane (MAM). *Biochim. Biophys. Acta* 1833, 213–224. doi:10.1016/j.bbamcr.2012.04.013

Ravanan, P., Sano, R., Talwar, P., Ogasawara, S., Matsuzawa, S., Cuddy, M., et al. (2011a). Synthetic triterpenoid cyano enone of methyl boswellate activates intrinsic, extrinsic, and endoplasmic reticulum stress cell death pathways in tumor cell lines. *Mol. Cancer Ther.* 10, 1635–1643. doi:10.1158/1535-7163.MCT-10-0887

Ravanan, P., Singh, S. K., Rao, G. S., and Kondaiah, P. (2011b). Growth inhibitory, apoptotic and anti-inflammatory activities displayed by a novel modified triterpenoid, cyano enone of methyl boswellates. *J. Biosci.* 36, 297–307. doi:10.1007/s12038-011-9056-7

Raven, J. F., Baltzis, D., Wang, S., Mounir, Z., Papadakis, A. I., Gao, H. Q., et al. (2008). PKR and PKR-like endoplasmic reticulum kinase induce the proteasome-dependent degradation of cyclin D1 via a mechanism requiring eukaryotic initiation factor 2alpha phosphorylation. *J. Biol. Chem.* 283, 3097–3108. doi:10.1074/jbc.M709677200

Rutkevich, L. A., and Williams, D. B. (2011). Participation of lectin chaperones and thiol oxidoreductases in protein folding within the endoplasmic reticulum. *Curr. Opin. Cell Biol.* 23, 157–166. doi:10.1016/j.ceb.2010.10.011

Salminen, A., Kauppinen, A., Suuronen, T., Kaarniranta, K., and Ojala, J. (2009). ER stress in Alzheimer's disease: a novel neuronal trigger for inflammation and Alzheimer's pathology. *J. Neuroinflammation* 6, 41. doi:10.1186/1742-2094-6-41

Sammels, E., Parys, J. B., Missiaen, L., De Smedt, H., and Bultynck, G. (2010). Intracellular Ca2+ storage in health and disease: a dynamic equilibrium. *Cell Calcium* 47, 297–314. doi:10.1016/j.ceca.2010.02.001

Santos, C. X., Tanaka, L. Y., Wosniak, J., and Laurindo, F. R. (2009). Mechanisms and implications of reactive oxygen species generation during the unfolded protein response: roles of endoplasmic reticulum oxidoreductases, mitochondrial electron transport, and NADPH oxidase. *Antioxid. Redox Signal.* 11, 2409–2427. doi:10.1089/ARS.2009.2625

Sawada, N., Yao, J., Hiramatsu, N., Hayakawa, K., Araki, I., Takeda, M., et al. (2008). Involvement of hypoxia-triggered endoplasmic reticulum stress in outlet obstruction-induced apoptosis in the urinary bladder. *Lab. Invest.* 88, 553–563. doi:10.1038/labinvest.2008.21

Scheuner, D., Song, B., McEwen, E., Liu, C., Laybutt, R., Gillespie, P., et al. (2001). Translational control is required for the unfolded protein response and in vivo glucose homeostasis. *Mol. Cell* 7, 1165–1176. doi:10.1016/S1097-2765(01)00265-9

Schroder, M., and Kaufman, R. J. (2005). The mammalian unfolded protein response. *Annu. Rev. Biochem.* 74, 739–789. doi:10.1146/annurev.biochem.73.011303.074134

Shamu, C. E., and Walter, P. (1996). Oligomerization and phosphorylation of the Ire1p kinase during intracellular signaling from the endoplasmic reticulum to the nucleus. *EMBO J.* 15, 3028–3039.

Shaulian, E., and Karin, M. (2001). AP-1 in cell proliferation and survival. *Oncogene* 20, 2390–2400. doi:10.1038/sj.onc.1204383

Sidrauski, C., and Walter, P. (1997). The transmembrane kinase Ire1p is a site-specific endonuclease that initiates mRNA splicing in the unfolded protein response. *Cell* 90, 1031–1039.

Smaili, S., Hirata, H., Ureshino, R., Monteforte, P. T., Morales, A. P., Muler, M. L., et al. (2009). Calcium and cell death signaling in neurodegeneration and aging. *An. Acad. Bras. Cienc.* 81, 467–475. doi:10.1590/S0001-37652009000300011

Smith, M. H., Ploegh, H. L., and Weissman, J. S. (2011). Road to ruin: targeting proteins for degradation in the endoplasmic reticulum. *Science* 334, 1086–1090. doi:10.1126/science.1209235

Sokka, A. L., Putkonen, N., Mudo, G., Pryazhnikov, E., Reijonen, S., Khiroug, L., et al. (2007). Endoplasmic reticulum stress inhibition protects against excitotoxic neuronal injury in the rat brain. *J. Neurosci.* 27, 901–908. doi:10.1523/JNEUROSCI.4289-06.2007

Stamler, J. S., Singel, D. J., and Loscalzo, J. (1992). Biochemistry of nitric oxide and its redox-activated forms. *Science* 258, 1898–1902. doi:10.1126/science.1281928

Szegezdi, E., Logue, S. E., Gorman, A. M., and Samali, A. (2006). Mediators of endoplasmic reticulum stress-induced apoptosis. *EMBO Rep.* 7, 880–885. doi:10.1038/sj.embor.7400779

Szul, T., and Sztul, E. (2011). COPII and COPI traffic at the ER-Golgi interface. *Physiology (Bethesda)* 26, 348–364. doi:10.1152/physiol.00017.2011

Tabas, I., and Ron, D. (2011). Integrating the mechanisms of apoptosis induced by endoplasmic reticulum stress. *Nat. Cell Biol.* 13, 184–190. doi:10.1038/ncb0311-184

Tadic, V., Prell, T., Lautenschlaeger, J., and Grosskreutz, J. (2014). The ER mitochondria calcium cycle and ER stress response as therapeutic targets in amyotrophic lateral sclerosis. *Front. Cell. Neurosci.* 8:147. doi:10.3389/fncel.2014.00147

Tam, A. B., Mercado, E. L., Hoffmann, A., and Niwa, M. (2012). ER stress activates NF-kappaB by integrating functions of basal IKK activity, IRE1 and PERK. *PLoS ONE* 7:e45078. doi:10.1371/journal.pone.0045078

Tamatani, M., Che, Y. H., Matsuzaki, H., Ogawa, S., Okado, H., Miyake, S., et al. (1999). Tumor necrosis factor induces Bcl-2 and Bcl-x expression through NFkappaB activation in primary hippocampal neurons. *J. Biol. Chem.* 274, 8531–8538. doi:10.1074/jbc.274.13.8531

Teng, X., Song, J., Zhang, G., Cai, Y., Yuan, F., Du, J., et al. (2011). Inhibition of endoplasmic reticulum stress by intermedin(1-53) protects against myocardial injury through a PI3 kinase-Akt signaling pathway. *J. Mol. Med. (Berl.)* 89, 1195–1205. doi:10.1007/s00109-011-0808-5

Tersey, S. A., Nishiki, Y., Templin, A. T., Cabrera, S. M., Stull, N. D., Colvin, S. C., et al. (2012). Islet beta-cell endoplasmic reticulum stress precedes the onset of type 1 diabetes in the nonobese diabetic mouse model. *Diabetes* 61, 818–827. doi:10.2337/db11-1293

Tsai, Y. C., and Weissman, A. M. (2010). The unfolded protein response, degradation from endoplasmic reticulum and cancer. *Genes Cancer* 1, 764–778. doi:10.1177/1947601910383011

Tu, B. P., and Weissman, J. S. (2004). Oxidative protein folding in eukaryotes: mechanisms and consequences. *J. Cell Biol.* 164, 341–346. doi:10.1083/jcb.200311055

Turrens, J. F. (2003). Mitochondrial formation of reactive oxygen species. *J. Physiol.* 552, 335–344. doi:10.1113/jphysiol.2003.049478

Uehara, T., Nakamura, T., Yao, D., Shi, Z. Q., Gu, Z., Ma, Y., et al. (2006). S-nitrosylated protein-disulphide isomerase links protein misfolding to neurodegeneration. *Nature* 441, 513–517. doi:10.1038/nature04782

Ullman, E., Fan, Y., Stawowczyk, M., Chen, H. M., Yue, Z., and Zong, W. X. (2008). Autophagy promotes necrosis in apoptosis-deficient cells in response to ER stress. *Cell Death Differ.* 15, 422–425. doi:10.1038/sj.cdd.4402234

Urano, F., Wang, X., Bertolotti, A., Zhang, Y., Chung, P., Harding, H. P., et al. (2000). Coupling of stress in the ER to activation of JNK protein kinases by transmembrane protein kinase IRE1. *Science* 287, 664–666. doi:10.1126/science.287.5453.664

Valko, M., Leibfritz, D., Moncol, J., Cronin, M. T., Mazur, M., and Telser, J. (2007). Free radicals and antioxidants in normal physiological functions and human disease. *Int. J. Biochem. Cell Biol.* 39, 44–84. doi:10.1016/j.biocel.2006.07.001

Van Huizen, R., Martindale, J. L., Gorospe, M., and Holbrook, N. J. (2003). P58IPK, a novel endoplasmic reticulum stress-inducible protein and potential negative regulator of eIF2alpha signaling. *J. Biol. Chem.* 278, 15558–15564. doi:10.1074/jbc.M212074200

Vang, S., Longley, K., Steer, C. J., and Low, W. C. (2014). The unexpected uses of urso- and tauroursodeoxycholic acid in the treatment of non-liver diseases. *Glob. Adv. Health Med.* 3, 58–69. doi:10.7453/gahmj.2014.017

Vannuvel, K., Renard, P., Raes, M., and Arnould, T. (2013). Functional and morphological impact of ER stress on mitochondria. *J. Cell. Physiol.* 228, 1802–1818. doi:10.1002/jcp.24360

Vattem, K. M., and Wek, R. C. (2004). Reinitiation involving upstream ORFs regulates ATF4 mRNA translation in mammalian cells. *Proc. Natl. Acad. Sci. U.S.A.* 101, 11269–11274. doi:10.1073/pnas.0400541101

Verfaillie, T., Garg, A. D., and Agostinis, P. (2013). Targeting ER stress induced apoptosis and inflammation in cancer. *Cancer Lett.* 332, 249–264. doi:10.1016/j.canlet.2010.07.016

Viana, R. J., Nunes, A. F., and Rodrigues, C. M. (2012). Endoplasmic reticulum enrollment in Alzheimer's disease. *Mol. Neurobiol.* 46, 522–534. doi:10.1007/s12035-012-8301-x

Vidal, R., Caballero, B., Couve, A., and Hetz, C. (2011). Converging pathways in the occurrence of endoplasmic reticulum (ER) stress in Huntington's disease. *Curr. Mol. Med.* 11, 1–12. doi:10.2174/156652411794474419

Voeltz, G. K., Rolls, M. M., and Rapoport, T. A. (2002). Structural organization of the endoplasmic reticulum. *EMBO Rep.* 3, 944–950. doi:10.1093/embo-reports/kvf202

Wallace, D. C. (2005). A mitochondrial paradigm of metabolic and degenerative diseases, aging, and cancer: a dawn for evolutionary medicine. *Annu. Rev. Genet.* 39, 359–407. doi:10.1146/annurev.genet.39.110304.095751

Wang, H. Q., and Takahashi, R. (2007). Expanding insights on the involvement of endoplasmic reticulum stress in Parkinson's disease. *Antioxid. Redox Signal.* 9, 553–561. doi:10.1089/ars.2006.1524

Wang, L., Tang, W., Jiang, T., Lu, P., Li, Y., Sun, A., et al. (2014). Endoplasmic reticulum stress is involved in the neuroprotective effect of propofol. *Neurochem. Res.* doi:10.1007/s11064-014-1369-0

Wolfson, J. J., May, K. L., Thorpe, C. M., Jandhyala, D. M., Paton, J. C., and Paton, A. W. (2008). Subtilase cytotoxin activates PERK, IRE1 and ATF6 endoplasmic reticulum stress-signalling pathways. *Cell. Microbiol.* 10, 1775–1786. doi:10.1111/j.1462-5822.2008.01164.x

Wu, S., Tan, M., Hu, Y., Wang, J. L., Scheuner, D., and Kaufman, R. J. (2004). Ultraviolet light activates NFkappaB through translational inhibition of IkappaBalpha synthesis. *J. Biol. Chem.* 279, 34898–34902. doi:10.1074/jbc.M405616200

Xu, K., and Zhu, X. P. (2012). Endoplasmic reticulum stress and prion diseases. *Rev. Neurosci.* 23, 79–84. doi:10.1515/rns.2011.062

Yamamoto, K., Sato, T., Matsui, T., Sato, M., Okada, T., Yoshida, H., et al. (2007). Transcriptional induction of mammalian ER quality control proteins is mediated by single or combined action of ATF6alpha and XBP1. *Dev. Cell* 13, 365–376. doi:10.1016/j.devcel.2007.07.018

Yamazaki, H., Hiramatsu, N., Hayakawa, K., Tagawa, Y., Okamura, M., Ogata, R., et al. (2009). Activation of the Akt-NF-kappaB pathway by subtilase cytotoxin through the ATF6 branch of the unfolded protein response. *J. Immunol.* 183, 1480–1487. doi:10.4049/jimmunol.0900017

Ye, J., Rawson, R. B., Komuro, R., Chen, X., Dave, U. P., Prywes, R., et al. (2000). ER stress induces cleavage of membrane-bound ATF6 by the same proteases that process SREBPs. *Mol. Cell* 6, 1355–1364. doi:10.1016/S1097-2765(00)00133-7

Yoneda, T., Imaizumi, K., Oono, K., Yui, D., Gomi, F., Katayama, T., et al. (2001). Activation of caspase-12, an endoplasmic reticulum (ER) resident caspase, through tumor necrosis factor receptor-associated factor 2-dependent mechanism in response to the ER stress. *J. Biol. Chem.* 276, 13935–13940. doi:10.1074/jbc.M010677200

Yorimitsu, T., Nair, U., Yang, Z., and Klionsky, D. J. (2006). Endoplasmic reticulum stress triggers autophagy. *J. Biol. Chem.* 281, 30299–30304. doi:10.1074/jbc.M607007200

Yoshida, H., Haze, K., Yanagi, H., Yura, T., and Mori, K. (1998). Identification of the cis-acting endoplasmic reticulum stress response element responsible for transcriptional induction of mammalian glucose-regulated proteins. Involvement of basic leucine zipper transcription factors. *J. Biol. Chem.* 273, 33741–33749.

Yoshida, H., Matsui, T., Yamamoto, A., Okada, T., and Mori, K. (2001a). XBP1 mRNA is induced by ATF6 and spliced by IRE1 in response to ER stress to produce a highly active transcription factor. *Cell* 107, 881–891. doi:10.1016/S0092-8674(01)00611-0

Yoshida, H., Okada, T., Haze, K., Yanagi, H., Yura, T., Negishi, M., et al. (2001b). Endoplasmic reticulum stress-induced formation of transcription factor complex ERSF including NF-Y (CBF) and activating transcription factors 6alpha and 6beta that activates the mammalian unfolded protein response. *Mol. Cell. Biol.* 21, 1239–1248. doi:10.1128/MCB.21.4.1239-1248.2001

Zhang, K., and Kaufman, R. J. (2008). From endoplasmic-reticulum stress to the inflammatory response. *Nature* 454, 455–462. doi:10.1038/nature07203

Zhang, K., Shen, X., Wu, J., Sakaki, K., Saunders, T., Rutkowski, D. T., et al. (2006). Endoplasmic reticulum stress activates cleavage of CREBH to induce a systemic inflammatory response. *Cell* 124, 587–599. doi:10.1016/j.cell.2005.11.040

Zhang, L., and Wang, A. (2012). Virus-induced ER stress and the unfolded protein response. *Front. Plant Sci.* 3:293. doi:10.3389/fpls.2012.00293

Zheng, M., Liu, J., Ruan, Z., Tian, S., Ma, Y., Zhu, J., et al. (2013). Intrahippocampal injection of Abeta1-42 inhibits neurogenesis and down-regulates IFN-gamma and NF-kappaB expression in hippocampus of adult mouse brain. *Amyloid* 20, 13–20. doi:10.3109/13506129.2012.755122

Zhong, J., Rao, X., Xu, J. F., Yang, P., and Wang, C. Y. (2012). The role of endoplasmic reticulum stress in autoimmune-mediated beta-cell destruction in type 1 diabetes. *Exp. Diabetes Res.* 2012, 238980. doi:10.1155/2012/238980

Zhou, A. X., and Tabas, I. (2013). The UPR in atherosclerosis. *Semin. Immunopathol.* 35, 321–332. doi:10.1007/s00281-013-0372-x

Zhou, L., Yang, D., Wu, D. F., Guo, Z. M., Okoro, E., and Yang, H. (2013). Inhibition of endoplasmic reticulum stress and atherosclerosis by 2-aminopurine in apolipoprotein e-deficient mice. *ISRN Pharmacol.* 2013, 847310. doi:10.1155/2013/847310

Zhu, Y., Fenik, P., Zhan, G., Sanfillipo-Cohn, B., Naidoo, N., and Veasey, S. C. (2008). Eif-2a protects brainstem motor neurons in a murine model of sleep apnea. *J. Neurosci.* 28, 2168–2178. doi:10.1523/JNEUROSCI.5232-07.2008

Zou, W., Yue, P., Khuri, F. R., and Sun, S. Y. (2008). Coupling of endoplasmic reticulum stress to CDDO-Me-induced up-regulation of death receptor 5 via a CHOP-dependent mechanism involving JNK activation. *Cancer Res.* 68, 7484–7492. doi:10.1158/0008-5472.CAN-08-1318

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 21 May 2014; accepted: 15 July 2014; published online: 29 July 2014.
Citation: Chaudhari N, Talwar P, Parimisetty A, Lefebvre d'Hellencourt C and Ravanan P (2014) A molecular web: endoplasmic reticulum stress, inflammation, and oxidative stress. Front. Cell. Neurosci. 8:213. doi: 10.3389/fncel.2014.00213
This article was submitted to the journal Frontiers in Cellular Neuroscience.

REVIEW ARTICLE-2

Secret Talk Between Adipose Tissue And Central Nervous System - An Emerging Frontier In The Neurodegenerative Research

Secret Talk between Adipose Tissue and Central Nervous System - An Emerging Frontier in the Neurodegenerative Research

Avinash Parimisetty, Nicolas Diotel, Christian Lefebvre d'Hellencourt*

INSERM U1188, Université de La Réunion, Plateforme CYROI, Saint-Denis de La Réunion, FRANCE

Address all correspondence: Prof. Christian Lefebvre d'Hellencourt
UMR 1188 Diabète athérothrombose Thérapies Réunion Océan Indien (DéTROI), Sainte-Clotilde, F-97490, France

Tel: (+262) 262 93 82 01 - Fax: (+262) 262 93 82 37

E-Mail: Christian.Lefebvre-d-Hellencourt@univ-reunion.fr

Grant Sponsor: Region La Reunion, Europe (CPER/FEDER)

ABSTRACT

It is an obsolete fact that adipose tissue just acts as a storage organ in the form of fat. Now the scene has completely changed with the discovery of leptin and by showing its long range effects in the brain that lead to fascinating discoveries in the adipokine research. Apart from its storage capacity, adipose tissue is well renowned to act as an endocrine organ, secreting an array of bioactive factors known as adipocytokines acting at physiological level and plays a vital role in whole body energy metabolism and as well in innate immune response. The global effect of these adipocytokines in the metabolic activities have been well established, but the impact of these adipocytokines, its potential neurodegeneration remains poorly defined.

Recently an association between adipose tissue (via their secreting factors i.e. adipocytokines) and CNS have been equated. As these factors are coupled with the regulation of inflammation and oxidative stress, there is a potential link with neurodegeneration. Many chronic neurodegenerative diseases such as Amyotrophic lateral sclerosis, Alzheimer and Parkinson diseases have been associated with inflammation and oxidative stress in the Central Nervous System (CNS). Inflammation and oxidative stress are two major physiological process involved in neurodegeneration. Glial cells are the most abundant cells of the CNS and maintains intimate interaction with neurons. Microglia and astrocytes are involved in the early and late onset of neuroinflammation and related neurodegenerative process in the brain. These glial cells triggers the release of multitude of cytokines that plays a key role in the defense and the damage of the CNS. Therefore the meticulous and painstaking balance between pro and anti-inflammatory cytokines are tightly controlled as dysregulation of this equilibrium may leads to progressive neurodegenerative disorders. Hence, manipulating and orchestrating these adipocytokines and glial cells as potential therapeutic targets could be an interesting lead to novel therapeutic strategy in order to counteract neurodegenerative disorders.

KEY WORDS: Obesity, White Adipose Tissue, Adipocytokines, CNS, Neuroinflammation, Neurodegeneration.

INTRODUCTION

Obesity and type 2 diabetes mellitus (T2DM) are main health issues in our modern societies and constitute greatest public health challenges of the 21st century (Awada et al., 2013b; (WHO), 2015b; a). The World Health Organization (WHO) reported that worldwide obesity has more than doubled since 1980 and more than 1.9 billion adults were overweight in 2014 ((WHO), 2015b). Following the same trends, WHO has predicted that diabetes will be the 7th leading cause of death in 2030, comprises 90% of Type 2 diabetes mainly resulting from excess body weight and physical inactivity (Alberti and Zimmet, 1998; Roglic et al., 2005; (WHO), 2015a). In parallel, 35.6 million people display dementia and 7.7 million new cases are reported every year, Alzheimer's disease (AD) being the main cause of dementia (Reisberg et al., 1997; (WHO), 2012). Interestingly, an increasing number of data recently highlight that metabolic syndrome, notably obesity and type 2 diabetes, are correlated with an increased risk to develop neurodegenerative disease such as AD, as well as neurological and neurovascular disorders (Gustafson et al., 2009; Arnoldussen et al., 2014; Kiliaan et al., 2014; Nguyen et al., 2014b). Consequently, adiposity has been proposed as an independent factor favoring the development of AD (Beydoun et al., 2008; Whitmer et al., 2008; Letra et al., 2014). Interestingly, it has been suggested that the misexpression of adipose-derived factors called adipokines or adipocytokines may disrupt directly or indirectly brain homeostasis and functions. Adipokines are semantically referring to cytokines secreted by adipose tissue. However, a certain number of adipose-derived factors are also referred as adipokines but correspond in fact to adipose-derived hormones.

In this review, we aimed at first describing the links between adiposity, adipokines levels and neurological disorders. Secondly, we will focus on adipokines signaling in the central nervous system (CNS), highlighting their potential effects on cognition, neurogenesis, and brain functioning. Finally, we will discuss how adipokines could disturb brain physiology and functions through blood brain barrier disruption resulting from increased inflammation and oxidative stress.

1 - Adipose Tissue: Not Just For Energy Storage Anymore

1.1 - Adipose tissue secretes adipokines

WAT was originally described to store energy in the form of fat. However, since the discovery of the leptin hormone in 1994, WAT is also recognized as a major endocrine organ secreting a wide variety of biologically active factors collectively called adipokines or adipocytokines (Lehr et al., 2012; Adamczak and Wiecek, 2013). To date, about hundred adipokines constituting the adipokinome have been documented to be released from white adipocytes (Chaldakov, 2007). Among the most studied adipokines, there are leptin, adiponectin, apelin, resistin, monocytes, and macrophage chemotactic protein 1 (MCP1), interleukin-1β (IL-1β), interleukin-6 (IL-6), interleukin-10 (IL-10), tumor-necrosis factor-alpha (TNF-α) and transforming growth factor (TGF)-β. In addition to adipokines, lipid-derived factors such as the lysophosphatidic acid are also important mediators produced by the fat tissue (Trayhurn and Wood, 2004; Ouchi et al., 2011). There are also a wide variety of others chemokines, cytokines, proteins involved in the fibrinolytic system, as well as lipids and lipid transporter proteins belonging to the adipokine family (Kershaw and Flier, 2004; Adamczak and Wiecek, 2013). The most important pro-inflammatory adipokines are leptin, TNFα and IL-6, while the two main anti-inflammatory ones corresponding to adiponectin and the secreted frizzled-related protein 5 (sFRP5) (Maeda et al., 2002; Xu et al., 2003; Ouchi et al., 2011). Adipokines exert pleiotropic effects on different tissues such as lung, skeletal muscle, heart, liver, blood vessels and regulate numerous physiological functions such as appetite, energy expenditure, insulin sensitivity and secretion, fat distribution, lipid and glucose metabolism, endothelial function, blood pressure, hemostasis, neuroendocrine functions and also immunity (Ahima et al., 2000; Trayhurn and Beattie, 2001; Trayhurn and Wood, 2004; Lehr et al., 2012; Leal Vde and Mafra, 2013; Bluher, 2014; Bluher and Mantzoros, 2015). So, the data generated over the last 20 years considerably change our view on adipose tissue as WAT plays a wide-ranging role in metabolic regulation and physiological homeostasis (Lehr et al., 2012; Adamczak and Wiecek, 2013).

1.2 - Adipokines and diseases: focus on neurological disorders and diseases

The dysregulation of adipokine production and/or levels has been correlated with several diseases and could notably promote and/or result in obesity-linked metabolic disorders (Maury and Brichard, 2010; Bluher and Mantzoros, 2015). Thus, low plasmatic leptin concentrations are associated with an increase risk for cardiovascular troubles (Ku et al., 2011). In contrast, higher plasmatic adiponectin levels seem to be associated with decreased risk for

developing TD2M (Spranger et al., 2003). Other data also show relationships between MCP-1 serum levels and insulin resistance, diabetic people exhibiting highest MCP-1 levels (Herder et al., 2006). In the same line of evidences, it appears that the inflammatory state of WAT in obese patients might be a key player linking high WAT mass to insulin resistance. Interestingly, an increasing number of studies reported links between metabolic disorders (i.e.: TD2M and obesity), brain homeostasis and functioning (Gustafson et al., 2003; Gustafson, 2006; Lee, 2011; Arnoldussen et al., 2014; Gianfrancesco et al., 2014; Letra et al., 2014; Nguyen et al., 2014a; Versini et al., 2014; Zhang and Tian, 2014). This concept according to which metabolic syndrome could have an effect on brain homeostasis and functions is relatively new and innovative. Initial studies demonstrated that a higher BMI and/or waist-to-hip ratio in middle-aged individuals is associated with a reduction in whole brain volume. Indeed, over the last decade, a number of magnetic resonance imaging (MRI) and computed tomography (CT) studies also reported alterations in brain morphology of overweight/obese individuals (Ward et al., 2005; Taki et al., 2008; Bruce-Keller et al., 2009). Studies notably documented a link between abdominal fat and reduced brain volume in healthy middle-aged adults notably the temporal lobe volume and the hippocampus (Gustafson et al., 2004; Debette et al., 2010). In a cross-sectional study of normal elderly individuals showing no sign of cognitive deficit, tensor-based morphometry also unveiled atrophy in the white and gray matter of the frontal lobes, anterior cingulate gyrus, hippocampus, and thalamus in both male and female subjects with a high BMI (BMI > 30) as compared to individuals with a normal BMI (18.5–25) (Raji et al., 2010). Upon further investigation, the brain volume reduction in gray and white matter was found to be associated with a common variant of the fat mass and obesity associated (*FTO*) gene (Ho et al., 2010a). In addition, a growing body of studies also show that obesity in mild-life is a predictor of mild cognitive impairment with aging and altered executive function and short-term memory compared to normal weight counterparts (Cournot et al., 2006; Lokken et al., 2009; Sabia et al., 2009; Nguyen et al., 2014a). Such data are also confirmed in rodents for which high fat diets result in impaired cognitive functions including a decrease in memory performance, learning and executive functions (Murray et al., 2009; McNeilly et al., 2011; Nguyen et al., 2014a). In addition, during the development of obesity in rodent models, it appears that neurochemical changes occurs in the brain altering cognition processes, reward neurocircuitry and stress responsiveness (Morris et al., 2014). Consequently, numerous studies described association between rich diets (sugar and/or fat) and cognitive defects in rodents and humans (Morris et al., 2014; Nguyen et al., 2014a) and it seems that such effects of diets could occur through the disruption of neurovascular function (Li et al., 2013; Lynch et al., 2013;

Morris et al., 2014). In addition, a linkage has been demonstrated between overweight, neuroinflammation and neurodegenerative diseases namely AD, Parkinson's disease (PD) and autoimmune nervous system diseases such as multiple sclerosis (Gustafson et al., 2003; Gustafson, 2006; Lee, 2011; Arnoldussen et al., 2014; Gianfrancesco et al., 2014; Letra et al., 2014; Versini et al., 2014; Zhang and Tian, 2014). Similarly, T2DM is associated to impaired cognition, peculiarly learning and memory deficits such as shown in rodents and humans while such effects are rarely observed in type 1 diabetes (McCrimmon et al., 2012; Zhou et al., 2014). This is peculiarly interesting given that T2DM patients are mostly overweight or obese compared to type 1 diabetic. Studies have revealed cortical and subcortical atrophy as well as hippocampal gray matter reduction of T2DM patients with cognitive impairment (Zhang et al., 2015). Interestingly, a recent study working on 80 T2DM patients and 80 healthy controls demonstrated that cognition impairment was correlated with reduced hippocampal CA1 size in the diabetic group (Zhang et al., 2015). Interestingly, diabetes is associated with an increased risk of Alzheimer's disease and vascular dementia, supported by increasing oxidative stress and inflammation and impaired insulin and amyloid metabolism (MacKnight et al., 2002; Haan, 2006; Whitmer, 2007; Biessels et al., 2008; Cheng et al., 2012; McCrimmon et al., 2012). TD2M people also display lower cerebral blood flow and neural slowing on recordings of sensory-evoked potentials (McCrimmon et al., 2012). Numerous studies performed on rodents also show an impact of diabetes on neurogenesis, depression and cognition (Ho et al., 2013).

Taken together, these data show that obesity and diabetes have negative effects on brain structures and/or functions. It also raises the question of the roles of adipokines in such neurological disorders. However, the rational of such events are still poorly understood. One interesting hypothesis is that abnormal adipokines concentrations, such as increase pro-inflammatory adipokines TNFα, resistin, leptin, IL-1β and also IL-6, could influence the blood brain barrier integrity and disrupt brain homeostasis through oxidative stress and inflammation (Ouchi et al., 2011; Letra et al., 2014). In the following part, we aim at further described effects of the main adipokines in the brain, regarding their transport in the central nervous system and their signaling.

2 - Adipokines and Targets in The Brain

As previously mentioned in this review, WAT is an important and a major endocrine secretory organ, releasing endocrine and paracrine signaling molecules called adipokines (Ouchi et al., 2003; Berg and Scherer, 2005). In this part, we will focus on the main adipokines (leptin, resistin, adiponectin, TNFα and also on a lipokine of interest, the lysophosphatidic acid), their targets in the brain and their potential impact on brain inflammation and functions.

2.1 - Leptin

Leptin is one of the most important adipose-derived hormones. Leptin which is mainly produced by adipocytes exert its effects both peripherally and centrally (Zhang et al., 1994; Trayhurn and Beattie, 2001; Trayhurn and Wood, 2004; Adamczak and Wiecek, 2013). This adipokine play key functions in regulating energy intake and expenditure, metabolism and behavior by directly acting on the CNS. Mice invalidating for leptin (ob/ob mice) display obesity, insulin resistance and hyperphagia showing notably the impact of this adipose-derived hormone on feeding behavior (Friedman and Halaas, 1998). Peripheral leptin exert its central effect through its binding at the level of choroid plexus leading to its transport across the blood brain barrier (Banks et al., 1996; Devos et al., 1996; Banks et al., 2000; Zlokovic et al., 2000). Such a transport involved leptin receptors and probably other mechanisms that are still poorly understood (Arnoldussen et al., 2014). However, leptin has been shown to be also locally and *de novo* produced in the brain, in the cerebellum, the cortex and the hypothalamus (Morash et al., 1999; Wilkinson et al., 2000; Brown et al., 2007; Brown et al., 2008), suggesting other specific and local functions for leptin than those previously described. Leptin receptors belong to the family of cytokine receptors, and at least five different isoforms have been identified in mouse: Ob-Ra to Ob-Re (Friedman and Halaas, 1998; Gorska et al., 2010). In the CNS, leptin receptors (Ob-R or LepR) were first described by Tartaglia and colleagues in choroid plexus and in the hypothalamus (Tartaglia et al., 1995b; Tartaglia, 1997). Among all Ob-R isoforms, only the full-length isoform (Ob-Rb) appears to fully transduce the activation signal at least in the brain and is essential for leptin's weight-reducing effects (Friedman and Halaas, 1998; Gorska et al., 2010). Ob-Rb is strongly and mainly expressed in the hypothalamic nuclei notably in the arcuate nucleus (ARC), the dorsomedial nucleus (DMH), paraventricular nucleus (PVN), the ventromedial hypothalamic nucleus (VMH) and the lateral hypothalamic nucleus (LH) (Mercer et al., 1996; Friedman and Halaas, 1998; Yi et al., 2013), but is also detected in the neocortex, the medulla and the cerebellum (Burguera et al., 2000). In addition, a weaker expression was also detected by *in situ* hybridization in the hippocampus and the thalamus

(Mercer et al., 1996). In the hypothalamus, the primary leptin targets are the orexigenic AgRP (Agouti-related peptide) neurons and the anorexigenic POMC (Pro-opiomelanocortin) neurons that are involved in feeding behavior. Thus, in the CNS, leptin activates anorexigenic POMC neurons through a neural network in the arcuate nucleus (Cowley et al., 2001). The appetite stimulating effects of AgRP/NPY are inhibited by leptin in the arcuate nucleus avoiding the release of orexigenic factors (Baskin et al., 1999; Enriori et al., 2007). Furthermore, leptin receptors were also documented to be expressed in glutamatergic and GABAergic neurons (Vong et al., 2011; Xu et al., 2013; Yi et al., 2013). Vong and colleagues (2011) show that the main effect of leptin are mediated by GABAergic neurons and only barely by glutamatergic neurons (Vong et al., 2011). However, it was recently shown that glutamate release mediates leptin action on energy expenditure (Xu et al., 2013). We realize now that the effects of leptin on these different neuronal types and brain nuclei are not so easy to understand as first though. In homeostatic conditions, leptin inhibits food intake and in extra-hypothalamic sites leptin act on neurogenesis, synaptogenesis, neuronal excitability and neuroprotection (Bouret, 2010; Paz-Filho et al., 2010b; Arnoldussen et al., 2014). Leptin was also shown to improve cognition and mood in depressed and anxious animal models, notably by improving long term potentiation (Arnoldussen et al., 2014). Leptin levels negatively correlated with the development of Alzheimer's disease in lean humans (Paz-Filho et al., 2010b; a) and leptin signaling seems to be dysregulated in Alzheimer's disease brains (Bonda et al., 2014). Interestingly, there are also positive correlations between plasma levels of leptin and body weight (Salbe et al., 2002; Fleisch et al., 2007). Additionally, in obese people, leptin resistance takes place impairing memory, neurogenesis and neuroprotection (Arnoldussen et al., 2014).

2.2 - Resistin

Resistin (or adipose tissue-specific secretory factor: ADSF) is a cysteine-rich adipose-derived peptide hormone, encoded by the *RETN* gene and known for its implication in inflammatory processes (Wang et al., 2002a; Ouchi et al., 2011). Its expression increases in parallel to adiposity (Degawa-Yamauchi et al., 2003; Vendrell et al., 2004; Lee et al., 2005) and is strongly related to insulin resistance notably in obese rodents (Park and Ahima, 2013). Interestingly, in humans, resistin is mainly expressed and secreted by macrophages while adipocytes are the main source in rodent (Park and Ahima, 2013). Resistin is known to play a key role in the CNS notably by regulating pituitary somatotrope cell functions (Broglio et al., 2005), affecting hypothalamic and peripheral insulin responsiveness, thermogenesis, feeding behavior and also by enhancing renal sympathetic nerve activity (Kosari et al., 2012; Yi and

Tschop, 2012; Kosari et al., 2013). However, the resistin receptor and the molecular mechanisms sustaining such effects are poorly understood and mainly unexplored until recently. Although resistin receptor has not be clearly identified, it has been shown that resistin administration modulates or activates several signaling pathway involving Gs protein-dependent mechanisms, the adenylate cyclase/cAMP/protein kinase A pathway, the phosphatidylinositol 3-kinase/Akt pathway, the protein kinase C, and extracellular Ca2+ signaling through L-type voltage-sensitive Ca2+ (Kosari et al. 2013; Rodríguez-Pacheco et al. 2009). More recently, an isoform of decorin (a small proteoglycan associated with collagen fibrils) was reported to be a resistin receptor on the surface of adipose progenitor cells. In addition, tyrosine kinase-like orphan receptor-1 (ROR1) was reported to mediate resistin effect on glucose uptake and adipogenesis in 3T3-L1 cells (Sánchez-Solana, Laborda, and Baladrón 2012), and that resistin utilizes the IGF-1R pathway in rheumatoid arthritis (Boström et al. 2011). Such puzzling data strongly suggest that resistin could potentially interact with different receptors depending on tissue and cell-types. Furthermore, resistin also regulates the synthesis and secretion of other pro-inflammatory cytokines TNF-α and IL-6 through nuclear factor-κB–dependent pathway in macrophage (Bokarewa et al., 2005; Silswal et al., 2005; Olefsky and Glass, 2010) .Recently, Toll-like receptor (TLR) 4 receptors were identified as potential receptor for resistin in the hypothalamus, leading to the activation of JNK and p38/MAPK pathways (Benomar et al., 2013). Interestingly, resistin was also reported to be expressed in the hypothalamus and the cortex and to inactivate hypothalamic neurons (Morash et al., 2002; Brown et al., 2005; Wilkinson et al., 2007). By modulating the activation of ERK1/2, resistin modulates the effects of central resistin on reducing thermogenesis (Kosari et al., 2013). In the rat brain, resistin is *de novo* produced suggesting specific roles for this local synthesis (Wilkinson et al., 2007). Interestingly, traumatic brain injury (TBI) increased *resistin* mRNA expression in the ipsilateral cortex without any effects on the contralateral hemisphere. However, resistin expression is up-regulated after TBI in the ipsi-and contralateral hippocampus (Brown et al., 2008). One explanation is that given TBI compromises the integrity of the blood brain barrier, it could result in the changes in gene expression in the contralateral side of the hippocampus by exposing the brain to circulating factors of peripheral origin (Brown et al., 2008). The increase of resistin expression following TBI (at 12 h post-injury), is in contrast to the delayed upregulation of resistin in hypoxic ischemic mouse brain (>7 days) (Wiesner et al., 2006). Thus resistin could participate in the acute responses to cerebral damage probably through inflammatory mechanisms. In a recent study, resistin was shown to not be related to cognitive function performance (Miralbell et al., 2013).

2.3 - Adiponectin

Adiponectin was discovered in 1995 by Scherer and collaborators (Scherer et al., 1995). It is one of the most important adipokines considering it's abundance in plasma relative to many other hormones (Matsuzawa, 2005; Thundyil et al., 2012b). Adiponectin self-associates into larger structures forming homotrimers that also self-associate and form hexamers or dodecamers. A globular fraction, named globular adiponectin, resulting from the cleavage of the full-length monomer was also documented (Waki et al., 2003). Adiponectin is mainly synthesized and secreted by adipocytes. However, it is now well admitted that adiponectin is expressed at the mRNA and/or protein level by the placenta, the liver, epithelial cells, osteoblasts, myocytes and also by pituitary cells (Wilkinson et al., 2007; Psilopanagioti et al., 2009; Thundyil et al., 2012b). Interestingly, some studies documented *adiponectin* transcript expression in the brain the diencephalon in chicken (Maddineni et al., 2005; Wilkinson et al., 2007), in the human pituitary (Psilopanagioti et al., 2009). In the pituitary, adiponectin could have a role in the release of somatotrophs and gonadotrophs (Thundyil et al., 2012b). It also modulates a wide range of metabolic processes such as body-weight regulation, glucose regulation, insulin sensitivity, lipid catabolism (fatty acid oxidation), endothelial function and also anti-atherogenic process (Berg et al., 2002; Okamoto et al., 2002; Stefan and Stumvoll, 2002; Whitehead et al., 2006; Thundyil et al., 2012b). Such effects are mediated by three different receptor types: adiponectin receptor 1 (ADIPOR1), adiponectin receptor 2 (ADIPOR2) and T-cadherin (CDH13) and involved different signaling pathway including notably AMPK, p38-MAPK, JNK, PPAR-α and NF-kB. These Receptors appear to be widely expressed in the mammalian brain including mouse, rat, human and pork their expression was documented in different brain structures such as the pituitary, the hypothalamus, in cortical and subcortical neurons (Degawa-Yamauchi et al., 2003; Yamauchi et al., 2003a; Fry et al., 2006; Hoyda et al., 2007; Psilopanagioti et al., 2009; Repunte-Canonigo et al., 2010; Thundyil et al., 2010; Thundyil et al., 2012b). In their recent review, Thundyil and colleagues (2012) documented adiponectin receptors expression in the central nervous system showing that AdipoR1 is mainly expressed in the hypothalamus, the brainstem and the pituitary gland while AdipoR2 seems to be mostly expressed in the cortex. Furthermore, AdipoR1 is strongly expressed in neurons and a bit in astrocytes while AdipoR2 is figured to be only weakly expressed in astrocytes and neurons (Thundyil et al., 2012b). However, until now, AdipoR expression in microglia is not really clear. Concerning T-cadherin receptor, it seems to be temporally and spatially expressed in different neuronal populations during axon growth

(Ranscht and Dours-Zimmermann, 1991). Furthermore, T-cadherin showed broad expression in the cerebral cortex, basal ganglia, amygdala, and hippocampus in the developing postnatal telencephalon of marmoset (Callithrix jacchus) (Matsunaga et al., 2013). In mouse, cdh13 was also shown to be expressed by projection neurons within the main and accessory olfactory bulbs. Interestingly, adiponectin deficiency is associated with exaggerated inflammatory response in critical illness or septic patients (Venkatesh et al., 2009; Hillenbrand et al., 2010; Hillenbrand et al., 2012). Recently, AdipoR1 and AdipoR2 expression was described in both U373 MG cell line and primary human astrocytes (Wan et al., 2014). It also appears that adiponectin induces a pro-inflammatory response in human astrocytes, increasing notably IL-6 and MCP-1 through NF-κB, p38MAPK and ERK1/2 pathways (Wan et al., 2014). In contrast, adiponectin was described to inhibit pro-inflammatory signal, notably by suppressing IL-6 release from BBB endothelial cells (Spranger et al., 2006). It results that adiponectin indirectly modulates inflammatory signaling across the BBB by negatively modulating Il-6 and TNFα release. *In vitro* experiment of hippocampal neurons reveals that adiponectin exerts neuroprotective effects through AMPK pathway (Qiu et al., 2011). Such neuroprotective effects of adiponectin is further reinforced by the fact that knock-out mice for adiponectin exhibit more brain damages after ischemic stroke to controls (Nishimura et al., 2008). This neuroprotective action acts through an endothelial nitric oxide synthase-dependent mechanism (Nishimura et al., 2008).

2.3 - Tumor necrosis factor α

TNFα is a pro-inflammatory adipokine well-known for its role in chronic peripheral and central inflammation (Thaler et al., 2012; Arnoldussen et al., 2014). TNFα is primarily produced as a transmembrane protein that self-associated into stable homotrimers (Kriegler et al., 1988; Tang et al., 1994). Such homotrimers could be cleave by the TNFα converting enzyme (TACE, also called ADAM17), allowing the release of secreted form of TNFα (Black et al., 1997). TNFα is mainly produced by macrophages and its expression is increased at the mRNA and protein levels in obese and in T2DM models (Hotamisligil et al., 1993). TNFα actions are mediated by two receptors: TNF-Receptor 1 (TNF-R1) and TNF-Receptor 2 (TNF-R2). TNFR1 is expressed in most tissues, and can be fully activated by both the membrane-bound and soluble trimeric forms of TNF, while TNFR2 is found only in cells of the immune system, and respond to the membrane-bound form of the TNF homotrimer (Arnoldussen et al., 2014). As most information regarding TNF signaling is derived from TNFR1, the role of TNFR2 is likely underestimated. In rodents, TNFα has been shown to be transported across the

BBB, but to be also locally produced by microglia, astrocytes and neurons in the brain (Lieberman et al., 1989; Chung and Benveniste, 1990; Morganti-Kossman et al., 1997). In the CNS, TNFα acts through TNF-receptors on neurons and astrocytes regulating a wide range of cellular processes such as cell-survival (Pickering et al., 2005; Montgomery and Bowers, 2012; Arnoldussen et al., 2014). More widely, TNFα exhibits pleiotropic effects with positive and negative outcomes on the brain. It notably acts on neurogenesis, synaptic transmission and plasticity (Arnoldussen et al., 2014). Thus, TNFα was notably described fort its neuroprotective roles on hippocampal neurons by suppressing accumulation of ROS and by maintaining intracellular levels of calcium (Barger et al., 1995). In addition, it modulates glutamatergic transmission (Beattie et al., 2002). Furthermore, TNFα favors neural progenitor cells survival by notably mediating anti-apoptotic signals via TNFR2 (Marchetti et al., 2004). In rat, TNFα appear to promote the survival of stroke-generated hippocampal and striatal neurons (Heldmann et al., 2005). In addition, TNFα knock-out mice show cognitive impairment (i.e: significant poorer learning, retention and spatial learning), suggesting a strong role for TNF-α on these mechanisms (Baune et al., 2008). Indeed, TNFα also exhibits a dark face, as reported in numerous other studies. It is notably involved in myelin damages (Selmaj and Raine, 1988), in favoring glutamate excitotoxicity (Hermann et al., 2001), in inhibition of long term potentiation in CA1 and in the dentate gyrus of the hippocampus in rat (Cunningham et al., 1996; Butler et al., 2004; Pickering et al., 2005) and in decreasing neurogenesis (Iosif et al., 2006; Lan et al., 2012).

Altogether, these data established that the roles of TNFα is not so easy to understand. This adipokine or locally produced cytokines could exhibit multiple faces exerting neuroprotective versus neurotoxic roles, pro versus anti-neurogenic effects according to the conditions (concentrations, physiological or pathological conditions…). Neuroinflammation and metabolic disorders such as obesity could act on these mechanisms through an excess of TNFα secretion.

2.4 - Lysophosphatidic acid (LPA)

Among the factors secreted by the adipose tissue, there are a lot of lipids from the lipokine family such as PGE2, anandamide and also lysophosphatidic acid (LPA). LPA is a bioactive signalling phospholipid acting on a wide range of biological processes including cell growth, migration and morphology (Frisca et al., 2012). LPA is detected in several biological fluids and tissues including the brain (Tokumura, 2004). It is synthesized from different enzymatic activities involving notably phospholipase A1 and A2, monoacylglycerol kinase,

but the main enzyme leading to LPA synthesis is autotaxin (Noguchi et al., 2009). Autotaxin is a multifunctional phosphodiesterase that converts lysophospholipids into LPA through its lysophospholipase D activity. To date, LPA effects are mediated through five G protein coupled receptors. However, additional receptors have been identified for their potential responsiveness to LPA (Noguchi et al., 2003; Kotarsky et al., 2006; Noguchi et al., 2009). Using knock-out mice for the five most known LPA receptors, it was shown that LPA plays key roles on inflammation (Zhao and Natarajan, 2013), angiogenesis (Chen et al., 2013), reproduction (Ye et al., 2005; Ye et al., 2008; Ye and Chun, 2010), brain development and neurogenesis (Estivill-Torrus et al., 2008; Matas-Rico et al., 2008). Indeed, LPA exert pleomorphic effects on neural progenitor cells from cortex, and notably calcium mediated conductance (Dubin et al., 2010). In the nervous system, neural progenitor cells, neurons, oligodendrocytes, Schwann cells, astrocytes and microglia have been documented for expressing different subsets of LPA receptors (Noguchi et al., 2009). It partially explains why LPA exerts a wide variety of effects on these different cell-types. Thus, LPA can favor proliferation and differentiation of neural progenitor cells as shown by treatment on *ex vivo* embryonic brain slice cultures resulting in an increase cell survival and differentiation (Kingsbury et al., 2003). In contrast, LPA promotes proliferation and differentiation in neurospheres (Svetlov et al., 2004; Fukushima et al., 2007). LPA also displays effect on cell morphology and neurite formation in both neural progenitors cells and neurons (Noguchi et al., 2009). It exhibits both cell-death and survival properties on neurons possibly due to differences in LPA concentration or signaling through different receptors (Holtsberg et al., 1998; Zheng et al., 2004; Zheng et al., 2005). For instance, it induces apoptosis and necrosis in hippocampal neurons (Holtsberg et al., 1998). LPA also exerts various effects on glial and microglial cells, by modulating intracellular calcium levels in oligodendrocytes, astrocytes and microglia (Noguchi et al., 2009). It notably favors astrocytes and microglia proliferation *in vitro* (Keller et al., 1997b; Moller et al., 2001). Interestingly, in human post-mortem brains LPA receptors 1-3 and autotaxin are only weakly expressed while LPAR2 is increased and autotaxin transcripts are decreased following brain injury. Such data also reinforce the fact that LPA signaling is involved in neurotrauma (Frugier et al., 2011).

DISCUSSION

It is well-known that T2DM is strongly associated with overweight and adiposity. In this review, we highlighted the striking correlations between metabolic syndrome and the prevalence of neurological disorders and dementia including AD. Adipose tissue was not initially envisioned as a source of inflammatory factors. However, it is now well accepted that adipose tissue is a key player in the development of inflammation associated with adiposity and consequently obesity (Weisberg et al., 2003). Thus, excess fat tissue in the obese environment contributes to a low-grade chronic inflammation (Greenberg and Obin, 2006) with elevated production of pro-inflammatory cytokines, such as TNFα, IL-6 and IL-1 (Tilg and Moschen, 2006; Moschen et al., 2007). In contrast, loss of adipose tissue is associated with a decrease in inflammation markers (Clement et al., 2004; Berg and Scherer, 2005). Interestingly, chronic and low-grade inflammation has been proposed to favor neurodegenerative diseases through the disruption of the BBB. Indeed, the blood brain barrier is a key interface linking systemic inflammation, neuroinflammation and neurodegeneration (Takeda et al., 2014), inflammatory factors being a main cause of the BBB disruption (Mauro et al., 2014). For instance, studies established positive correlations between mid-life adiposity in women with disruption of BBB integrity, showing that overweight/obesity could favor the onset of vascular disorders increasing BBB permeability later in life (Gustafson et al., 2007). In the same line of evidence, rats fed with Western diet, known for promoting diabetes and obesity, display a leakier BBB due to the decreased expression of tight junctions (Kanoski et al., 2010). Kanoski and colleagues also show that a primary cerebral target following BBB disruption is the hippocampus, well-known for its involvement in cognitive processes (Kanoski et al., 2010). This is of peculiar interest given that AD patients display hippocampal atrophy and disruption of fronto-hippocampal connections early in the course of the disease (Whitwell, 2010; Remy et al., 2015; Wisse et al., 2015). This is further reinforced by the fact that AD in human and rodent models is strongly linked to an increase permeability of the BBB (Ujiie et al., 2003; Bowman et al., 2007). Consequently, the chronic low-grade inflammation that takes place in obese and diabetic people could favor brain inflammation and degeneration through BBB disruption. While the causal nature of these processes leading to neurodegeneration has not been definitively established, it is widely accepted that neuroinflammation and oxidative stress responses occur with clinical manifestation of the disease. In this review we notably described the negative impact of pro-inflammatory adipokines (TNFα, IL-6 and leptin) on brain homeostasis and functions. In addition, pro-inflammatory adipokines plays a major role in the

production of reactive oxygen species (ROS) (Laurikka et al., 2014; Matsuda and Shimomura, 2014). Due to its ability to secrete adipokines that promote ROS production, WAT has been regarded as an independent factor provoking oxidative stress (Furukawa et al., 2004; Vachharajani and Granger, 2009; Fernandez-Sanchez et al., 2011). Exposure to obese state for a long time in a host system, down-regulates and depletes the activity of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx), these enzymes being found to be significantly lowered compared with healthy persons which in turn leads to the development of obesity-related health problems (Ozata et al., 2002). In addition to this levels of vitamin A, levels of serum antioxidants, such as vitamin E, vitamin C, and β-carotene, as well as glutathione, are also decreased in obesity (Vincent et al., 2005). When compared to the normal or lean individuals, obese individuals exhibit high levels of biomarkers of oxidative damage and inflammation such as C-reactive protein, LDL oxidation, and Triglyceride levels (Pihl et al., 2006). Thus, apart from inflammation, which is quite well-known to be one of the critical factors that damages the brain, production of ROS which exceeds the antioxidant defenses in the host system is another factor can also result in brain damages. Cytokines produced by the monocytes and macrophages in WAT are the potent stimulators for the production of reactive oxygen and nitrogen species which generates oxidative stress. Adipose tissue also has the secretory capacity of angiotensin II, which stimulates nicotinamide adenine dinucleotide phosphate (NADPH) oxidase activity. NADPH oxidase comprises the major route for ROS production in adipocytes (Morrow, 2003). Thus, obesity results in an increased oxidative stress status that can lead to neural dysfunction and death (Rodriguez-Rodriguez et al., 2014; Ruszkiewicz and Albrecht, 2015). It has been reported that obesity may induce systemic oxidative stress and, in turn, oxidative stress is associated with an irregular production of adipokines, which contributes to the development of the metabolic syndrome (Esposito et al., 2006). In parallel, oxidative stress is implicated in numerous neurological diseases and/or disorders such as AD, PD, amyotrophic lateral sclerosis (ALS), multiple sclerosis (MS), cerebral ischemia/reperfusion injury and TBI, promoting neurodegeneration (Freeman and Keller, 2012). An increasing number of studies using, *in vitro* models and knock-out animals demonstrate that oxidative stress disrupt the BBB permeability (Lochhead et al., 2010; Freeman and Keller, 2012; Enciu et al., 2013).

Taken together, these data suggest that in pathological conditions adipokines released by WAT promote inflammation and ROS production that may disrupt the BBB permeability and could directly or indirectly act on different brain structures, the hippocampus being one of the most sensitive area. It could explain why metabolic syndrome is associated with

hippocampus atrophy and an increase risk to develop dementia such as AD. One main issue in people suffering from metabolic syndrome should be to struggle against inflammation and reduce oxidative stress in order to decrease their potential effects on brain neurodegeneration and their adverse effects.

CREDITS

This work was supported by the grants from Conseil Régional de La Réunion and Europe (CPER/FEDER). AP is funded by fellowships from 'Conseil Régional de La Réunion'. ND and CLH were supported by the grants from Europe (CPER/FEDER).

REFERENCES

(WHO) WHO. 2012. Dementia. http://wwwwhoint/mediacentre/factsheets/fs362/en/.

(WHO) WHO. 2015a. Diabetes. http://wwwwhoint/mediacentre/factsheets/fs312/en/.

(WHO) WHO. 2015b. Obesity and overweight.
http://wwwwhoint/mediacentre/factsheets/fs311/en/.

Adamczak M, Wiecek A. 2013. The adipose tissue as an endocrine organ. Seminars in nephrology 33(1):2-13.

Ahima RS, Saper CB, Flier JS, Elmquist JK. 2000. Leptin regulation of neuroendocrine systems. Front Neuroendocrinol 21(3):263-307.

Alberti KG, Zimmet PZ. 1998. Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. Diabetic medicine : a journal of the British Diabetic Association 15(7):539-553.

Arnoldussen IA, Kiliaan AJ, Gustafson DR. 2014. Obesity and dementia: Adipokines interact with the brain. European neuropsychopharmacology : the journal of the European College of Neuropsychopharmacology.

Awada R, Parimisetty A, Lefebvre d'Hellencourt C. 2013. Influence of obesity on neurodegenerative diseases. Neurodegenerative Diseases Chapter 16:381-401.

Banks WA, Clever CM, Farrell CL. 2000. Partial saturation and regional variation in the blood-to-brain transport of leptin in normal weight mice. American journal of physiology Endocrinology and metabolism 278(6):E1158-1165.

Banks WA, Kastin AJ, Huang W, Jaspan JB, Maness LM. 1996. Leptin enters the brain by a saturable system independent of insulin. Peptides 17(2):305-311.

Barger SW, Horster D, Furukawa K, Goodman Y, Krieglstein J, Mattson MP. 1995. Tumor necrosis factors alpha and beta protect neurons against amyloid beta-peptide toxicity: evidence for involvement of a kappa B-binding factor and attenuation of peroxide and Ca2+ accumulation. Proceedings of the National Academy of Sciences of the United States of America 92(20):9328-9332.

Baskin DG, Hahn TM, Schwartz MW. 1999. Leptin sensitive neurons in the hypothalamus. Hormone and metabolic research = Hormon- und Stoffwechselforschung = Hormones et metabolisme 31(5):345-350.

Baune BT, Wiede F, Braun A, Golledge J, Arolt V, Koerner H. 2008. Cognitive dysfunction in mice deficient for TNF- and its receptors. American journal of medical genetics Part B, Neuropsychiatric genetics : the official publication of the International Society of Psychiatric Genetics 147B(7):1056-1064.

Beattie EC, Stellwagen D, Morishita W, Bresnahan JC, Ha BK, Von Zastrow M, Beattie MS, Malenka RC. 2002. Control of synaptic strength by glial TNFalpha. Science 295(5563):2282-2285.

Benomar Y, Gertler A, De Lacy P, Crepin D, Ould Hamouda H, Riffault L, Taouis M. 2013. Central resistin overexposure induces insulin resistance through Toll-like receptor 4. Diabetes 62(1):102-114.

Berg AH, Combs TP, Scherer PE. 2002. ACRP30/adiponectin: an adipokine regulating glucose and lipid metabolism. Trends in endocrinology and metabolism: TEM 13(2):84-89.

Berg AH, Scherer PE. 2005. Adipose tissue, inflammation, and cardiovascular disease. Circulation research 96(9):939-949.

Beydoun MA, Beydoun HA, Wang Y. 2008. Obesity and central obesity as risk factors for incident dementia and its subtypes: a systematic review and meta-analysis. Obesity reviews : an official journal of the International Association for the Study of Obesity 9(3):204-218.

Biessels GJ, Deary IJ, Ryan CM. 2008. Cognition and diabetes: a lifespan perspective. Lancet Neurol 7(2):184-190.
Black RA, Rauch CT, Kozlosky CJ, Peschon JJ, Slack JL, Wolfson MF, Castner BJ, Stocking KL, Reddy P, Srinivasan S, Nelson N, Boiani N, Schooley KA, Gerhart M, Davis R, Fitzner JN, Johnson RS, Paxton RJ, March CJ, Cerretti DP. 1997. A metalloproteinase disintegrin that releases tumour-necrosis factor-alpha from cells. Nature 385(6618):729-733.
Bluher M. 2014. Adipokines - removing road blocks to obesity and diabetes therapy. Molecular metabolism 3(3):230-240.
Bluher M, Mantzoros CS. 2015. From leptin to other adipokines in health and disease: facts and expectations at the beginning of the 21st century. Metabolism: clinical and experimental 64(1):131-145.
Bokarewa M, Nagaev I, Dahlberg L, Smith U, Tarkowski A. 2005. Resistin, an adipokine with potent pro-inflammatory properties. Journal of immunology 174(9):5789-5795.
Bonda DJ, Stone JG, Torres SL, Siedlak SL, Perry G, Kryscio R, Jicha G, Casadesus G, Smith MA, Zhu X, Lee HG. 2014. Dysregulation of leptin signaling in Alzheimer disease: evidence for neuronal leptin resistance. Journal of neurochemistry 128(1):162-172.
Bouret SG. 2010. Neurodevelopmental actions of leptin. Brain research 1350:2-9.
Bowman GL, Kaye JA, Moore M, Waichunas D, Carlson NE, Quinn JF. 2007. Blood-brain barrier impairment in Alzheimer disease: stability and functional significance. Neurology 68(21):1809-1814.
Broglio C, Gómez A, Durán E, Ocaña FM, Jiménez-Moya F, Rodríguez F, Salas C. 2005. Hallmarks of a common forebrain vertebrate plan: specialized pallial areas for spatial, temporal and emotional memory in actinopterygian fish. Brain research bulletin 66(4-6):277-281.
Brown R, Imran SA, Belsham DD, Ur E, Wilkinson M. 2007. Adipokine gene expression in a novel hypothalamic neuronal cell line: resistin-dependent regulation of fasting-induced adipose factor and SOCS-3. Neuroendocrinology 85(4):232-241.
Brown R, Thompson HJ, Imran SA, Ur E, Wilkinson M. 2008. Traumatic brain injury induces adipokine gene expression in rat brain. Neuroscience letters 432(1):73-78.
Brown R, Wiesner G, Ur E, Wilkinson M. 2005. Pituitary resistin gene expression is upregulated in vitro and in vivo by dexamethasone but is unaffected by rosiglitazone. Neuroendocrinology 81(1):41-48.
Bruce-Keller AJ, Keller JN, Morrison CD. 2009. Obesity and vulnerability of the CNS. Biochimica et biophysica acta 1792(5):395-400.
Burguera B, Couce ME, Long J, Lamsam J, Laakso K, Jensen MD, Parisi JE, Lloyd RV. 2000. The long form of the leptin receptor (OB-Rb) is widely expressed in the human brain. Neuroendocrinology 71(3):187-195.
Butler MP, O'Connor JJ, Moynagh PN. 2004. Dissection of tumor-necrosis factor-alpha inhibition of long-term potentiation (LTP) reveals a p38 mitogen-activated protein kinase-dependent mechanism which maps to early-but not late-phase LTP. Neuroscience 124(2):319-326.
Chaldakov GN. 2007. The Adipobiology of Disease. Immunology, Endocrine & Metabolic Agents in Medicinal Chemistry (Formerly Current Medicinal Chemistry - Immunology, Endocrine and Metabolic Agents) Volume 7, Number 2, April 2007,: pp. 105-105.
Chen Y, Ramakrishnan DP, Ren B. 2013. Regulation of angiogenesis by phospholipid lysophosphatidic acid. Frontiers in bioscience 18:852-861.
Cheng G, Huang C, Deng H, Wang H. 2012. Diabetes as a risk factor for dementia and mild cognitive impairment: a meta-analysis of longitudinal studies. Internal medicine journal 42(5):484-491.

Chung IY, Benveniste EN. 1990. Tumor necrosis factor-alpha production by astrocytes. Induction by lipopolysaccharide, IFN-gamma, and IL-1 beta. Journal of immunology 144(8):2999-3007.

Clement K, Viguerie N, Poitou C, Carette C, Pelloux V, Curat CA, Sicard A, Rome S, Benis A, Zucker JD, Vidal H, Laville M, Barsh GS, Basdevant A, Stich V, Cancello R, Langin D. 2004. Weight loss regulates inflammation-related genes in white adipose tissue of obese subjects. FASEB J 18(14):1657-1669.

Cournot M, Marquie JC, Ansiau D, Martinaud C, Fonds H, Ferrieres J, Ruidavets JB. 2006. Relation between body mass index and cognitive function in healthy middle-aged men and women. Neurology 67(7):1208-1214.

Cowley MA, Smart JL, Rubinstein M, Cerdan MG, Diano S, Horvath TL, Cone RD, Low MJ. 2001. Leptin activates anorexigenic POMC neurons through a neural network in the arcuate nucleus. Nature 411(6836):480-484.

Cunningham AJ, Murray CA, O'Neill LA, Lynch MA, O'Connor JJ. 1996. Interleukin-1 beta (IL-1 beta) and tumour necrosis factor (TNF) inhibit long-term potentiation in the rat dentate gyrus in vitro. Neuroscience letters 203(1):17-20.

Debette S, Beiser A, Hoffmann U, Decarli C, O'Donnell CJ, Massaro JM, Au R, Himali JJ, Wolf PA, Fox CS, Seshadri S. 2010. Visceral fat is associated with lower brain volume in healthy middle-aged adults. Ann Neurol 68(2):136-144.

Degawa-Yamauchi M, Bovenkerk JE, Juliar BE, Watson W, Kerr K, Jones R, Zhu Q, Considine RV. 2003. Serum resistin (FIZZ3) protein is increased in obese humans. The Journal of clinical endocrinology and metabolism 88(11):5452-5455.

Devos R, Richards JG, Campfield LA, Tartaglia LA, Guisez Y, van der Heyden J, Travernier J, Plaetinck G, Burn P. 1996. OB protein binds specifically to the choroid plexus of mice and rats. Proceedings of the National Academy of Sciences of the United States of America 93(11):5668-5673.

Dubin AE, Herr DR, Chun J. 2010. Diversity of lysophosphatidic acid receptor-mediated intracellular calcium signaling in early cortical neurogenesis. The Journal of neuroscience : the official journal of the Society for Neuroscience 30(21):7300-7309.

Enciu AM, Gherghiceanu M, Popescu BO. 2013. Triggers and effectors of oxidative stress at blood-brain barrier level: relevance for brain ageing and neurodegeneration. Oxid Med Cell Longev 2013:297512.

Enriori PJ, Evans AE, Sinnayah P, Jobst EE, Tonelli-Lemos L, Billes SK, Glavas MM, Grayson BE, Perello M, Nillni EA, Grove KL, Cowley MA. 2007. Diet-induced obesity causes severe but reversible leptin resistance in arcuate melanocortin neurons. Cell metabolism 5(3):181-194.

Esposito K, Ciotola M, Schisano B, Misso L, Giannetti G, Ceriello A, Giugliano D. 2006. Oxidative stress in the metabolic syndrome. J Endocrinol Invest 29(9):791-795.

Estivill-Torrus G, Llebrez-Zayas P, Matas-Rico E, Santin L, Pedraza C, De Diego I, Del Arco I, Fernandez-Llebrez P, Chun J, De Fonseca FR. 2008. Absence of LPA1 signaling results in defective cortical development. Cerebral cortex 18(4):938-950.

Fernandez-Sanchez A, Madrigal-Santillan E, Bautista M, Esquivel-Soto J, Morales-Gonzalez A, Esquivel-Chirino C, Durante-Montiel I, Sanchez-Rivera G, Valadez-Vega C, Morales-Gonzalez JA. 2011. Inflammation, oxidative stress, and obesity. International journal of molecular sciences 12(5):3117-3132.

Fleisch AF, Agarwal N, Roberts MD, Han JC, Theim KR, Vexler A, Troendle J, Yanovski SZ, Yanovski JA. 2007. Influence of serum leptin on weight and body fat growth in children at high risk for adult obesity. The Journal of clinical endocrinology and metabolism 92(3):948-954.

Freeman LR, Keller JN. 2012. Oxidative stress and cerebral endothelial cells: regulation of the blood-brain-barrier and antioxidant based interventions. Biochimica et biophysica acta 1822(5):822-829.
Friedman JM, Halaas JL. 1998. Leptin and the regulation of body weight in mammals. Nature 395(6704):763-770.
Frisca F, Sabbadini RA, Goldshmit Y, Pebay A. 2012. Biological effects of lysophosphatidic acid in the nervous system. International review of cell and molecular biology 296:273-322.
Frugier T, Crombie D, Conquest A, Tjhong F, Taylor C, Kulkarni T, McLean C, Pebay A. 2011. Modulation of LPA receptor expression in the human brain following neurotrauma. Cellular and molecular neurobiology 31(4):569-577.
Fry M, Smith PM, Hoyda TD, Duncan M, Ahima RS, Sharkey KA, Ferguson AV. 2006. Area postrema neurons are modulated by the adipocyte hormone adiponectin. The Journal of neuroscience : the official journal of the Society for Neuroscience 26(38):9695-9702.
Fukushima N, Shano S, Moriyama R, Chun J. 2007. Lysophosphatidic acid stimulates neuronal differentiation of cortical neuroblasts through the LPA1-G(i/o) pathway. Neurochemistry international 50(2):302-307.
Furukawa S, Fujita T, Shimabukuro M, Iwaki M, Yamada Y, Nakajima Y, Nakayama O, Makishima M, Matsuda M, Shimomura I. 2004. Increased oxidative stress in obesity and its impact on metabolic syndrome. The Journal of clinical investigation 114(12):1752- 1761.
Gianfrancesco MA, Acuna B, Shen L, Briggs FB, Quach H, Bellesis KH, Bernstein A, Hedstrom AK, Kockum I, Alfredsson L, Olsson T, Schaefer C, Barcellos LF. 2014. Obesity during childhood and adolescence increases susceptibility to multiple sclerosis after accounting for established genetic and environmental risk factors. Obesity research & clinical practice 8(5):e435-447.
Gorska E, Popko K, Stelmaszczyk-Emmel A, Ciepiela O, Kucharska A, Wasik M. 2010. Leptin receptors. European journal of medical research 15 Suppl 2:50-54.
Greenberg AS, Obin MS. 2006. Obesity and the role of adipose tissue in inflammation and metabolism. Am J Clin Nutr 83(2):461S-465S.
Gustafson D. 2006. Adiposity indices and dementia. Lancet Neurol 5(8):713-720.
Gustafson D, Lissner L, Bengtsson C, Bjorkelund C, Skoog I. 2004. A 24-year follow-up of body mass index and cerebral atrophy. Neurology 63(10):1876-1881.
Gustafson D, Rothenberg E, Blennow K, Steen B, Skoog I. 2003. An 18-year follow-up of overweight and risk of Alzheimer disease. Arch Intern Med 163(13):1524-1528.
Gustafson DR, Backman K, Waern M, Ostling S, Guo X, Zandi P, Mielke MM, Bengtsson C, Skoog I. 2009. Adiposity indicators and dementia over 32 years in Sweden. Neurology 73(19):1559-1566.
Gustafson DR, Karlsson C, Skoog I, Rosengren L, Lissner L, Blennow K. 2007. Mid-life adiposity factors relate to blood-brain barrier integrity in late life. J Intern Med 262(6):643-650.
Haan MN. 2006. Therapy Insight: type 2 diabetes mellitus and the risk of late-onset Alzheimer's disease. Nature clinical practice Neurology 2(3):159-166.
Heldmann U, Thored P, Claasen JH, Arvidsson A, Kokaia Z, Lindvall O. 2005. TNF-alpha antibody infusion impairs survival of stroke-generated neuroblasts in adult rat brain. Experimental neurology 196(1):204-208.
Herder C, Baumert J, Thorand B, Koenig W, de Jager W, Meisinger C, Illig T, Martin S, Kolb H. 2006. Chemokines as risk factors for type 2 diabetes: results from the MONICA/KORA Augsburg study, 1984-2002. Diabetologia 49(5):921-929.

Hermann GE, Rogers RC, Bresnahan JC, Beattie MS. 2001. Tumor necrosis factor-alpha induces cFOS and strongly potentiates glutamate-mediated cell death in the rat spinal cord. Neurobiology of disease 8(4):590-599.

Higdon JV, Frei B. 2003. Obesity and oxidative stress: a direct link to CVD? Arterioscler Thromb Vasc Biol 23(3):365-367.

Hillenbrand A, Knippschild U, Weiss M, Schrezenmeier H, Henne-Bruns D, Huber-Lang M, Wolf AM. 2010. Sepsis induced changes of adipokines and cytokines - septic patients compared to morbidly obese patients. BMC surgery 10:26.

Hillenbrand A, Weiss M, Knippschild U, Wolf AM, Huber-Lang M. 2012. Sepsis-Induced Adipokine Change with regard to Insulin Resistance. International journal of inflammation 2012:972368.

Ho AJ, Stein JL, Hua X, Lee S, Hibar DP, Leow AD, Dinov ID, Toga AW, Saykin AJ, Shen L, Foroud T, Pankratz N, Huentelman MJ, Craig DW, Gerber JD, Allen AN, Corneveaux JJ, Stephan DA, DeCarli CS, DeChairo BM, Potkin SG, Jack CR, Jr., Weiner MW, Raji CA, Lopez OL, Becker JT, Carmichael OT, Thompson PM. 2010. A commonly carried allele of the obesity-related FTO gene is associated with reduced brain volume in the healthy elderly. Proceedings of the National Academy of Sciences of the United States of America 107(18):8404-8409.

Ho N, Sommers MS, Lucki I. 2013. Effects of diabetes on hippocampal neurogenesis: links to cognition and depression. Neuroscience and biobehavioral reviews 37(8):1346-1362.

Holtsberg FW, Steiner MR, Keller JN, Mark RJ, Mattson MP, Steiner SM. 1998. Lysophosphatidic acid induces necrosis and apoptosis in hippocampal neurons. Journal of neurochemistry 70(1):66-76.

Hotamisligil GS, Shargill NS, Spiegelman BM. 1993. Adipose expression of tumor necrosis factor-alpha: direct role in obesity-linked insulin resistance. Science 259(5091):87-91.

Hoyda TD, Fry M, Ahima RS, Ferguson AV. 2007. Adiponectin selectively inhibits oxytocin neurons of the paraventricular nucleus of the hypothalamus. The Journal of physiology 585(Pt 3):805-816.

Iosif RE, EkDahl CT, Ahlenius H, Pronk CJ, Bonde S, Kokaia Z, Jacobsen SE, Lindvall O. 2006. Tumor necrosis factor receptor 1 is a negative regulator of progenitor proliferation in adult hippocampal neurogenesis. The Journal of neuroscience : the official journal of the Society for Neuroscience 26(38):9703-9712.

Kanoski SE, Zhang Y, Zheng W, Davidson TL. 2010. The effects of a high-energy diet on hippocampal function and blood-brain barrier integrity in the rat. Journal of Alzheimer's disease : JAD 21(1):207-219.

Keller JN, Steiner MR, Holtsberg FW, Mattson MP, Steiner SM. 1997. Lysophosphatidic acid-induced proliferation-related signals in astrocytes. Journal of neurochemistry 69(3):1073- 1084.

Khan NI, Naz L, Yasmeen G. 2006. Obesity: an independent risk factor for systemic oxidative stress. Pak J Pharm Sci 19(1):62-65.

Kiliaan AJ, Arnoldussen IA, Gustafson DR. 2014. Adipokines: a link between obesity and dementia? Lancet Neurol 13(9):913-923.

Kingsbury MA, Rehen SK, Contos JJ, Higgins CM, Chun J. 2003. Non-proliferative effects of lysophosphatidic acid enhance cortical growth and folding. Nature neuroscience 6(12):1292-1299.

Kosari S, Camera DM, Hawley JA, Stebbing M, Badoer E. 2013. ERK1/2 in the brain mediates the effects of central resistin on reducing thermogenesis in brown adipose tissue. International journal of physiology, pathophysiology and pharmacology 5(3):184-189.

Kosari S, Rathner JA, Badoer E. 2012. Central resistin enhances renal sympathetic nerve activity via phosphatidylinositol 3-kinase but reduces the activity to brown adipose

tissue via extracellular signal-regulated kinase 1/2. Journal of neuroendocrinology 24(11):1432- 1439.

Kotarsky K, Boketoft A, Bristulf J, Nilsson NE, Norberg A, Hansson S, Owman C, Sillard R, Leeb-Lundberg LM, Olde B. 2006. Lysophosphatidic acid binds to and activates GPR92,a G protein-coupled receptor highly expressed in gastrointestinal lymphocytes. The Journal of pharmacology and experimental therapeutics 318(2):619-628.

Kriegler M, Perez C, DeFay K, Albert I, Lu SD. 1988. A novel form of TNF/cachectin is a cell surface cytotoxic transmembrane protein: ramifications for the complex physiology of TNF. Cell 53(1):45-53.

Ku IA, Farzaneh-Far R, Vittinghoff E, Zhang MH, Na B, Whooley MA. 2011. Association of low leptin with cardiovascular events and mortality in patients with stable coronary artery disease: the Heart and Soul Study. Atherosclerosis 217(2):503-508.

Lan X, Chen Q, Wang Y, Jia B, Sun L, Zheng J, Peng H. 2012. TNF-alpha affects human cortical neural progenitor cell differentiation through the autocrine secretion of leukemia inhibitory factor. PloS one 7(12):e50783.

Laurikka A, Vuolteenaho K, Toikkanen V, Rinne T, Leppanen T, Tarkka M, Laurikka J, Moilanen E. 2014. Adipocytokine resistin correlates with oxidative stress and myocardial injury in patients undergoing cardiac surgery. European journal of cardio-thoracic surgery : official journal of the European Association for Cardio-thoracic Surgery 46(4):729-736.

Leal Vde O, Mafra D. 2013. Adipokines in obesity. Clinica chimica acta; international journal of clinical chemistry 419:87-94.

Lee EB. 2011. Obesity, leptin, and Alzheimer's disease. Annals of the New York Academy of Sciences 1243:15-29.

Lee JH, Bullen JW, Jr., Stoyneva VL, Mantzoros CS. 2005. Circulating resistin in lean, obese, and insulin-resistant mouse models: lack of association with insulinemia and glycemia. American journal of physiology Endocrinology and metabolism 288(3):E625-632.

Lehr S, Hartwig S, Sell H. 2012. Adipokines: a treasure trove for the discovery of biomarkers for metabolic disorders. Proteomics Clinical applications 6(1-2):91-101.

Letra L, Santana I, Seica R. 2014. Obesity as a risk factor for Alzheimer's disease: the role of adipocytokines. Metabolic brain disease 29(3):563-568.

Li W, Prakash R, Chawla D, Du W, Didion SP, Filosa JA, Zhang Q, Brann DW, Lima VV, Tostes RC, Ergul A. 2013. Early effects of high-fat diet on neurovascular function and focal ischemic brain injury. American journal of physiology Regulatory, integrative and comparative physiology 304(11):R1001-1008.

Lieberman AP, Pitha PM, Shin HS, Shin ML. 1989. Production of tumor necrosis factor and other cytokines by astrocytes stimulated with lipopolysaccharide or a neurotropic virus. Proceedings of the National Academy of Sciences of the United States of America 86(16):6348-6352.

Lochhead JJ, McCaffrey G, Quigley CE, Finch J, DeMarco KM, Nametz N, Davis TP. 2010. Oxidative stress increases blood-brain barrier permeability and induces alterations in occludin during hypoxia-reoxygenation. Journal of cerebral blood flow and metabolism official journal of the International Society of Cerebral Blood Flow and Metabolism 30(9):1625-1636.

Lokken KL, Boeka AG, Austin HM, Gunstad J, Harmon CM. 2009. Evidence of executive dysfunction in extremely obese adolescents: a pilot study. Surgery for obesity and related diseases : official journal of the American Society for Bariatric Surgery 5(5):547-552.

Lynch CM, Kinzenbaw DA, Chen X, Zhan S, Mezzetti E, Filosa J, Ergul A, Faulkner JL, Faraci FM, Didion SP. 2013. Nox2-derived superoxide contributes to cerebral vascular dysfunction in diet-induced obesity. Stroke 44(11):3195-3201.

MacKnight C, Rockwood K, Awalt E, McDowell I. 2002. Diabetes mellitus and the risk of dementia, Alzheimer's disease and vascular cognitive impairment in the Canadian Study of Health and Aging. Dementia and geriatric cognitive disorders 14(2):77-83.

Maddineni S, Metzger S, Ocon O, Hendricks G, 3rd, Ramachandran R. 2005. Adiponectin gene is expressed in multiple tissues in the chicken: food deprivation influences adiponectin messenger ribonucleic acid expression. Endocrinology 146(10):4250-4256.

Maeda N, Shimomura I, Kishida K, Nishizawa H, Matsuda M, Nagaretani H, Furuyama N, Kondo H, Takahashi M, Arita Y, Komuro R, Ouchi N, Kihara S, Tochino Y, Okutomi K, Horie M, Takeda S, Aoyama T, Funahashi T, Matsuzawa Y. 2002. Diet-induced insulin resistance in mice lacking adiponectin/ACRP30. Nature medicine 8(7):731-737.

Marchetti L, Klein M, Schlett K, Pfizenmaier K, Eisel UL. 2004. Tumor necrosis factor (TNF)-mediated neuroprotection against glutamate-induced excitotoxicity is enhanced by N-methyl-D-aspartate receptor activation. Essential role of a TNF receptor 2-mediated phosphatidylinositol 3-kinase-dependent NF-kappa B pathway. The Journal of biological chemistry 279(31):32869-32881.

Matas-Rico E, Garcia-Diaz B, Llebrez-Zayas P, Lopez-Barroso D, Santin L, Pedraza C, Smith-Fernandez A, Fernandez-Llebrez P, Tellez T, Redondo M, Chun J, De Fonseca FR, Estivill-Torrus G. 2008. Deletion of lysophosphatidic acid receptor LPA1 reduces neurogenesis in the mouse dentate gyrus. Molecular and cellular neurosciences 39(3):342-355.

Matsuda M, Shimomura I. 2014. Roles of adiponectin and oxidative stress in obesity-associated metabolic and cardiovascular diseases. Reviews in endocrine & metabolic disorders 15(1):1-10.

Matsunaga E, Nambu S, Oka M, Iriki A. 2013. Differential cadherin expression in the developing postnatal telencephalon of a New World monkey. J Comp Neurol 521(17):4027-4060.

Matsuzawa Y. 2005. Adiponectin: Identification, physiology and clinical relevance in metabolic and vascular disease. Atherosclerosis Supplements 6(2):7-14.

Mauro C, De Rosa V, Marelli-Berg F, Solito E. 2014. Metabolic syndrome and the immunological affair with the blood-brain barrier. Frontiers in immunology 5:677.

Maury E, Brichard SM. 2010. Adipokine dysregulation, adipose tissue inflammation and metabolic syndrome. Molecular and cellular endocrinology 314(1):1-16.

McCrimmon RJ, Ryan CM, Frier BM. 2012. Diabetes and cognitive dysfunction. Lancet 379(9833):2291-2299.

McNeilly AD, Williamson R, Sutherland C, Balfour DJ, Stewart CA. 2011. High fat feeding promotes simultaneous decline in insulin sensitivity and cognitive performance in a delayed matching and non-matching to position task. Behavioural brain research 217(1):134-141.

Mercer JG, Hoggard N, Williams LM, Lawrence CB, Hannah LT, Trayhurn P. 1996. Localization of leptin receptor mRNA and the long form splice variant (Ob-Rb) in mouse hypothalamus and adjacent brain regions by in situ hybridization. FEBS letters 387(2-3):113-116.

Miralbell J, Lopez-Cancio E, Lopez-Oloriz J, Arenillas JF, Barrios M, Soriano-Raya JJ, Galan A, Caceres C, Alzamora M, Pera G, Toran P, Davalos A, Mataro M. 2013. Cognitive patterns in relation to biomarkers of cerebrovascular disease and vascular risk factors. Cerebrovascular diseases 36(2):98-105.

Moller T, Contos JJ, Musante DB, Chun J, Ransom BR. 2001. Expression and function of lysophosphatidic acid receptors in cultured rodent microglial cells. The Journal of biological chemistry 276(28):25946-25952.
Montgomery SL, Bowers WJ. 2012. Tumor necrosis factor-alpha and the roles it plays in homeostatic and degenerative processes within the central nervous system. Journal of neuroimmune pharmacology : the official journal of the Society on NeuroImmune Pharmacology 7(1):42-59.
Morash B, Li A, Murphy PR, Wilkinson M, Ur E. 1999. Leptin gene expression in the brain and pituitary gland. Endocrinology 140(12):5995-5998.
Morash BA, Willkinson D, Ur E, Wilkinson M. 2002. Resistin expression and regulation in mouse pituitary. FEBS letters 526(1-3):26-30.
Morganti-Kossman MC, Lenzlinger PM, Hans V, Stahel P, Csuka E, Ammann E, Stocker R, Trentz O, Kossmann T. 1997. Production of cytokines following brain injury: beneficial and deleterious for the damaged tissue. Molecular psychiatry 2(2):133-136.
Morris MJ, Beilharz JE, Maniam J, Reichelt AC, Westbrook RF. 2014. Why is obesity such a problem in the 21st century? The intersection of palatable food, cues and reward pathways, stress, and cognition. Neuroscience and biobehavioral reviews.
Moschen AR, Kaser A, Enrich B, Mosheimer B, Theurl M, Niederegger H, Tilg H. 2007. Visfatin, an adipocytokine with pro-inflammatory and immunomodulating properties. Journal of immunology 178(3):1748-1758.
Murray AJ, Knight NS, Cochlin LE, McAleese S, Deacon RM, Rawlins JN, Clarke K. 2009. Deterioration of physical performance and cognitive function in rats with short-term high-fat feeding. FASEB J 23(12):4353-4360.
Nguyen JC, Killcross AS, Jenkins TA. 2014a. Obesity and cognitive decline: role of inflammation and vascular changes. Frontiers in neuroscience 8:375.
Nguyen S, Major K, Demonet JF, Smith C, Rubli E, Humbert M, Bula C. 2014b. [Diabetes and dementia: the dangerous liaisons?]. Revue medicale suisse 10(449):2090-2092, 2094-2096.
Nishimura M, Izumiya Y, Higuchi A, Shibata R, Qiu J, Kudo C, Shin HK, Moskowitz MA, Ouchi N. 2008. Adiponectin prevents cerebral ischemic injury through endothelial nitric oxide synthase dependent mechanisms. Circulation 117(2):216-223.
Noguchi K, Herr D, Mutoh T, Chun J. 2009. Lysophosphatidic acid (LPA) and its receptors. Current opinion in pharmacology 9(1):15-23.
Noguchi K, Ishii S, Shimizu T. 2003. Identification of p2y9/GPR23 as a novel G protein-coupled receptor for lysophosphatidic acid, structurally distant from the Edg family. The Journal of biological chemistry 278(28):25600-25606.
Okamoto Y, Kihara S, Ouchi N, Nishida M, Arita Y, Kumada M, Ohashi K, Sakai N, Shimomura I, Kobayashi H, Terasaka N, Inaba T, Funahashi T, Matsuzawa Y. 2002. Adiponectin reduces atherosclerosis in apolipoprotein E-deficient mice. Circulation 106(22):2767-2770.
Olefsky JM, Glass CK. 2010. Macrophages, inflammation, and insulin resistance. Annual review of physiology 72:219-246.
Ouchi N, Kihara S, Funahashi T, Matsuzawa Y, Walsh K. 2003. Obesity, adiponectin and vascular inflammatory disease. Current opinion in lipidology 14(6):561-566.
Ouchi N, Parker JL, Lugus JJ, Walsh K. 2011. Adipokines in inflammation and metabolic disease. Nature reviews Immunology 11(2):85-97.
Ozata M, Mergen M, Oktenli C, Aydin A, Sanisoglu SY, Bolu E, Yilmaz MI, Sayal A, Isimer A, Ozdemir IC. 2002. Increased oxidative stress and hypozincemia in male obesity. Clin Biochem 35(8):627-631.

Park HK, Ahima RS. 2013. Resistin in rodents and humans. Diabetes & metabolism journal 37(6):404-414.
Paz-Filho G, Wong ML, Licinio J. 2010a. Leptin levels and Alzheimer disease. JAMA : the journal of the American Medical Association 303(15):1478; author reply 1478-1479.
Paz-Filho G, Wong ML, Licinio J. 2010b. The procognitive effects of leptin in the brain and their clinical implications. International journal of clinical practice 64(13):1808-1812.
Pickering M, Cumiskey D, O'Connor JJ. 2005. Actions of TNF-alpha on glutamatergic synaptic transmission in the central nervous system. Experimental physiology 90(5):663-670.
Pihl E, Zilmer K, Kullisaar T, Kairane C, Magi A, Zilmer M. 2006. Atherogenic inflammatory and oxidative stress markers in relation to overweight values in male former athletes. International journal of obesity 30(1):141-146.
Psilopanagioti A, Papadaki H, Kranioti EF, Alexandrides TK, Varakis JN. 2009. Expression of adiponectin and adiponectin receptors in human pituitary gland and brain. Neuroendocrinology 89(1):38-47.
Qiu G, Wan R, Hu J, Mattson MP, Spangler E, Liu S, Yau SY, Lee TM, Gleichmann M, Ingram DK, So KF, Zou S. 2011. Adiponectin protects rat hippocampal neurons against excitotoxicity. Age 33(2):155-165.
Raji CA, Ho AJ, Parikshak NN, Becker JT, Lopez OL, Kuller LH, Hua X, Leow AD, Toga AW, Thompson PM. 2010. Brain structure and obesity. Hum Brain Mapp 31(3):353-364.
Ranscht B, Dours-Zimmermann MT. 1991. T-cadherin, a novel cadherin cell adhesion molecule in the nervous system lacks the conserved cytoplasmic region. Neuron 7(3):391-402.
Reisberg B, Burns A, Brodaty H, Eastwood R, Rossor M, Sartorius N, Winblad B. 1997. Diagnosis of Alzheimer's disease. Report of an International Psychogeriatric Association Special Meeting Work Group under the cosponsorship of Alzheimer's disease International, the European Federation of Neurological Societies, the World Health Organization, and the World Psychiatric Association. International psychogeriatrics / IPA 9 Suppl 1:11-38.
Remy F, Vayssiere N, Saint-Aubert L, Barbeau E, Pariente J. 2015. White matter disruption at the prodromal stage of Alzheimer's disease: Relationships with hippocampal atrophy and episodic memory performance. NeuroImage Clinical 7:482-492.
Repunte-Canonigo V, Berton F, Cottone P, Reifel-Miller A, Roberts AJ, Morales M, Francesconi W, Sanna PP. 2010. A potential role for adiponectin receptor 2 (AdipoR2) in the regulation of alcohol intake. Brain research 1339:11-17.
Rodriguez-Rodriguez A, Egea-Guerrero JJ, Murillo-Cabezas F, Carrillo-Vico A. 2014. Oxidative stress in traumatic brain injury. Current medicinal chemistry 21(10):1201-1211.
Roglic G, Unwin N, Bennett PH, Mathers C, Tuomilehto J, Nag S, Connolly V, King H. 2005. The burden of mortality attributable to diabetes: realistic estimates for the year 2000. Diabetes care 28(9):2130-2135.
Ruszkiewicz J, Albrecht J. 2015. Changes in the mitochondrial antioxidant systems in neurodegenerative diseases and acute brain disorders. Neurochemistry international.
Sabia S, Kivimaki M, Shipley MJ, Marmot MG, Singh-Manoux A. 2009. Body mass index over the adult life course and cognition in late midlife: the Whitehall II Cohort Study. Am J Clin Nutr 89(2):601-607.
Salbe AD, Weyer C, Lindsay RS, Ravussin E, Tataranni PA. 2002. Assessing risk factors for obesity between childhood and adolescence: I. Birth weight, childhood adiposity, parental obesity, insulin, and leptin. Pediatrics 110(2 Pt 1):299-306.

Scherer PE, Williams S, Fogliano M, Baldini G, Lodish HF. 1995. A novel serum protein similar to C1q, produced exclusively in adipocytes. The Journal of biological chemistry 270(45):26746-26749.

Selmaj K, Raine CS. 1988. Tumor necrosis factor mediates myelin damage in organotypic cultures of nervous tissue. Annals of the New York Academy of Sciences 540:568-570.

Silswal N, Singh AK, Aruna B, Mukhopadhyay S, Ghosh S, Ehtesham NZ. 2005. Human resistin stimulates the pro-inflammatory cytokines TNF-alpha and IL-12 in macrophages by NF-kappaB-dependent pathway. Biochemical and biophysical research communications 334(4):1092-1101.

Spranger J, Kroke A, Mohlig M, Bergmann MM, Ristow M, Boeing H, Pfeiffer AF. 2003. Adiponectin and protection against type 2 diabetes mellitus. Lancet 361(9353):226-228.

Spranger J, Verma S, Gohring I, Bobbert T, Seifert J, Sindler AL, Pfeiffer A, Hileman SM, Tschop M, Banks WA. 2006. Adiponectin does not cross the blood-brain barrier but modifies cytokine expression of brain endothelial cells. Diabetes 55(1):141-147.

Stefan N, Stumvoll M. 2002. Adiponectin--its role in metabolism and beyond. Hormone and metabolic research = Hormon- und Stoffwechselforschung = Hormones et metabolisme 34(9):469-474.

Svetlov SI, Ignatova TN, Wang KK, Hayes RL, English D, Kukekov VG. 2004. Lysophosphatidic acid induces clonal generation of mouse neurospheres via proliferation of Sca-1- and AC133-positive neural progenitors. Stem cells and development 13(6):685-693.

Takeda S, Sato N, Morishita R. 2014. Systemic inflammation, blood-brain barrier vulnerability and cognitive/non-cognitive symptoms in Alzheimer disease: relevance to pathogenesis and therapy. Frontiers in aging neuroscience 6:171.

Taki Y, Kinomura S, Sato K, Inoue K, Goto R, Okada K, Uchida S, Kawashima R, Fukuda H. 2008. Relationship between body mass index and gray matter volume in 1,428 healthy individuals. Obesity (Silver Spring) 16(1):119-124.

Tang P, Hung MC, Klostergaard J. 1994. TNF cytotoxicity: effects of HER-2/neu expression and inhibitors of ADP-ribosylation. Lymphokine and cytokine research 13(2):117-123.

Tartaglia LA. 1997. The leptin receptor. The Journal of biological chemistry 272(10):6093-6096.

Tartaglia LA, Dembski M, Weng X, Deng N, Culpepper J, Devos R, Richards GJ, Campfield LA, Clark FT, Deeds J, Muir C, Sanker S, Moriarty A, Moore KJ, Smutko JS, Mays GG, Wool EA, Monroe CA, Tepper RI. 1995. Identification and expression cloning of a leptin receptor, OB-R. Cell 83(7):1263-1271.

Thaler JP, Yi CX, Schur EA, Guyenet SJ, Hwang BH, Dietrich MO, Zhao X, Sarruf DA, Izgur V, Maravilla KR, Nguyen HT, Fischer JD, Matsen ME, Wisse BE, Morton GJ, Horvath TL, Baskin DG, Tschop MH, Schwartz MW. 2012. Obesity is associated with hypothalamic injury in rodents and humans. The Journal of clinical investigation 122(1):153-162.

Thundyil J, Pavlovski D, Sobey CG, Arumugam TV. 2012. Adiponectin receptor signalling in the brain. British journal of pharmacology 165(2):313-327.

Thundyil J, Tang SC, Okun E, Shah K, Karamyan VT, Li YI, Woodruff TM, Taylor SM, Jo DG, Mattson MP, Arumugam TV. 2010. Evidence that adiponectin receptor 1 activation exacerbates ischemic neuronal death. Experimental & translational stroke medicine 2(1):15.

Tilg H, Moschen AR. 2006. Adipocytokines: mediators linking adipose tissue, inflammation and immunity. Nature reviews Immunology 6(10):772-783.

Tokumura A. 2004. Metabolic pathways and physiological and pathological significances of lysolipid phosphate mediators. Journal of cellular biochemistry 92(5):869-881.
Trayhurn P, Beattie JH. 2001. Physiological role of adipose tissue: white adipose tissue as an endocrine and secretory organ. The Proceedings of the Nutrition Society 60(3):329-339.
Trayhurn P, Wood IS. 2004. Adipokines: inflammation and the pleiotropic role of white adipose tissue. The British journal of nutrition 92(3):347-355.
Ujiie M, Dickstein DL, Carlow DA, Jefferies WA. 2003. Blood-brain barrier permeability precedes senile plaque formation in an Alzheimer disease model. Microcirculation 10(6):463-470.
Vachharajani V, Granger DN. 2009. Adipose tissue: a motor for the inflammation associated with obesity. IUBMB Life 61(4):424-430.
Vendrell J, Broch M, Vilarrasa N, Molina A, Gomez JM, Gutierrez C, Simon I, Soler J, Richart C. 2004. Resistin, adiponectin, ghrelin, leptin, and pro-inflammatory cytokines: relationships in obesity. Obesity research 12(6):962-971.
Venkatesh B, Hickman I, Nisbet J, Cohen J, Prins J. 2009. Changes in serum adiponectin concentrations in critical illness: a preliminary investigation. Critical care 13(4):R105.
Versini M, Jeandel PY, Rosenthal E, Shoenfeld Y. 2014. Obesity in autoimmune diseases: not a passive bystander. Autoimmunity reviews 13(9):981-1000.
Vincent HK, Vincent KR, Bourguignon C, Braith RW. 2005. Obesity and postexercise oxidative stress in older women. Medicine and science in sports and exercise 37(2):213-219.
Vong L, Ye C, Yang Z, Choi B, Chua S, Jr., Lowell BB. 2011. Leptin action on GABAergic neurons prevents obesity and reduces inhibitory tone to POMC neurons. Neuron 71(1):142-154.
Waki H, Yamauchi T, Kamon J, Ito Y, Uchida S, Kita S, Hara K, Hada Y, Vasseur F, Froguel P, Kimura S, Nagai R, Kadowaki T. 2003. Impaired multimerization of human adiponectin mutants associated with diabetes. Molecular structure and multimer formation of adiponectin. The Journal of biological chemistry 278(41):40352-40363.
Wan Z, Mah D, Simtchouk S, Klegeris A, Little JP. 2014. Globular adiponectin induces a pro-inflammatory response in human astrocytic cells. Biochemical and biophysical research communications 446(1):37-42.
Wang H, Chu WS, Hemphill C, Elbein SC. 2002. Human resistin gene: molecular scanning and evaluation of association with insulin sensitivity and type 2 diabetes in Caucasians. The Journal of clinical endocrinology and metabolism 87(6):2520-2524.
Ward MA, Carlsson CM, Trivedi MA, Sager MA, Johnson SC. 2005. The effect of body mass index on global brain volume in middle-aged adults: a cross sectional study. BMC Neurol 5:23.
Weisberg SP, McCann D, Desai M, Rosenbaum M, Leibel RL, Ferrante AW, Jr. 2003. Obesity is associated with macrophage accumulation in adipose tissue. The Journal of clinical investigation 112(12):1796-1808.
Whitehead JP, Richards AA, Hickman IJ, Macdonald GA, Prins JB. 2006. Adiponectin--a key adipokine in the metabolic syndrome. Diabetes, obesity & metabolism 8(3):264-280.
Whitmer RA. 2007. Type 2 diabetes and risk of cognitive impairment and dementia. Current neurology and neuroscience reports 7(5):373-380.
Whitmer RA, Gustafson DR, Barrett-Connor E, Haan MN, Gunderson EP, Yaffe K. 2008. Central obesity and increased risk of dementia more than three decades later. Neurology 71(14):1057-1064.
Whitwell JL. 2010. Progression of atrophy in Alzheimer's disease and related disorders. Neurotoxicity research 18(3-4):339-346.

Wiesner G, Brown RE, Robertson GS, Imran SA, Ur E, Wilkinson M. 2006. Increased expression of the adipokine genes resistin and fasting-induced adipose factor in hypoxic/ischaemic mouse brain. Neuroreport 17(11):1195-1198.
Wilkinson M, Brown R, Imran SA, Ur E. 2007. Adipokine gene expression in brain and pituitary gland. Neuroendocrinology 86(3):191-209.
Wilkinson M, Morash B, Ur E. 2000. The brain is a source of leptin. Frontiers of hormone research 26:106-125.
Wisse LE, Reijmer YD, Ter Telgte A, Kuijf HJ, Leemans A, Luijten PR, Koek HL, Geerlings MI, Biessels GJ. 2015. Hippocampal Disconnection in Early Alzheimer's Disease: A 7 Tesla MRI Study. Journal of Alzheimer's disease : JAD.
Xu A, Wang Y, Keshaw H, Xu LY, Lam KS, Cooper GJ. 2003. The fat-derived hormone adiponectin alleviates alcoholic and nonalcoholic fatty liver diseases in mice. The Journal of clinical investigation 112(1):91-100.
Xu Y, Kim ER, Zhao R, Myers MG, Jr., Munzberg H, Tong Q. 2013. Glutamate release mediates leptin action on energy expenditure. Molecular metabolism 2(2):109-115.
Yamauchi T, Kamon J, Ito Y, Tsuchida A, Yokomizo T, Kita S, Sugiyama T, Miyagishi M, Hara K, Tsunoda M, Murakami K, Ohteki T, Uchida S, Takekawa S, Waki H, Tsuno NH, Shibata Y, Terauchi Y, Froguel P, Tobe K, Koyasu S, Taira K, Kitamura T, Shimizu T, Nagai R, Kadowaki T. 2003. Cloning of adiponectin receptors that mediate antidiabetic metabolic effects. Nature 423(6941):762-769.
Ye X, Chun J. 2010. Lysophosphatidic acid (LPA) signaling in vertebrate reproduction. Trends in endocrinology and metabolism: TEM 21(1):17-24.
Ye X, Hama K, Contos JJ, Anliker B, Inoue A, Skinner MK, Suzuki H, Amano T, Kennedy G, Arai H, Aoki J, Chun J. 2005. LPA3-mediated lysophosphatidic acid signalling in embryo implantation and spacing. Nature 435(7038):104-108.
Ye X, Skinner MK, Kennedy G, Chun J. 2008. Age-dependent loss of sperm production in mice via impaired lysophosphatidic acid signaling. Biology of reproduction 79(2):328-336.
Yi CX, Meyer CW, Jastroch M. 2013. Leptin action in the brain: How (and when) it makes fat burn. Molecular metabolism 2(2):63-64.
Yi CX, Tschop MH. 2012. Brain-gut-adipose-tissue communication pathways at a glance. Disease models & mechanisms 5(5):583-587.
Zhang P, Tian B. 2014. Metabolic syndrome: an important risk factor for Parkinson's disease. Oxid Med Cell Longev 2014:729194.
Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM. 1994. Positional cloning of the mouse obese gene and its human homologue. Nature 372(6505):425-432.
Zhang YW, Zhang JQ, Liu C, Wei P, Zhang X, Yuan QY, Yin XT, Wei LQ, Cui JG, Wang J. 2015. Memory Dysfunction in Type 2 Diabetes Mellitus Correlates with Reduced Hippocampal CA1 and Subiculum Volumes. Chinese medical journal 128(4):465-471.
Zhao Y, Natarajan V. 2013. Lysophosphatidic acid (LPA) and its receptors: role in airway inflammation and remodeling. Biochimica et biophysica acta 1831(1):86-92.
Zheng ZQ, Fang XJ, Qiao JT. 2004. Dual action of lysophosphatidic acid in cultured cortical neurons: survival and apoptogenic. Sheng li xue bao : [Acta physiologica Sinica] 56(2):163-171.
Zheng ZQ, Fang XJ, Zhang Y, Qiao JT. 2005. Neuroprotective effect of lysophosphatidic acid on AbetaP31-35-induced apoptosis in cultured cortical neurons. Sheng li xue bao : [Acta physiologica Sinica] 57(3):289-294.
Zhou H, Liu J, Ren L, Liu W, Xing Q, Men L, Song G, Du J. 2014. Relationship between [corrected] spatial memory in diabetic rats and protein kinase Cgamma, caveolin-1 in

the hippocampus and neuroprotective effect of catalpol. Chinese medical journal 127(5):916-923.

Zlokovic BV, Jovanovic S, Miao W, Samara S, Verma S, Farrell CL. 2000. Differential regulation of leptin transport by the choroid plexus and blood-brain barrier and high affinity transport systems for entry into hypothalamus and across the blood-cerebrospinal fluid barrier. Endocrinology 141(4):1434-1441.

Autotaxin and Adiponectin Expression In Neuroinflammation and It's Effects On Microglial Cells

Avinash Parimisetty, Rana Awada, Dorothee Girard, Aurelie Catan, Cynthia Planesse, Philippe Rondeau, Nicolas Diotel, Christian Lefebvre d'Hellencourt

EA 4516 – Groupe d'Etude sur l'Inflammation Chronique et l'Obésité (GEICO), Plateforme CYROI, Université de La Réunion- 15, Avenue René Cassin 97490 Saint Denis, France

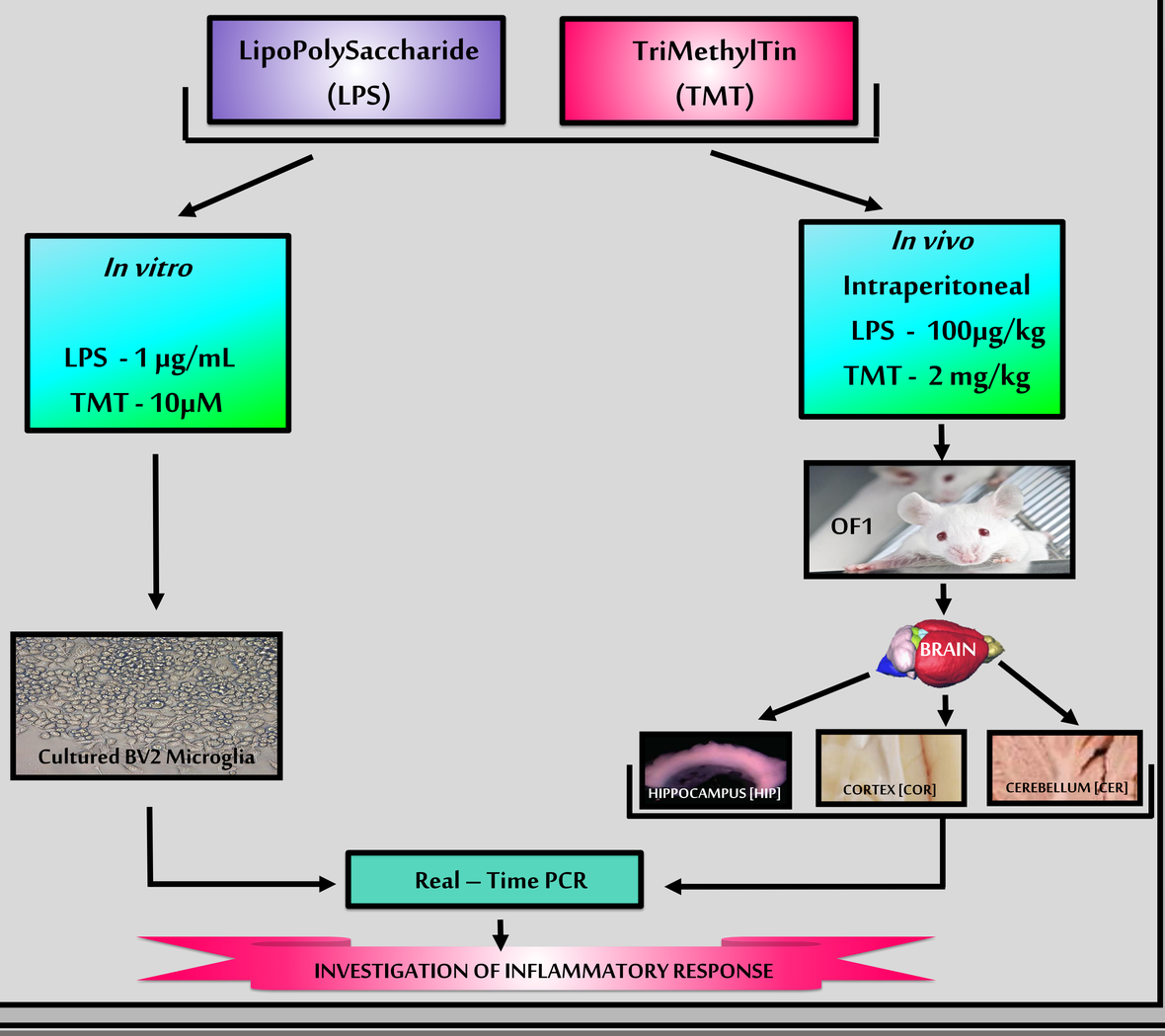
INTRODUCTION

Neuroinflammation is an intricate and tightly regulated biological process that eventuates inside the central nervous system (CNS) in response to host injury [1]. Many chronic neurodegenerative diseases such as amyotrophic lateral sclerosis, alzheimer's and parkinson's diseases have been associated with inflammation in the CNS. Glial cells are the most abundant cells of the brain, in which microglial cells were found to be the key players involved in neurodegeneration.The balance between pro and anti-inflammatory cytokines are tightly controlled as dysregulation of this equilibrium may lead to progressive neurodegenerative disorders [2]. Autotaxin (ATX) and adiponectin (ADIPO) have anti-inflammatory properties, but the precise mechanisms mediating this response in the CNS remains to be determined.

RESEARCH TASK

To characterize the expression and effects of Autotaxin and Adiponectin in mouse CNS under inflammatory settings.

EXPERIMENTAL DESIGN



KEY FINDINGS

IN VIVO

LPS Response

CYTOKINE RESPONSE IN THE BRAIN	EXPRESSION PEAK	LOCALITY OF THE BRAIN
TNF-α	2H	HIP - COR - CER
iNOS	4H	HIP - COR - CER
Adiponectin	6H - 120H - 24H	HIP - COR - CER
Autotaxin	No Significant changes	HIP - COR - CER

TMT Response

CYTOKINE RESPONSE IN THE BRAIN	EXPRESSION PEAK	LOCALITY OF THE BRAIN
TNF-α	24H	HIP
iNOS	4H	HIP
Adiponectin	192H	HIP
Autotaxin	120H	HIP

IN VITRO

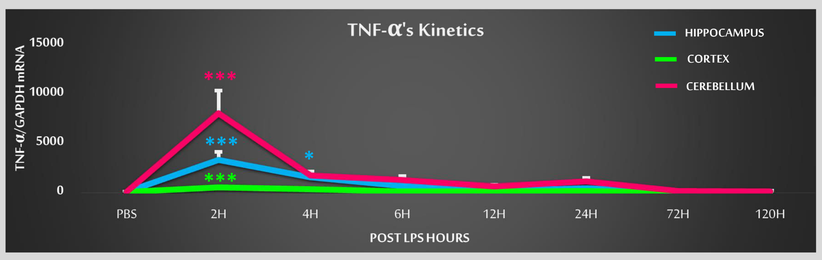
- ✓ Autotaxin expression was significantly enhanced in LPS or TMT treated BV2.
- ✓ Autotaxin down-regulated the pro-inflammatory cytokine TNFα.
- ✓ Autotaxin up-regulated the anti-inflammatory cytokine IL-10.

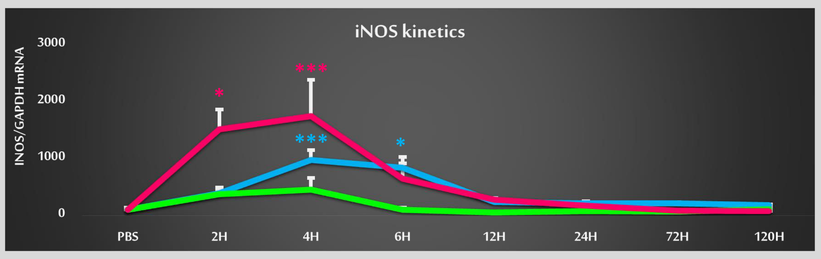
Experiments with Adiponectin overexpressing BV2 are in progress.....

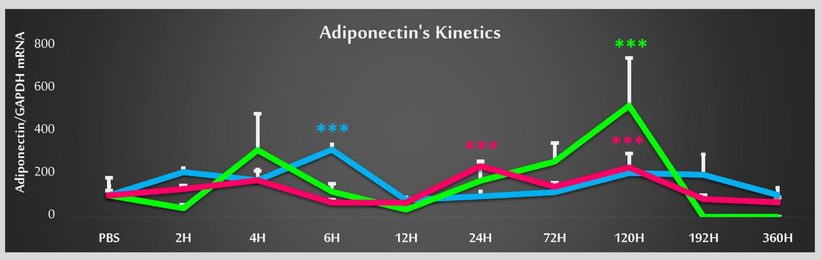
RESULTS

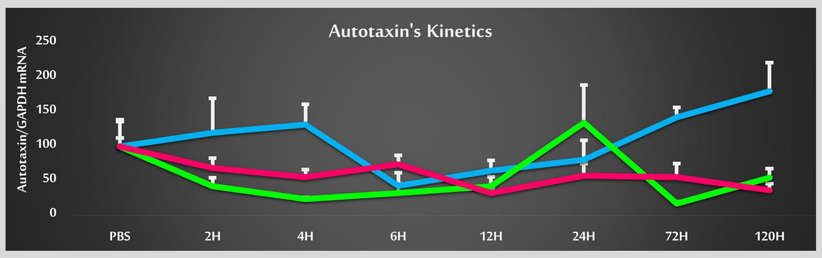
DYNAMIC EXPRESSION OF INFLAMMATORY MARKERS - A TIME FRAME *IN VIVO* KINETIC STUDY

Neuroinflammatory LPS Model

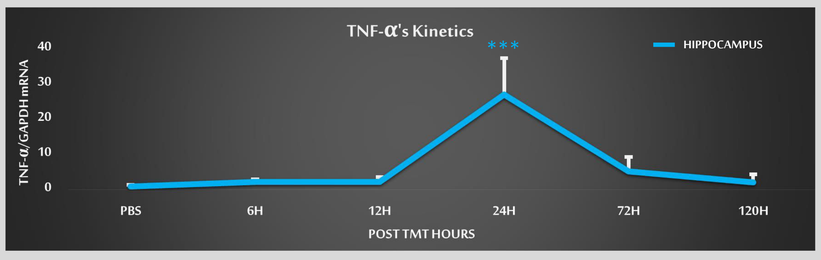


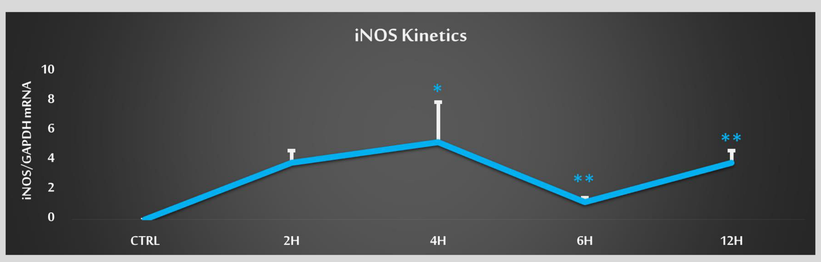


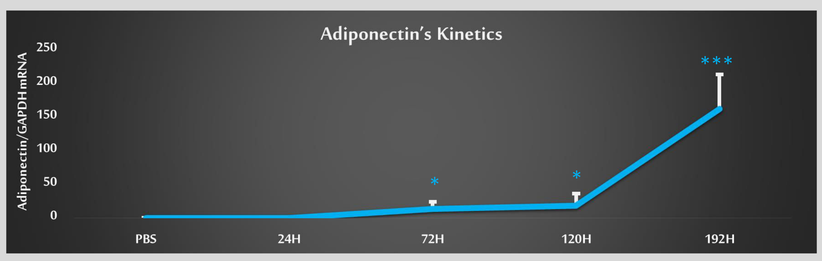


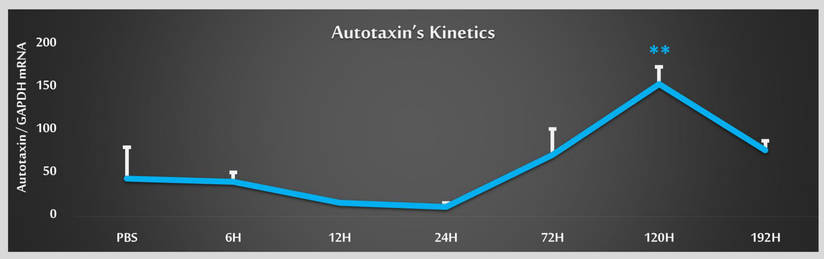


Neurodegenerative TMT Model

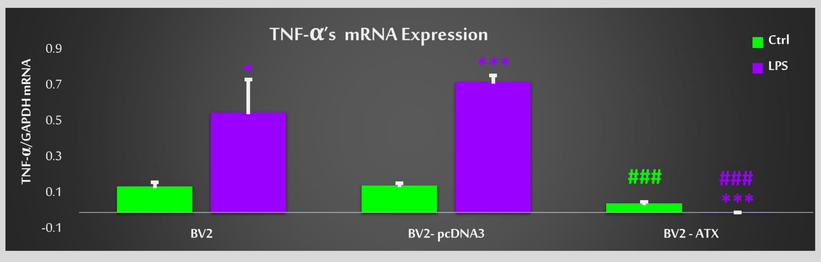


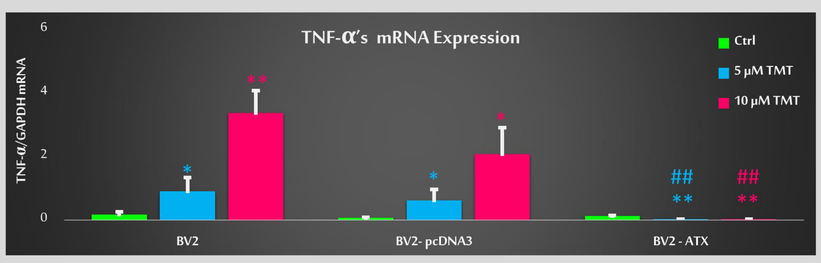


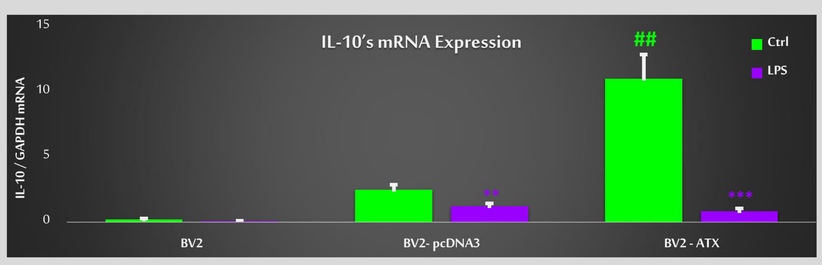


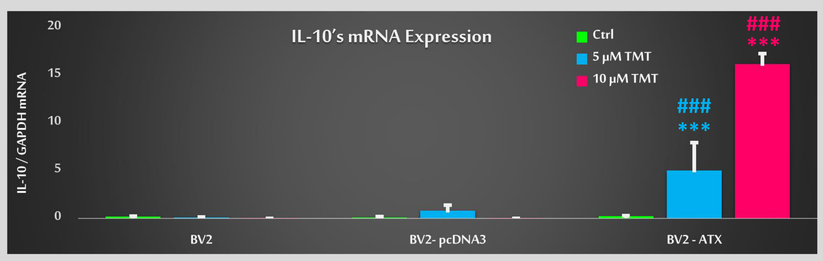


IN VITRO Study









STATISTICAL RELEVANCE

Graph Pad PRISM 5.01

In vivo: One way ANOVA followed by Tukey Test — N Size = 3-8 — Probability value: * p<0.05; ** p<0.01; *** p<0.001

In vitro: *One way and* Two way ANOVA — N Size = 3 — Probability value: * #p<0.05; ** ##p<0.01; *** ###p<0.001

[* Signifies - Difference with control]; [# Signifies - Difference with the corresponding control]

CONCLUSION

- Peripheral infection could induce a transient neuroinflammatory response in three distinct regions of the brain (HIP-COR-CER) that involved inflammatory cytokines and oxidative stress. [*In vivo*]
- Autotaxin could have anti-inflammatory effects on microglia stimulated or not by LPS and TMT [*In vitro*]
- Depending on the factor produced, pathways for these anti-inflammatory effects may be different.

Further investigations are under progress which involves the treatment of mice with these factors. This should give us better insight into the molecular mechanisms in regulating the brain inflammation.

CITATIONS

1. Gendelman HE. 2002. Neural immunity: Friend or foe? J Neurovirol 8(6):474-479.
2. Drachman, D. A. (2005). "Do we have brain to spare?" Neurology 64(12): 2004-2005.

ACKNOWLEDGEMENT Region La Reunion, Universite de La Reunion and [CPER/FEDER] Europe.

Corresponding Author MaverikAvinash@gmail.com

Abstract

Globally obesity is one of the greatest public health challenges of 21st century, and is considered a major health risk factor. Obesity is responsible for the onset of various kinds of disorders including diabetes, cardiovascular diseases and cancer. Adipose tissue (AT) is a highly active endocrine organ which has intense secretory activity producing an assortment of over 600 factors that have versatile biological activities. Some of these factors are named adipocytokines and have gain an intensive focus on current metabolic and disease recent research. Accumulating data on adipocytokine research strongly suggest that adipose tissue is the key player in promoting chronic inflammation. Many chronic neurodegenerative diseases such as Amyotrophic lateral sclerosis, Alzheimer's and Parkinson's diseases have been associated with inflammation in the Central Nervous System (CNS) in which microglia and astrocytes (glial cells) play a decisive role. Autotaxin (ATX) and Adiponectin (ADIPO) are mediators secreted by the AT. The role of these mediators in metabolic activities have been well studied but the potential role of these adipocyte secreted factors and its precise mechanisms in CNS vulnerability remains to be determined.

Here we used, *in vivo,* two distinct inflammatory stimuli, lipopolysaccharide (LPS) and trimethyltin (TMT), to characterize the expression of inflammatory mediators in mouse CNS. Acute intraperitoneal (ip) injection of LPS (100µg/Kg bwt) mimics gram negative bacterial infection, while acute ip injection of organometal TMT (2mg/kg bwt), induces hippocampal neurodegeneration. Microglia and astrocytes are the major source of inflammatory factors in the brain. To investigate, *in vitro,* the role of ATX and ADIPO in inflammatory and oxidative stress condition, we generated stable over-expressing transfectant in murine microglia BV2 cells for ATX and murine astrocyte CLTT cells for ADIPO. BV2 and CLTT stably transfected overexpressing clones were treated with LPS (1 µg/mL) and H_2O_2 (100µM).

Our *in vivo* results demonstrated that ATX and ADIPO were expressed in the brain and LPS induced a transient neuroinflammatory response in three distinct regions of the brain hippocampus (HIP), cortex (COR) and cerebellum (CER). Besides this it was also found that with this mild dosage of 100 µg LPS/Kg bwt of mice, microglia and astrocytes were not activated in the brain **(Project-1)**. Our *in vitro* results authenticate the anti-inflammatory effects of ATX in microglial cells demonstrated by the downregulation of microglial activation markers (CD11b, CD14, CD80 and CD86) and pro-inflammatory cytokine expression and secretion (TNF-α and IL-6) **(Project-2).** Likewise, ADIPO put forth its anti-oxidant role in astrocyte cells mediated via significant mitigation of ROS, and as well by the significant down and upregulation of pro-oxidative inducible nitric oxide synthase (iNOS) and cyclooxygenase-2(COX-2) and anti-oxidative enzymes mRNA expression levels superoxide dismutase (SOD) and catalase (CAT) respectively **(Project-3)**.

Overall these results suggest that peripheral inflammation induced by infection will not induce neurodegeneration (unless a massive infection) but could prime the glial cells and make them more responsive to the next stimulation. ATX and ADIPO may play a role in the regulation of neuroinflammation by regulating glial activation in stressed situations. Further investigations will be needed to better understand the molecular mechanisms regulating brain inflammation and lead to new therapeutic strategies to combat neurodegenerative diseases.

Key words: Adipose Tissue, Autotaxin, Adiponectin, Neuroinflammation, Neurodegeneration.

Résumé

L'obésité est l'un des plus grands défis de santé publique du 21ème siècle et est considérée comme un facteur de risque majeur pour la santé. L'obésité est responsable de l'apparition de divers troubles, notamment du diabète, des maladies cardiovasculaires et de certains cancers. Le tissu adipeux (TA) est un organe endocrine très actif qui a une activité sécrétoire intense produisant un assortiment de plus de 600 facteurs qui ont des activités biologiques variées. Certains de ces facteurs sont appelés adipocytokines et font l'objet d'un intérêt particulier dans les recherches récentes sur le métabolisme et les pathologies associées. De nombreuses données sur les adipocytokines suggèrent fortement que le tissu adipeux est un élément clé dans le développement d'une inflammation chronique. De nombreuses maladies neurodégénératives chroniques telles que la sclérose latérale amyotrophique, la maladie d'Alzheimer et la maladie de Parkinson ont été associées à une inflammation du système nerveux central (SNC), dans lequel la microglie et les astrocytes (cellules gliales) jouent un rôle déterminant. L'autotaxin (ATX) et l'adiponectine (ADIPO) sont des médiateurs sécrétées par le TA. Le rôle de ces médiateurs dans les activités métaboliques a été bien étudié, mais leur rôle potentiel ainsi que les mécanismes précis dans la vulnérabilité du CNS restent à déterminer.

Ici, nous proposons d'utiliser, *in vivo*, deux stimuli inflammatoires distincts le lipopolysaccharide (LPS) et le triméthylétain (TMT) pour caractériser l'expression de médiateurs de l'inflammation du SNC chez la souris. Une injection intrapéritonéale (ip) aiguë de LPS (100 µg/kg de poids corporel) mime une infection bactérienne Gram négative, tandis que l'injection ip aiguë de TMT (2 mg/kg de poids corporel), induit une neurodégénérescence hippocampique. Les microglies et les astrocytes sont les principales sources de facteurs inflammatoires dans le cerveau. Afin de rechercher, *in vitro*, le rôle de l'ATX et de l'ADIPO sur ces cellules dans un état inflammatoire et de stress oxydatif, nous avons généré des tansfectants stables sur-exprimant l'ATX dans des cellules microgliales murines (BV2) et l'ADIPO dans des cellules astrocytaires murines (CLTT). Les clones BV2 et CLTT surexprimant ces facteurs ont été traitées avec du LPS (1 µg/ml) et du H_2O_2 (100µM).

Nos résultats *in vivo* ont démontré que l'ATX et l'ADIPO sont exprimés dans le cerveau et que le LPS pourrait induire une réponse neuroinflammatoire transitoire dans trois régions distinctes du cerveau l'hippocampe (HIP), le cortex (COR) et le cervelet (CER). En outre, il a été également constaté qu'à cette dose modérée de 100µg de LPS / kg de poids corporel de la souris, la microglie et les astrocytes ne sont pas activés dans le cerveau **(Projet-1)**. Nos résultats *in vitro* démontrent les effets anti-inflammatoires de l'ATX dans les cellules microgliales observables par la baisse d'expression des marqueurs d'activation microgliale (CD11b, CD14, CD80 et CD86) et d'expression et de production de cytokines pro-inflammatoires (TNF-α et IL-6) **(Project-2)**. De même, nous avons montré que l'ADIPO a un rôle anti-oxydant dans les astrocytes via l'atténuation significative de ROS, une inhibition d'enzymes pro-oxydantes (iNOS et la COX-2) et une régulation positive d'enzymes anti-oxydantes (SOD et CAT) **(Projet-3)**.

Dans l'ensemble, ces résultats suggèrent qu'une inflammation périphérique induite par une infection ne provoque pas de neurodégénérescence (à moins d'une infection importante), mais pourrait sensibiliser les cellules gliales et augmenter leur réponse à la stimulation suivante. L'ATX et l'ADIPO pourraient jouer un rôle dans la régulation de la neuroinflammation en régulant l'activation gliale dans un contexte de stress. Des travaux supplémentaires seront nécessaires afin de mieux comprendre les mécanismes moléculaires régulant l'inflammation du SNC et aboutir à de nouvelles stratégies thérapeutiques pour combattre les maladies neurodégénératives.

Mots clés: tissu adipeux, autotaxin, adiponectine, neuroinflammation, neurodégénérescence.