DR ROBERTO COSIMO MELCANGI (Orcid ID : 0000-0003-0861-8967)

PROFESSOR GEORGE E. BARRETO (Orcid ID : 0000-0002-6644-1971)

Article type : Review Article

Lipotoxicity, neuroinflammation, glial cells and estrogenic compounds

Oscar Hidalgo-Lanussa[a], Eliana Baez-Jurado[a], Valentina Echeverria[b,c], Ghulam Md Ashraf[d], Amirhossein Sahebkar[e,f,g], Luis Miguel Garcia-Segura[h], Roberto C. Melcangi[i], George E. Barreto[a,j*]

a. Departamento de Nutrición y Bioquímica, Facultad de Ciencias, Pontificia Universidad Javeriana, Bogotá D.C., Colombia.

b. Facultad de Ciencias de la Salud, Universidad San Sebastián, Concepción, Chile

c. Bay Pines VA Healthcare System, Research and Development, Bay Pines, Florida, USA

d. King Fahd Medical Research Center, King Abdulaziz University, Jeddah, Saudi Arabia

e. Neurogenic Inflammation Research Center, Mashhad University of Medical Sciences, Mashhad, Iran

f. Biotechnology Research Center, Pharmaceutical Technology Institute, Mashhad University of Medical Sciences, Mashhad, Iran

g. School of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran

h. Instituto Cajal, CSIC, Madrid and Centro de Investigación Biomédica en Red Fragilidad y Envejecimiento Saludable (CIBERFES), Instituto de Salud Carlos III, Madrid, Spain

i. Dipartimento di Scienze Farmacologiche e Biomolecolari, Università degli Studi di Milano, Milano, Italy

j. Department of Biological Sciences, University of Limerick, Limerick, Ireland

Running title: Glial cells, hormones and lipotoxicity

*Corresponding author: George E. Barreto, M.Sc., Ph.D. Email: gesbarreto@gmail.com

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/jne.12776

Abstract

The high concentrations of free fatty acids as a consequence of obesity and overweight have become risk factors for the development of different diseases including neurodegenerative ailments. Free fatty acids (FAs) are strongly related to inflammatory events, causing cellular and tissue alterations in the brain, including cell death, deficits in neurogenesis and gliogenesis and cognitive decline. It has been reported that people with a high body mass index have a higher risk of suffering from Alzheimer's disease. Hormones such as estradiol not only have beneficial effects on brain tissue but also exert some adverse effects on peripheral tissues including the ovary and breast. For this reason, some studies have evaluated the protective effect of estrogen receptor (ER) agonists with more specific tissue activities, such as the neuroactive steroid tibolone. Activation of ERs positively affects the expression of pro-survival factors and cell signaling pathways, thus promoting cell survival. This review aims to discuss the relationship between lipotoxicity and the development of neurodegenerative diseases. We also elaborate on the cellular and molecular mechanisms involved in neuroprotection induced by estrogens.

Keywords: Estrogens; obesity; neurodegenerative diseases; microglia; neurosteroids; lipotoxicity

1. Introduction

Obesity and consumption of high-fat diets significantly increase the levels of free fatty acids in plasma, as well as the susceptibility to chronic inflammatory processes (1). Overweight, as a consequence, represents a common pathophysiological basis for many pathologies including chronic degenerative diseases, and constitutes one of the most important public health problems nowadays (2). A diet rich in simple sugars and saturated fatty acids reduces the expression of brain-derived neurotrophic factor (BDNF), and low BDNF levels are associated with insulin resistance and metabolic syndrome (3). The fat ingested with food has a dual function in the body. First, the energetic function of ingested fat is associated with the regulation of gene expression that affects the metabolism of lipids, carbohydrates and proteins, as well as cell growth and differentiation (4). Second, fatty acids interact with the genome through different mechanisms that include: i) alteration of various transcription factors such as proliferator-activated receptor (PPAR), insulin and liver X receptor (LXR),

hepatocyte nuclear factor 4 (HNF4), nuclear factor-kappa B (NF-κB) and sterol regulatory element-binding proteins (SREBPs); ii) regulation of enzymes such as cyclooxygenase, protein kinase C, superoxide dismutase and catalase, and iii) changes in the the structure of cell membrane, affecting G protein-coupled or tyrosine kinase receptors (5).

Metabolic alterations modify the correct functioning of the central nervous system (CNS) by changing the neuronal and glial environment (6). Also, the progression of CNS diseases is a consequence of the occurrence of exacerbated inflammatory processes that lead to continuous and systematic deterioration of brain tissues (7). Recent studies have suggested that people with obesity are more prone to develop cognitive pathologies such as Alzheimer's disease (AD), among others (8), leading to the generation of a large number of inflammatory processes as a consequence of the excess of free fatty acids (9-11).

Decades ago, it was possible to characterize the cellular components responsible for the regulation and control of inflammation in the nervous system (12). The glial component, mainly astrocytes and microglia, are the main cells responsible for the regulation of inflammation (13). Acute activation of glia modulates neuroinflammatory response in a protective manner. On the contrary, chronic glial activation promotes neurodegeneration *via* secretion of inflammatory factors involved in neuronal loss (14). The regulatory mechanism is an extremely complex process determined by the transduction of inflammatory-type signals mediated by cytokines and chemokines (15). Despite the large body of information about the inflammatory mechanisms, it is not yet completely clear how the regulation of inflammation is carried out and how it can be altered in pathological events (16). However, previous studies support the assertion that modulation of exacerbated inflammatory processes may hold a therapeutic potential for many diseases of the nervous system. Inflammation can be regulated through the elimination and detoxification of neurotransmitters such as glutamate, and intracellular messengers by the glial cells. These cells are able to regulate glutamate levels under physiological and pathological conditions, and contribute to oxidative stress reduction, synaptic homeostatic maintenance and the regulation of inflammatory signals (17, 18). In this regard, neuroactive steroids could play a critical role in the regulation of inflammatory processes in a pathological context, mainly by affecting the immunomodulatory function of astrocytes and microglia. Estrogens can positively regulate the expression of inflammatory

mediators (cytokines and chemokines) produced by microglia and other glial cells that are involved in neuroinflammation and neurodegeneration (19).

It is clear that the brain is a steroidogenic tissue (20), and a target of endogenous and exogenous steroids. It is noteworthy that neuroactive steroids are steroids that come from peripheral glands (*i.e.* steroid hormones), which have effects on nervous tissue, while neurosteroids are those directly synthesised in the nervous system. It has been identified that treatment with estradiol regulates the expression of endoplasmic reticulum stress proteins (11, 21, 22), protects mitochondria (22-25) and regulates the expression and activation of membrane estrogen receptors, in addition to reducing the activity of proapoptotic proteases such as calpain and caspase-3 in glial cells under different metabolic challenges (26). Although palmitic acid may induce toxicity in glial cells (27, 28) and estrogenic compounds attenuate PA-induced neuroinflammation (29-32), the signaling mechanisms involved in these estrogenic actions are not fully explored and deserve further studies. This review highlights the inflammatory processes evoked by free fatty acids in glial cells. We also discuss the mechanisms of action of neuroactive steroids in the regulation of inflammation in glial cells as well as the therapeutic options that attenuate degeneration in the nervous system caused by glial reactivity.

2. Metabolism of fatty acids and cellular mechanisms during injury

Fatty acids (FAs) are an essential source of energy and ATP for the maintenance of cellular functions (33). Excess FAs, glucose and other nutrients can be efficiently stored as adipose tissue (34). Triglycerides provide more than twice the energy in the form of ATP compared to carbohydrates or proteins (35) since their catabolism generates more reducing equivalents ($FADH_2$ and $NADH_2$) and acetyl-CoA during β-oxidation in the mitochondria.

Fatty acids are fundamental for cellular functioning as they are used as energy sources through β-oxidation under strict enzymatic control at the mitochondrial and peroxisomal levels (36). It has been described that FAs can be cytotoxic when the regulation of their metabolism is not adequate, with characteristic physiopathological consequences such as the induction of damage in various organs (e.g liver) that can culminate in non-alcoholic fatty liver disease, non-alcoholic steatohepatitis, liver cirrhosis and hepatocellular carcinoma (37).

At the pancreatic level, FA-associated toxicity may lead to dysfunction of β cells. At the cardiac level, it may lead to the loss of myocardial contractility with consequent heart failure, while it may act as a precursor of neuroinflammatory processes in the brain (38).

Lipotoxicity is a phenomenon characterized by increased levels of FAs. Also, increased amounts of FAs can induce the activation of various metabolic pathways that cause the uncoupling of cellular metabolism, with the generation of signaling cascades related to the start of programmed cell death and finally cellular energetic failure (39). In obesity, excessive consumption of foods rich in carbohydrates and excessive release of FAs by adipose tissue exceed the storage limit and the capacity of oxidation in different tissues (40). In this regard, FAs are redirected to harmful non-oxidative metabolic pathways, which are linked with the intracellular accumulation of toxic metabolites such as ROS (41).

The oxidation of FAs increases the proportion of acetyl coenzyme A/coenzyme A and $NADH/NAD^+$ in mitochondria, which results in the inactivation of pyruvate dehydrogenase (42). Inactivation of this enzyme leads to the accumulation of citrate and subsequent inactivation of the enzyme phosphofructokinase. In turn, this latter inactivation leads to the accumulation of glucose-6-phosphate, which then stimulates the synthesis of glycogen and the inhibition of hexokinase, resulting in the inhibition of glucose uptake (43).

A large amount of FAs available at the cellular level that is not used by cellular activity can negatively alter the process of β-oxidation and affect cell/energy balance. When this process is impaired, the FAs are metabolized by alternative routes and are degraded or incorporated into other molecules (44). FAs can have damaging cellular effects by increasing the activity of the serine palmitoyl transferase that catalyzes the condensation of palmitoyl coenzyme A and serine to form dihydrosphingosine. This is the first step for the *de novo* synthesis of ceramide, a molecule involved in the regulation of cellular processes such as cellular differentiation and proliferation, in addition to cellular apoptosis, through the expression of the antiapoptotic molecule Bcl2 (45). The accumulation of metabolites involved in the synthesis of ceramides, along with other catabolic products of FAs triggers a signaling network between the endoplasmic reticulum, the cell nucleus and the mitochondria. For example, FA alters important apoptotic factors of the ER such as the inositol 1 recruiting

protein (IRE1), transcription activating factor 6 (ATF6) and protein kinases like the PKR of the endoplasmic reticulum (PERK) (46). The intracellular accumulation of palmitic acid and stearic acid causes the activation of apoptosis *via* IRE1 and PERK in humans with non-alcoholic steatohepatitis (47). The mitochondria are the main cellular compartment affected by FAs, whereby the increase of mitochondrial β-oxidation, the induction of cytochrome P450 and leukocyte infiltration result in the generation of oxidative stress and nitrogenfree radicals (48). Several studies on obesity and non-alcoholic steatohepatitis in male patients have revealed an increase in the expression of metabolites derived from oxidative stress caused by the excess of FAs (49). Some of these metabolites include 4-nitrotyrosine, hydroxynonenal, substances reactive to thiobarbituric acid (lipoperoxidation markers) and 8-hydroxydeoxyguanosine (a marker of DNA damage), among other biomarkers of oxidative stress (50).

Several investigations have shown that toll-like receptors (TLR), which are responsible for transducing FA-induced signaling, recognize the pathogens and respond by activating the innate immune response (51, 52). In macrophage cultures of female C57BL/6 mice, lauric acid could activate TLR4 receptor and dimerize with TLR2 (53). TLR4 can also be activated by palmitic acid, thereby triggering the translocation to the nucleus of NF-κB and subsequent overexpression of the pro-inflammatory cytokines tumor necrosis factor-α (TNF-α) and IL-6 (54). These cytokines, in turn, contribute to the inflammatory process associated with obesity (55) (Figure 1).

Previous studies have reported an association between high-fat diets and cognitive decline in humans (56). In addition, a higher probability of suffering from dementia has been associated with the development of inflammatory processes and obesity (22). Sedentary lifestyle, lack of physical activity and unhealthy eating habits have been cataloged as determining factors in the development of neurodegenerative diseases due to the inflammatory response that is generated in the brain (57). Furthermore, these factors may influence the outcome of other types of injuries that alter brain function, such as traumatic brain injury (TBI), thereby producing alterations in the cerebral energy machinery that prevent tissue repair and delay the recovery of cerebral function (58). Indeed, obesity, characterized by insulin resistance and the presence of neuroinflammatory processes,

increases the consequences of TBI (59). This occurs due to the transient alteration of glucose demands and the presence of high concentrations of free FAs that is a characteristic of obese patients (60).

Some studies have reported an increase in the levels of saturated FAs in post-mortem human brains with TBI (61, 62). In studies of male and female mice that underwent TBI, it was found that obesity increases the activation of glial cells (astrocytes and microglia), the expression of neuroinflammatory factors and the impairments of learning and memory processes (63). All these alterations suggest that obese people have a reduced ability of the injured brain to respond with neuroprotective and anti-inflammatory actions. Therefore, obesity can be a contributing factor triggering neuroinflammation (64). Also, it has been well established that estrogens play an essential role in the prevention of body weight gain (65). There is a strong relationship between the development of obesity, diabetes and the metabolic syndrome with a dramatic drop in the circulating levels of 17 β-estradiol (E2) in post-menopausal women (66). Treatment with E2 significantly reduces the risk of developing these pathologies (67) because the action of E2 on estrogen receptors (ERs) triggers the activation of intracellular signals that counteract the inflammatory process (68). However, these therapeutic benefits provided by E2 can be accompanied by negative side effects such as reproductive endocrine toxicity (69) and the development of breast cancer (70). Due to the above-mentioned side effects, administration of estrogen in post-menopausal women is not free from controversy and alternative compounds, such as tibolone, are being used in clinical practice (see section 5).

3. Lipotoxicity, glial cells, neurodegeneration and neuroinflammation

The release and presence of a large amount of saturated FAs in the bloodstream can affect brain function. Development of inflammatory processes in the nervous system contributes is a decisive risk factor for neurodegenerative diseases. These changes in the cerebral environment contribute to the activation of microglia as a mechanism of defense and repair; however, the presence of exacerbated and prolonged processes can trigger a deregulation in microglial activation, which will stimulate additional release of inflammatory factors by these cells. This can culminate in the elevated levels of several pro-inflammatory cytokines and induction of a chronic neuroinflammatory environment, a situation that is associated with

many neurodegenerative disorders such as age-related macular degeneration, Alzheimer's disease (AD), multiple sclerosis, Parkinson's disease (PD), Huntington's disease and tauopathies (71).

The increase in life expectancy in some countries is directly related to the increase in the prevalence of AD (72). AD is mainly associated with aging, with this being the main risk factor in the onset of this neurodegenerative pathology (73) but a higher risk has also been found in patients with obesity. Together with alterations in oxidative stress and mitochondrial dysfunction (74), the progression of AD is directly linked to alterations in local immune responses, characterized by inflammation and activation of astrocytes and microglia (75). Indeed, neuroinflammation is involved in multiple pathological mechanisms of AD and pro-inflammatory mediators such as IL-1β, IL-6 and TNFα have been shown to be associated with the disease (76).

Chronic inflammation in AD is a general observation (77), while oxidative stress precedes neuronal damage (78). Other inflammatory mediators such as IL-1β, transforming growth factor-β (TGF-β) and inducible cyclo-oxygenase (COX-2) have been found to be upregulated in AD (76). Most studies and experimental evidence indicate that a pro-inflammatory environment promotes the development of AD, while anti-inflammatory treatment decreases its progression (79, 80).

Neuroinflammation may be neuroprotective at early stages but has chronic negative effects if the stimulus responsible for inflammation persists, causing over-activation of microglia and increased release of cytokines (81). For example, amyloid plaques are surrounded by glia that secrete pro-inflammatory molecules including TNF-α, IL-1β and MCP-1, which in turn increase neuronal sensitivity to free radicals and accelerate neurodegeneration (82). Another factor that favours neuroinflammation is inducible nitric oxide synthase (iNOS), which has been found to be increased in the AD brains (83). The effects of treatment with non-steroidal anti-inflammatory drugs (NSAIDs) (84) and estrogens on the inflammatory processes involved in different diseases have been widely reported. (85). Due to the low estrogen levels that have been found in obese patients with increased levels of free fatty acids, hormonal supplements have been used (33, 40) and this replacement therapy significantly decreased the risk of developing the disease (47) (Figure 2).

PD is another neurodegenerative disease affected by the neuroinflammatory processes activated by FAs, and is the second most common neurodegenerative disorder after AD. It affects 1-2% of the general population above 65 years of age (86). One of the markers of neurodegeneration in PD is the abnormal accumulation of α-synuclein protein in neurons, which triggers the activation of glial cells and the progression of inflammation (87). Reactive microglia have been observed in the *substantia nigra* of patients with PD, which suggests that this inflammatory process could aggravate neurodegeneration (88). Indeed, active microglia and, to a lesser extent, reactive astrocytes are associated with neuronal loss. This is due to the release of TNF-α, IL-1β and IL-6, prostaglandins (PGE2, PGD2), reactive oxygen species (ROS), and nitric oxide (NO) (89). However, analysis of neuronal loss in patients with PD demonstrates that it is a heterogeneous process since neurons containing neuromelanin are predominantly involved (90). This leads to an increase in the pro-inflammatory molecules TNF-α, IL-6 and NO that trigger neuroinflammatory processes. It is noteworthy that neuromelanin has a crucial role in microglial activation, which leads to a vicious circle of neuronal death (91).

4. Estrogens and the brain

Estrogens play a vital role in both male and female reproductive physiology, stimulating cell growth and differentiation in various tissues such as breast, uterus, vagina, ovary, testis, epididymis and prostate (92). It has been shown that estrogens are of great importance in cardiovascular physiology; for example, the risk of cardiovascular disease is lower in women than in men before menopause (93). After menopause, the levels of E2, the estrogen that predominates in the circulation before menopause, decreases to the level found in men of similar age and can sometimes be even lower (94). Estrogens are required for neuronal growth and differentiation and are known to affect cognitive and mood functions. In addition, estrogens can be useful in preventing or delaying the onset of degenerative diseases of the nervous system. Most actions of estrogens in the brain are thought to be mediated by activation of estrogen receptor alpha (ERα), estrogen receptor beta (ERβ) or GPER, a G protein-coupled ER, and this topic is discussed below.

4.1 Role of estrogenic compounds in glial cell function and inflammation

ERs are proteins belonging to the superfamily of nuclear receptors, which also include other receptors for steroid hormones, the vitamin D receptor, retinoids, thyroid hormone, and some orphan receptors. ERs were identified approximately 40 years ago when in 1962 the presence of estrogen-binding sites in different tissues of rats was described. Two isoforms are currently known, ERα and ERβ (95). The ERs present a diverse subcellular localization and can be detected in the cytoplasm (96), nucleus (97), mitochondria and endoplasmic reticulum (98). Both receptors (ERα and ERβ) have different functions according to the tissue where they exert their actions. The classical theory of action of steroid hormones states that the steroid receptor is activated by binding to the ligand (in this case E2), and acts as a transcription factor by binding to DNA and stimulating gene transcription. Because these nuclear receptors exert their actions in the nucleus, their mode of action is so called genomic (99). The transcription stimulated by the ER is regulated in a tissue-specific manner. However, in addition to its canonical response, estradiol can induce effects quickly and independently of genomic mechanisms (100). The non-genomic mode of action of estradiol (and in general for all steroid hormones) is a rapid process that does not require the synthesis of new proteins, and is mediated by receptors localized in the membrane (101). The rapid estrogenic action includes the regulation of ion fluxes, discharge of secretory vesicles and activation of protein kinases associated with the membrane (102). In 1967, the idea that estradiol could induce its cellular effects through non-genomic mechanisms was introduced, as evidenced by the increase in the production of cyclic AMP (cAMP) *in vivo* (103, 104). Subsequently, it was demonstrated that E2 increases the intracellular concentration of Ca^{2+} induced by glucose and cyclic GMP (cGMP) in beta cells of the pancreas (105).

In the last decade, evidence has emerged about the role of G protein-coupled receptor for estrogen (GPER) in the metabolic regulation of the nervous system (106). The GPER is a receptor coupled to the G protein of 7 transmembrane domains, while its expression and distribution has been demonstrated in a wide variety of cells and tissues (107). The GPER is located in the plasma membrane but many investigations have identified it in other subcellular compartments, particularly in the endoplasmic reticulum and the Golgi apparatus (108), suggesting possible role of estrogen in these organelles.

It has been shown that estradiol binds to the GPER and activates multiple signaling pathways (107). It is known that GPER exerts its effects through rapid non-genomic signaling and transcriptional activation (109, 110). The mechanisms of action of this receptor depend on the type of tissue as well as the abundance and expression of receptors. The rapid non-genomic signaling in response to E2 through GPER can activate multiple signaling pathways, including MAPK, PI3K, PKC, Ca^{2+} and adenylyl cyclase (111-113), demonstrating activation of some survival and metabolic downstream pathways. Depending on the cellular context, the GPER can mediate proliferative and survival responses (114). Although the mechanisms of action of the GPER are still unclear, it has been described that it is also a target of other molecules with estrogenic activity such as tamoxifen, which has been related to the regulation of functions in the central nervous system (115).

There is a key regulatory role of the estrogenic response in inflammatory processes (116, 117). In inflammatory *in vitro* and *in vivo* models, it has been shown that administration of estradiol and Selective Estrogen Receptor Modulators (SERMs) decreases the expression of the proinflammatory cytokines IL-1β, IL-10 and TNFα (118, 119). Raloxifene, another SERM, has also been shown to protect mitochondria in astrocytic cells exposed to glucose deprivation (120). On the other hand, administration of estradiol can modulate the mitogenic stimulation of B and T lymphocytes, and as a consequence, increase the production of immunoglobulins such as IgM. Indeed, epidemiological studies have shown that women of any age experience lower rates of infection and mortality associated with inflammatory processes compared to men (121).

The relationship between the estrogenic response and inflammation is also associated with the differential activity of the estrogen receptor subtypes (122). For example, in patients with rheumatoid arthritis, a density analysis of ERα and ERβ in synoviocytes showed a greater presence of ERβ (121). The same trend is observed in patients with systemic lupus erythematosus, where T lymphocytes have a higher concentration of $ER\beta^+$ lymphocytes than $ER\alpha^+$ lymphocytes (122). In the case of the nervous system, inflammation can be triggered by conditions such as oxidative stress, endotoxins, mechanical damage and chronic neurodegenerative diseases (123). Inflammation can be acute or chronic and the estrogenic response can influence or modulate the two types of response, which can involve

cellular components such as microglia and astrocytes (124-126). It has been shown that human astrocytes can secrete molecules associated with inflammation such as interleukins and chemotactic factors such as MCP-1, MIP-1α and inducible factor IP-10 (127). De Marinis *et al.* reported that astrocytes are actively involved in the secretion of pro-inflammatory molecules IL-6 and INF-γ (116, 128, 129). Additionally, estradiol has the ability to decrease the secretion of these inflammatory molecules in an oxidative model mediated by H_2O_2 following stimulation with LPS, whose response is associated with the NF-κB pathway (130). This response is similar in astrocytes by reducing the release of proinflammatory molecules in metabolic dysfunction models (131), such as stimulation with palmitic acid (27) (Figure 3).

Some neurosteroids such as DHEA modulate microglial activation and immune response *in vitro* (132). In *in vivo* experiments, neuroactive steroids inhibited TNF-α and INFγ in astrocytes and microglia (133). In contrast, progesterone (PROG) did not inhibit the immune responses of microglia (85) but prevented morphological changes associated with activated microglia phenotype (134). Additionally, other studies reported that PROG did not have anti-inflammatory effects on mixed cultures of astrocytes and microglia but inhibited microglial proliferation in separate cultures (135).

Both central and peripheral steroids participate in the activation and protection of the brain, in particular during the aging process and after an injury (136). The neuroprotective actions of steroids have been reported in several studies, in which it is shown that these compounds have a highly specific affinity to their nuclear receptors and mediate several protective functions, among which are the increase in the levels of anti-inflammatory factors, anti-apoptotic factors, and decrease in the expression of inflammatory mediators (137). Pregnenolone and dehydroepiandrosterone are known to promote the survival and differentiation of neuronal cells grown from embryonic rat brain (138). In a similar way, estradiol, tamoxifen and raloxifene reduced the activation of microglia in male and female rats after peripheral inflammation induced by the administration of LPS. This latter effect was proposed to be mediated by a mechanism that may involve ER in microglia, thereby improving cell survival depending on the sex (139).

The classical mechanism of action of steroid hormones in the brain is through interaction with their intracellular receptors (ERs, progesterone and androgen receptors) (140, 141). The expression of these receptors by glial cells allows them to partially mediate the action of estrogenic compounds in the brain (118). Indeed, the response induced by each receptor stimulats cell survival, differentiation and connectivity of neurons and glial cells in the brain, including the medulla (102). Also, neurosteroids play an important role in the development of the prenatal CNS, while regulating behavior and neuroendocrine signaling in the adult brain (142). Although many of the signaling pathways activated by estrogen are known, the anti-inflammatory molecular mechanisms are not completely clear (143).

Many of the neurodegenerative diseases are characterized by the development of brain inflammation as a consequence of injuries or metabolic damage (59). As mentioned above, inflammation in the nervous system can be triggered by conditions such as oxidative stress, endotoxins, cytokine secretion, mechanical injury and chronic neurodegenerative pathologies (12). This inflammation can be acute or chronic, and the cellular components of the nervous system such as microglia or astroglia can respond to estrogenic activity by modulating these inflammatory processes (126).

Body weight increases in several conditions associated with hormonal decline. In women, estrogens favor the deposition of fat in some parts of the body; however, after menopause the fat subcellular localization changes, being more similar to men (144). The adipose tissue is capable of producing estrogens through the aromatization of androgens and an increase in the blood concentration of estrogens has been observed in men with obesity but this is not evident in women (145). It has been demonstrated that ERα is the main isoform that participates in the control of weight by estrogens and the absence of ERα produces hyperpalsia and hypertrophy of the adipocytes (146).

Taking into account alterations in plasma estrogen levels, the increase in free saturated FAs and the infiltration of proinflammatory agents is presented as the ideal scenario for the development of neurodegenerative diseases. Estrogens play a fundamental role in the prevention of obesity (147). Studies in female mice showed a weight gain and hyperadiposity after ovariectomy, which eliminates endogenous estrogens. Interestingly, treatment with

estradiol reduced the development of this obese phenotype (148), demonstrating that these effects on the homeostasis of body weight are mediated mainly by the activation of ER (149). Humans or mice with mutations in the ER gene (*ESR1*) are obese (150). Likewise, estradiol therapy was found to have no effect in mice subjected to ER suppression (151).

The estrogenic response associated with inflammatory processes has been found to have the ability to decrease the secretion of inflammatory molecules and reduce the production of ROS (152). In this regard, it was reported that this mechanism was associated with the response of ER through the activation of the inflammatory pathway NF-κB (153) (154), as well as the expression and modification of some proteins such as neuroglobin (24). For instance, neuroglobin is a monomeric globin protein of about 150 amino acids (155) that has the capacity to transport and store oxygen owing to the heme group it contains (156). Hormonal regulation of neuroglobin and protective mechanisms are discussed in more details below.

4.2 Signaling mechanisms associated with estrogen-induced neuroprotection

Neuroglobin has been studied in different models of damage such as focal cerebral ischemia, β-amyloid peptide-mediated toxicity, anoxia, and glucose and oxygen deprivation (157). The presence of neuroglobin, once thought to be expressed only in neurons, has recently been discovered in glial cells. Although the neuroprotective effects of neuroglobin have been identified in these models, its molecular mechanisms of action are not yet well established (158) (159). Several investigations have found that estrogens and androgens can regulate the expression of neuroglobin, hence the modulation of this protein by estrogenic activity (160) could be partly responsible for the protective effects of estradiol in models of oxidative stress (157).

Despite its broad spectrum of protective effects, the molecular mechanisms of action of neuroglobin are poorly understood (161). It has recently been shown that neuroglobin is a hormone-inducible protein (162). Neuroglobin has the potential to be a hypoxia signaling molecule, free radical scavenger or NADH oxidase capable of supporting anaerobic glycolysis (161). Several lines of evidence regarding neuroglobin's tendency to auto-oxidate, its low concentration in the brain (~ 1μM) and its low affinity for oxygen, suggest that neuroglobin

could play different roles ranging from oxygen storage to facilitating its diffusion (163, 164). Additionally, photoactivation experiments of NADH/FMN that induce a reducing state in neuroglobin suggest that this protein participates in the elimination of oxygen radicals, mainly through reacting with NO to form a peroxynitrite radical that converts to the more stable form nitrite (165). The previous mechanism suggests a protective role for neuroglobin in the regulation of highly reactive species such as peroxynitrite (166). Under conditions of normoxia, neuroglobin is bound to oxygen while under hypoxic conditions, according to deoxygenation experiments, neuroglobin adopts a hexacoordinated structure (163, 167). This structure can be a signal indicating the low levels of oxygen in the cellular environment, triggering events to protect the cell from death (116). Therefore, overexpression of neuroglobin could increase survival in conditions of low oxygen levels. Additionally, hypoxia produces a pH decrease and this condition also favors the hexacoordinated conformation of neuroglobin (167, 168). Interestingly, neuroglobin is a hormone-regulated protein (24, 32, 130, 160), suggesting that estrogenic compounds (estradiol and tibolone) or androgens (testosterone) can modulate its expression. The protective actions of tibolone are discussed in the next section.

5. Tibolone: a neuroactive steroid and regulator of estrogenic activity

The family of drugs with selective tissue-specific estrogenic activity is called STEAR (169). The most representative molecule of this family of drugs is tibolone (170). Tibolone is considered as a synthetic steroid with estrogenic, progestogenic and androgenic activity (171). Tibolone is prescribed for the treatment of climacteric symptoms in post-menopausal women (172).

Tibolone has a 3-keto-δ5-10 configuration, a 7α-methyl substituent, and a 17α-ethynyl group that *per se* cannot explain its combined effects in different tissues like vagina, bones and brain (170). Additionally, this compound mimics the activity of estradiol through neither an aromatic ring nor the 3-OH substituent, which is necessary to act as an agonist of ER (77). Its similar activity to estradiol is probably due to the hydroxyl groups and isomeric metabolites.

Tibolone is rapidly metabolized in the body to three different metabolites: a) 3α- and b) 3β-OH-tibolone, metabolized by the enzymes 3α/β hydroxy-steroid dehydrogenase - HSD, respectively, and c) δ4-tibolone also called δ4-isomer, metabolized by the enzyme 3β-HSD-isomerase (77), which explain the combined activity of tibolone. Specifically, clinical evidence has indicated that 3α- and 3β-OH-tibolone metabolites are responsible for the estrogenic activity, while δ4-tibolone is associated with the progestogenic and androgenic activity, determined by its ability to interact with androgen and progesterone receptors (169). Similar to one of the predominant forms of estrogen (estradiol), tibolone is metabolized to weak sulfated estrogenic forms that serve as a substrate of sulphatase-type enzymes for the permanent production of estrogenic metabolites in different tissues (173).

Tibolone and its metabolites have different types of activities (progestogenic, androgenic and estrogenic) in different tissue types (liver, bone, breast tissue and brain, among others), depending on the selective modulation of the receptors with which they interact (174). In studies where an estrogenic compound (E2) was used in models of cerebral ischemia in mice, it was demonstrated that the activation of the ERβ is neuroprotective against the damage (175). Similar to this work, a study investigated the neuroprotective effects of estradiol and an ERα ligand using a model of multiple sclerosis. In the absence of anti-inflammatory effects, it was found that treatments based on the ERβ would potentially allow the development of neuroprotective strategies for different neurodegenerative diseases, whereas the use of ERα ligands should be restricted due to side effects in the womb and uterus (176). In contrast, other studies reported neuroprotection mediated by the ERα through signaling mechanisms mediated exclusively by astrocytes (177).

In addition to estrogenic compounds, progestogens and androgens have been studied in animal models for neurodegenerative diseases showing their neuroprotective potential. In the case of progestogens, it has been reported that their synthesis can be induced by estradiol in astrocytes (178), suggesting not only the importance of estradiol on progesterone synthesis, but also their combined action with other steroids. The progestogenic activity of tibolone is of great interest given that protective effects mediated by progesterone (pre- and post-insult) have been reported in different models that mimic the physiopathological conditions of important neurodegenerative diseases such as oxidative insults by glutamate cytotoxicity and

glucose deprivation, among others (179). In the CNS, different brain areas such as the hippocampus, cerebral cortex, spinal cord and sciatic nerve exhibit this protective effect of tibolone (180) (Table 1).

Interestingly, tibolone's actions in the brain are partly mediated by ERβ. Tibolone has been shown to induce protective effects in both microglia and astroglial cells upon different inflammatoty stimuli (23, 24, 29-32). For example, astrocytic cells exposed to glucose deprivation present more fragmented nuclei and increased levels of oxidative stress. Treatment of cells with tibolone (10nM) or DPN (an ERβ agonist) but not with PPT (an ERα agonist) preserved mitochondrial function and improved the outcome in a similar fashion (24), suggesting an estrogenic action of tibolone in astrocytes. However, after blocking ERβ using an antagonist, protection with tibolone in astrocytic cells deprived of glucose is dampened and more cell death is observed (24). Similarly, in BV-2 microglial cells exposed to lipotoxicity with palmitic acid, cells treated with tibolone are rescued by a mechanism that involves ERβ and neuroglobin. In this regard, several studies have shown that neuroglobin expression is increased in both astrocytes and microglial cells after treatment with estradiol, testosterone and tibolone, suggesting that this protein is hormonally regulated. Blocking the expression of neuroglobin using siRNA reduced the protective effects of tibolone in microglia and astrocytes subjected to metabolic dysfunction (24, 32). It has also been shown that tibolone induces protection in glial cells by epigenetic mechanisms, and these include the regulation of interleukin-6, miRNA, TERT, DNA methylation and telomeric complex (29, 30).

6. Conclusions

The excess of free saturated FAs in the circulation has direct effects on the homeostasis and functioning of the CNS. Free saturated FAs have consequences on different cells of the brain and their compartments. The main effects are evidenced at the mitochondrial level where an increase in oxidative stress contributes to the activation of inflammatory mechanisms that could lead to cell death. In the last decade and as shown in this review, treatment with neuroactive steroids has been of great interest due to the fundamental protective role that they have on the CNS. Neuroactive steroids can be characterized by their antioxidant and antiapoptotic properties as well as their capacity to decrease glial activity. These protective

effects on the survival and modulation of astrocytes and microglia are both dependent on and independent of the estrogen receptors, which trigger the activation of signaling pathways and transcription factors of genes involved in the inflammatory response and cell protection. Therefore, neuroactive steroids, such as tibolone and estradiol, are promising therapeutic candidates for the treatment of pathologies that affect the CNS to attenuate the progression of diseases related to exacerbated inflammatory processes. Future clinical studies on the use of steroids in the CNS diseases are warranted.

Conflict of interest

The authors declare no conflict of interest.

Authors contributions

Conceived and designed the idea: OHL, GEB, LMGS, RCM

Wrote the manuscript: OHL, GEB, LMGS, RCM, EBJ, VE, GMA, AS.

All the authors approved the final manuscript and submission.

Table 1. Studies on the relationship between fatty acid and estrogen in the brain.

References	In vitro/in vivo model	Aims	Results
(16)	Microglial cell line BV-2 and primary microglia	Effect of palmitic acid and stearic acid on microglial activation and their relationship with Alzheimer's disease	Palmitic acid activated microglia and stimulated the TLR4 / NF-κB pathway promoting the production of proinflammatory mediators
(27)	Primary astrocytes derived from male and female Wistar rat hippocampus	Effects of estradiol in astrocytes exposed to palmitic acid, with important sex-related outcomes	PA increased the ER stress and induced cell death. Estradiol (E2) increased the levels of protective factors in astrocytes of both sexes, with a clear sex dirmorphic response
(31)	T98G human astrocytic	Effect of tibolone on mitochondrial function in human astrocytes exposed to palmitic acid	Tibolone improved cell survival and preserved mitochondrial membrane potential in astrocytic cells treated with palmitic acid.
(10)	Mouse BV-2 microglia cells, exposed to LPS and palmitic acid	Effects of free fatty acids on the microglial response to inflammatory stimuli	Palmitic acid induced the alternative activation of microglia cells, affected the mRNA levels of the proinflammatory cytokines Ia-1β and IL-6.
(181)	Human neuroblastoma cells SH-SY5Y and human glioblastoma cells T98G	Neuroinflammation and reactive gliosis are associated with the elevation of saturated fatty acids related to the high-fat diet	Palmitic acid induced apoptosis by increasing oxidative stress in neurons and astrocytes.
(182)	Ovariectomized C57BL/6 female mice subjected to brain injury with subsequent treatment with tibolone	Evaluated the effect of tibolone on reactive gliosis in the cerebral cortex after brain injury in ovariectomized adult female mice	Tibolone exerted beneficial homeostatic actions in the cerebral cortex after acute brain injury
(183)	High-fat diets (HFDs) in Hypothalamic neurons and astrocytes of male C57BL/6 mice	Demonstrate that hypothalamic PGC-1α regulates ERα and inflammation in vivo	PGC-1α depletion with ERα overexpression significantly inhibited PA-induced inflammation, confirming that ERα is a critical determinant of the anti-inflammatory response

Figure Legends

Figure 1. Fatty acids induce inflammation in the nervous system and attenuate the signaling pathway of insulin in obesity. The excess of fatty acids produce inflammation principally by means of three mechanisms. **a)** Increase in lipotoxicity when the FA concentration exceeds the oxidative requirements, increasing the levels of diacylglycerol (DAG) and ceramide. DAG activates protein kinase C (PKC) that activates the nuclear factor inhibitor NF-kB (IkB) kinase (IKKβ-NF-kB) pathway, causing inflammation. A high concentration of ceramides activates the nodule-type receptor that contains a pirine domain (NLRP3), modulating the release of interleukin (IL)-1β and also leads to the activation of the protein phosphatase 2A (PP2A), attenuating the signaling pathway of insulin. **b)** Palmitic acid dysregulates the function of the endoplasmic reticulum (ER) producing stress and inflammation via NF-kB, N-terminal c-Jun (JNK) and NLRP3 inflammasome, leading to further generation of reactive oxygen species (ROS) in mitochondria. **c)** Palmitic acid activates the toll-like receptor (TLR)-4 through fetuin A; likewise high-fat diets increase lipopolysaccharide levels (LPS), an activator of this receptor, which leads to an increase in the activity of the IKKβ-NF-kB pathway.

Figure 2. Effect of fatty acids (FA) on the central nervous system. Metabolic diseases such as obesity are characterized by the accumulation of multiple metabolites that generate alterations on physiological conditions. The excess of fatty acids can contribute to an increase in inflammatory processes directly affecting the brain. The increase in the BBB permeability facilitates the entry of FA and subsequent recognition by brain cells (neurons, astrocytes, microglia), triggering different response mechanisms that contribute to the appearance of a neuroinflammatory environment, characterized by secretion of inflammatory cytokines, the activation of glial cells and neuronal death among others. The activation of microglia implies morphological and functional changes from a chronic inflammation, whereby through signaling pathways and transcription factors related to apoptotic processes and cell death contribute to the risk of developing neurodegenerative diseases.

Figure 3. Neurosteroids and neuroactive steroids have protective effects on the CNS cells. Treatment with estradiol, SERMs and STEARs has protective effects on the integrity of the cerebral microenvironment. Different investigations have reported that these molecules recover mitochondrial functions, oxidative stress, reduce the release of inflammatory factors (chemokines, cytokines, etc.) and attenuate the expression of proinflammatory and pro-apoptotic proteins and genes. This protection is due to the activation of estrogen receptors and GPER, among others, where through genomic and non-genomic mechanisms they activate the anti-inflammatory response and cell survival.

References

1. Karelis AD, Faraj M, Bastard JP, St-Pierre DH, Brochu M, Prud'homme D, Rabasa-Lhoret R. The metabolically healthy but obese individual presents a favorable inflammation profile. *J Clin Endocrinol Metab*. 2005; 90(7): 4145-50.

2. De Zwaan M, Mitchell JE, Howell LM, Monson N, Swan-Kremeier L, Roerig JL, Kolotkin RL, Crosby RD. Two measures of health-related quality of life in morbid obesity. *Obes Res*. 2002; 10(11): 1143-51.

3. Mattson MP, Chan SL, Duan W. Modification of brain aging and neurodegenerative disorders by genes, diet, and behavior. *Physiol Rev*. 2002; 82(3): 637-72.

4. Jump DB. Fatty acid regulation of gene transcription. *Crit Rev Clin Lab Sci*. 2004; 41(1): 41-78.

5. Sampath H, Ntambi JM. Polyunsaturated fatty acid regulation of genes of lipid metabolism. *Annu Rev Nutr*. 2005; 25317-40.

6. Pekny M, Pekna M. Astrocyte reactivity and reactive astrogliosis: costs and benefits. *Physiol Rev*. 2014; 94(4): 1077-98.

7. Pedraza-Alva G, Perez-Martinez L, Valdez-Hernandez L, Meza-Sosa KF, Ando-Kuri M. Negative regulation of the inflammasome: keeping inflammation under control. *Immunol Rev*. 2015; 265(1): 231-57.

8. Moser VA, Uchoa MF, Pike CJ. TLR4 inhibitor TAK-242 attenuates the adverse neural effects of diet-induced obesity. *J Neuroinflammation*. 2018; 15(1): 306.

9. Colton CA. Heterogeneity of microglial activation in the innate immune response in the brain. *J Neuroimmune Pharmacol*. 2009; 4(4): 399-418.

10. Tracy LM, Bergqvist F, Ivanova EV, Jacobsen KT, Iverfeldt K. Exposure to the saturated free fatty acid palmitate alters BV-2 microglia inflammatory response. *J Mol Neurosci*. 2013; 51(3): 805-12.

11. Martin-Jimenez CA, Garcia-Vega A, Cabezas R, Aliev G, Echeverria V, Gonzalez J, Barreto GE.

Astrocytes and endoplasmic reticulum stress: A bridge between obesity and neurodegenerative diseases. *Prog Neurobiol*. 2017; 15845-68.

12. Amor S, Peferoen LA, Vogel DY, Breur M, van der Valk P, Baker D, van Noort JM. Inflammation in neurodegenerative diseases--an update. *Immunology*. 2014; 142(2): 151-66.

13. Karve IP, Taylor JM, Crack PJ. The contribution of astrocytes and microglia to traumatic brain injury. *Br J Pharmacol*. 2016; 173(4): 692-702.

14. Haversen L, Danielsson KN, Fogelstrand L, Wiklund O. Induction of proinflammatory cytokines by long-chain saturated fatty acids in human macrophages. *Atherosclerosis*. 2009; 202(2): 382-93.

15. Niu G, Li J, Wang H, Ren Y, Bai J. Associations of A-FABP with Anthropometric and Metabolic Indices and Inflammatory Cytokines in Obese Patients with Newly Diagnosed Type 2 Diabetes. *Biomed Res Int*. 2016; 20169382092.

16. Wang Z, Liu D, Wang F, Liu S, Zhao S, Ling EA, Hao A. Saturated fatty acids activate microglia via Toll-like receptor 4/NF-kappaB signalling. *Br J Nutr*. 2012; 107(2): 229-41.

17. Arevalo MA, Santos-Galindo M, Acaz-Fonseca E, Azcoitia I, Garcia-Segura LM. Gonadal hormones and the control of reactive gliosis. *Horm Behav*. 2013; 63(2): 216-21.

18. Sochocka M, Diniz BS, Leszek J. Inflammatory Response in the CNS: Friend or Foe? *Mol Neurobiol*. 2016.

19. Nilsen J, Brinton RD. Mitochondria as therapeutic targets of estrogen action in the central nervous system. *Curr Drug Targets CNS Neurol Disord*. 2004; 3(4): 297-313.

20. Giatti S, Garcia-Segura LM, Barreto GE, Melcangi RC. Neuroactive steroids, neurosteroidogenesis and sex. *Prog Neurobiol*. 2018.

21. Avila MF, Torrente D, Cabezas R, Morales L, Garcia-Segura LM, Gonzalez J, Barreto GE. Structural insights from GRP78-NF-kappaB binding interactions: a computational approach to understand a possible neuroprotective pathway in brain injuries. *J Theor Biol*. 2014; 34543-51.

22. Martin-Jimenez CA, Gaitan-Vaca DM, Echeverria V, Gonzalez J, Barreto GE. Relationship Between Obesity, Alzheimer's Disease, and Parkinson's Disease: an Astrocentric View. *Mol Neurobiol*. 2016.

23. Avila Rodriguez M, Garcia-Segura LM, Cabezas R, Torrente D, Capani F, Gonzalez J, Barreto GE. Tibolone protects T98G cells from glucose deprivation. *J Steroid Biochem Mol Biol*. 2014; 144 Pt B294-303.

24. Avila-Rodriguez M, Garcia-Segura LM, Hidalgo-Lanussa O, Baez E, Gonzalez J, Barreto GE. Tibolone protects astrocytic cells from glucose deprivation through a mechanism involving estrogen receptor beta and the upregulation of neuroglobin expression. *Mol Cell Endocrinol*. 2016; 43335-46.

25. Martin-Jimenez C, Gaitan-Vaca DM, Areiza N, Echeverria V, Ashraf GM, Gonzalez J, Sahebkar A, Garcia-Segura LM, Barreto GE. Astrocytes Mediate Protective Actions of Estrogenic Compounds after Traumatic Brain Injury. *Neuroendocrinology*. 2019; 108(2): 142-60.

26. Sribnick EA, Ray SK, Banik NL. Estrogen prevents glutamate-induced apoptosis in C6 glioma

cells by a receptor-mediated mechanism. *Neuroscience*. 2006; 137(1): 197-209.

27. Frago LM, Canelles S, Freire-Regatillo A, Argente-Arizon P, Barrios V, Argente J, Garcia-Segura LM, Chowen JA. Estradiol Uses Different Mechanisms in Astrocytes from the Hippocampus of Male and Female Rats to Protect against Damage Induced by Palmitic Acid. *Front Mol Neurosci*. 2017; 10330.

28. Ortiz-Rodriguez A, Acaz-Fonseca E, Boya P, Arevalo MA, Garcia-Segura LM. Lipotoxic Effects of Palmitic Acid on Astrocytes Are Associated with Autophagy Impairment. *Mol Neurobiol*. 2019; 56(3): 1665-80.

29. Gonzalez-Giraldo Y, Forero DA, Echeverria V, Garcia-Segura LM, Barreto GE. Tibolone attenuates inflammatory response by palmitic acid and preserves mitochondrial membrane potential in astrocytic cells through estrogen receptor beta. *Mol Cell Endocrinol*. 2019; 48665-78.

30. Gonzalez-Giraldo Y, Garzon-Benitez AV, Forero DA, Barreto GE. TERT inhibition leads to reduction of IL6 expression induced by palmitic acid and interferes with the protective effects of tibolone in an astrocytic cell model. *Journal of neuroendocrinology*. 2019e12768.

31. Gonzalez-Giraldo Y, Garcia-Segura LM, Echeverria V, Barreto GE. Tibolone Preserves Mitochondrial Functionality and Cell Morphology in Astrocytic Cells Treated with Palmitic Acid. *Mol Neurobiol*. 2018; 55(5): 4453-62.

32. Hidalgo-Lanussa O, Avila-Rodriguez M, Baez-Jurado E, Zamudio J, Echeverria V, Garcia-Segura LM, Barreto GE. Tibolone Reduces Oxidative Damage and Inflammation in Microglia Stimulated with Palmitic Acid through Mechanisms Involving Estrogen Receptor Beta. *Mol Neurobiol*. 2018; 55(7): 5462-77.

33. Large V, Peroni O, Letexier D, Ray H, Beylot M. Metabolism of lipids in human white adipocyte. *Diabetes Metab*. 2004; 30(4): 294-309.

34. Xu C, Xu GH. [Adipose triglyceride lipase regulates adipocyte lipolysis]. *Sheng Li Ke Xue Jin Zhan*. 2008; 39(1): 10-4.

35. Miyoshi H, Perfield JW, 2nd, Souza SC, Shen WJ, Zhang HH, Stancheva ZS, Kraemer FB, Obin MS, Greenberg AS. Control of adipose triglyceride lipase action by serine 517 of perilipin A globally regulates protein kinase A-stimulated lipolysis in adipocytes. *J Biol Chem*. 2007; 282(2): 996-1002.

36. Li K, Wahlqvist ML, Li D. Nutrition, One-Carbon Metabolism and Neural Tube Defects: A Review. *Nutrients*. 2016; 8(11).

37. Varin A, Thomas C, Ishibashi M, Menegaut L, Gautier T, Trousson A, Bergas V, de Barros JP, Narce M, Lobaccaro JM, Lagrost L, Masson D. Liver X receptor activation promotes polyunsaturated fatty acid synthesis in macrophages: relevance in the context of atherosclerosis. *Arterioscler Thromb Vasc Biol*. 2015; 35(6): 1357-65.

38. Schonfeld P, Reiser G. Inhibition of beta-oxidation is not a valid therapeutic tool for reducing oxidative stress in conditions of neurodegeneration. *J Cereb Blood Flow Metab*. 2016.

39. Hauck AK, Bernlohr DA. Oxidative stress and lipotoxicity. *J Lipid Res*. 2016; 57(11): 1976-86.

40. Zlobine I, Gopal K, Ussher JR. Lipotoxicity in obesity and diabetes-related cardiac dysfunction. *Biochim Biophys Acta*. 2016; 1860(10): 1555-68.

41. Ruiz-Ramirez A, Lopez-Acosta O, Barrios-Maya MA, El-Hafidi M. Cell Death and Heart Failure in Obesity: Role of Uncoupling Proteins. *Oxid Med Cell Longev*. 2016; 20169340654.

42. Randle PJ, Garland PB, Hales CN, Newsholme EA. The glucose fatty-acid cycle. Its role in insulin sensitivity and the metabolic disturbances of diabetes mellitus. *Lancet*. 1963; 1(7285): 785-9.

43. Gong ZG, Zhang J, Xu YJ. Metabolomics Reveals that Momordica charantia Attenuates Metabolic Changes in Experimental Obesity. *Phytother Res*. 2016.

44. Gries FA, Oberdisse K. [Lipid metabolism disorders and diabetes mellitus]. *Dtsch Med Wochenschr*. 1970; 95(14): 727-34.

45. Unger RH. Lipotoxic diseases. *Annu Rev Med*. 2002; 53319-36.

46. Puri P, Mirshahi F, Cheung O, Natarajan R, Maher JW, Kellum JM, Sanyal AJ. Activation and dysregulation of the unfolded protein response in nonalcoholic fatty liver disease. *Gastroenterology*. 2008; 134(2): 568-76.

47. Henkel A, Green RM. The unfolded protein response in fatty liver disease. *Semin Liver Dis*. 2013; 33(4): 321-9.

48. Madan K, Bhardwaj P, Thareja S, Gupta SD, Saraya A. Oxidant stress and antioxidant status among patients with nonalcoholic fatty liver disease (NAFLD). *J Clin Gastroenterol*. 2006; 40(10): 930-5.

49. Yesilova Z, Ozata M, Oktenli C, Bagci S, Ozcan A, Sanisoglu SY, Uygun A, Yaman H, Karaeren N, Dagalp K. Increased acylation stimulating protein concentrations in nonalcoholic fatty liver disease are associated with insulin resistance. *Am J Gastroenterol*. 2005; 100(4): 842-9.

50. Yesilova Z, Yaman H, Oktenli C, Ozcan A, Uygun A, Cakir E, Sanisoglu SY, Erdil A, Ates Y, Aslan M, Musabak U, Erbil MK, Karaeren N, Dagalp K. Systemic markers of lipid peroxidation and antioxidants in patients with nonalcoholic Fatty liver disease. *Am J Gastroenterol*. 2005; 100(4): 850-5.

51. Lee JY, Zhao L, Youn HS, Weatherill AR, Tapping R, Feng L, Lee WH, Fitzgerald KA, Hwang DH. Saturated fatty acid activates but polyunsaturated fatty acid inhibits Toll-like receptor 2 dimerized with Toll-like receptor 6 or 1. *J Biol Chem*. 2004; 279(17): 16971-9.

52. Leszek J, Barreto GE, Gasiorowski K, Koutsouraki E, Avila-Rodrigues M, Aliev G. Inflammatory Mechanisms and Oxidative Stress as Key Factors Responsible for Progression of Neurodegeneration: Role of Brain Innate Immune System. *CNS Neurol Disord Drug Targets*. 2016; 15(3): 329-36.

53. Shi H, Kokoeva MV, Inouye K, Tzameli I, Yin H, Flier JS. TLR4 links innate immunity and fatty acid-induced insulin resistance. *J Clin Invest*. 2006; 116(11): 3015-25.

54. Hwang DH, Kim JA, Lee JY. Mechanisms for the activation of Toll-like receptor 2/4 by saturated fatty acids and inhibition by docosahexaenoic acid. *Eur J Pharmacol*. 2016; 78524-35.

55. Pal D, Dasgupta S, Kundu R, Maitra S, Das G, Mukhopadhyay S, Ray S, Majumdar SS, Bhattacharya S. Fetuin-A acts as an endogenous ligand of TLR4 to promote lipid-induced insulin resistance. *Nat Med*. 2012; 18(8): 1279-85.

56. Begin ME, Plourde M, Pifferi F, Cunnane SC. What Is the Link between Docosahexaenoic

Acid, Cognitive Impairment, and Alzheimer's Disease in the Elderly? In: Montmayeur JP, le Coutre J, eds. *Fat Detection: Taste, Texture, and Post Ingestive Effects*. Boca Raton (FL) 2010.

57. Sandu RE, Buga AM, Uzoni A, Petcu EB, Popa-Wagner A. Neuroinflammation and comorbidities are frequently ignored factors in CNS pathology. *Neural Regen Res*. 2015; 10(9): 1349-55.

58. Peters ME. Traumatic brain injury (TBI) in older adults: aging with a TBI versus incident TBI in the aged. *Int Psychogeriatr*. 2016; 28(12): 1931-4.

59. Corrigan F, Mander KA, Leonard AV, Vink R. Neurogenic inflammation after traumatic brain injury and its potentiation of classical inflammation. *J Neuroinflammation*. 2016; 13(1): 264.

60. Lee JH, Wei ZZ, Cao W, Won S, Gu X, Winter M, Dix TA, Wei L, Yu SP. Regulation of therapeutic hypothermia on inflammatory cytokines, microglia polarization, migration and functional recovery after ischemic stroke in mice. *Neurobiol Dis*. 2016; 96248-60.

61. Fraser T, Tayler H, Love S. Low-temperature improved-throughput method for analysis of brain fatty acids and assessment of their post-mortem stability. *J Neurosci Methods*. 2008; 169(1): 135-40.

62. Nasaruddin ML, Holscher C, Kehoe P, Graham SF, Green BD. Wide-ranging alterations in the brain fatty acid complement of subjects with late Alzheimer's disease as detected by GC-MS. *Am J Transl Res*. 2016; 8(1): 154-65.

63. Martins IV, Rivers-Auty J, Allan SM, Lawrence CB. Mitochondrial Abnormalities and Synaptic Loss Underlie Memory Deficits Seen in Mouse Models of Obesity and Alzheimer's Disease. *J Alzheimers Dis*. 2016.

64. Karelina K, Sarac B, Freeman LM, Gaier KR, Weil ZM. Traumatic brain injury and obesity induce persistent central insulin resistance. *Eur J Neurosci*. 2016; 43(8): 1034-43.

65. Taylor LE, Sullivan JC. Sex Differences in Obesity-Induced Hypertension and Vascular Dysfunction: A Protective Role for Estrogen in Adipose Tissue Inflammation? *Am J Physiol Regul Integr Comp Physiol*. 2016ajpregu 00202 2016.

66. Alvarado-Garcia A, Hernandez-Quijano T, Hernandez-Valencia M, Negrin-Perez MC, Rios-Castillo B, Valencia-Perez GU, Vital-Reyes VS, Basavilvazo-Rodriguez MA, Torres-Arreola LP, Ortiz-Luna GF, Sanchez-Aguirre F, Montano-Uscanga A. [Clinical practice guideline. Diagnosis and treatment of postmenopausal and perinemopausia]. *Rev Med Inst Mex Seguro Soc*. 2015; 53(2): 214-25.

67. Han S, Zhao B, Pan X, Song Z, Liu J, Gong Y, Wang M. Estrogen receptor variant ER-alpha36 is involved in estrogen neuroprotection against oxidative toxicity. *Neuroscience*. 2015; 310224-41.

68. Cheng HY, Hung SH, Chu PJ. Rescue from Sexually Dimorphic Neuronal Cell Death by Estradiol and PI3 Kinase Activity. *Cell Mol Neurobiol*. 2016; 36(5): 767-75.

69. Sjogren LL, Morch LS, Lokkegaard E. Hormone replacement therapy and the risk of endometrial cancer: A systematic review. *Maturitas*. 2016; 9125-35.

70. Zarate A, Hernandez-Valencia M, Saucedo R, Basurto L, Manuel-Apolinar L. [Current position about the use of estrogen therapy in women during the climacteric period]. *Rev Med Inst Mex*

Seguro Soc. 2014; 52(1): 66-9.

71. Farlow MR, Andreasen N, Riviere ME, Vostiar I, Vitaliti A, Sovago J, Caputo A, Winblad B, Graf A. Long-term treatment with active Abeta immunotherapy with CAD106 in mild Alzheimer's disease. *Alzheimers Res Ther*. 2015; 7(1): 23.

72. Miller AA, Spencer SJ. Obesity and neuroinflammation: a pathway to cognitive impairment. *Brain Behav Immun*. 2014; 4210-21.

73. Ferri CP, Prince M, Brayne C, Brodaty H, Fratiglioni L, Ganguli M, Hall K, Hasegawa K, Hendrie H, Huang Y, Jorm A, Mathers C, Menezes PR, Rimmer E, Scazufca M. Global prevalence of dementia: a Delphi consensus study. *Lancet*. 2005; 366(9503): 2112-7.

74. Growdon ME, Schultz AP, Dagley AS, Amariglio RE, Hedden T, Rentz DM, Johnson KA, Sperling RA, Albers MW, Marshall GA. Odor identification and Alzheimer disease biomarkers in clinically normal elderly. *Neurology*. 2015; 84(21): 2153-60.

75. Lopategui Cabezas I, Herrera Batista A, Penton Rol G. The role of glial cells in Alzheimer disease: potential therapeutic implications. *Neurologia*. 2014; 29(5): 305-9.

76. Luterman JD, Haroutunian V, Yemul S, Ho L, Purohit D, Aisen PS, Mohs R, Pasinetti GM. Cytokine gene expression as a function of the clinical progression of Alzheimer disease dementia. *Arch Neurol*. 2000; 57(8): 1153-60.

77. Eikelenboom P, van Gool WA. Neuroinflammatory perspectives on the two faces of Alzheimer's disease. *J Neural Transm (Vienna)*. 2004; 111(3): 281-94.

78. Lee JU, Kang DI, Zhu WL, Shin SY, Hahm KS, Kim Y. Solution structures and biological functions of the antimicrobial peptide, arenicin-1, and its linear derivative. *Biopolymers*. 2007; 88(2): 208-16.

79. Broe GA, Grayson DA, Creasey HM, Waite LM, Casey BJ, Bennett HP, Brooks WS, Halliday GM. Anti-inflammatory drugs protect against Alzheimer disease at low doses. *Arch Neurol*. 2000; 57(11): 1586-91.

80. Melton LM, Keith AB, Davis S, Oakley AE, Edwardson JA, Morris CM. Chronic glial activation, neurodegeneration, and APP immunoreactive deposits following acute administration of double-stranded RNA. *Glia*. 2003; 44(1): 1-12.

81. Wyss-Coray T, Yan F, Lin AH, Lambris JD, Alexander JJ, Quigg RJ, Masliah E. Prominent neurodegeneration and increased plaque formation in complement-inhibited Alzheimer's mice. *Proc Natl Acad Sci U S A*. 2002; 99(16): 10837-42.

82. Combs CK, Bates P, Karlo JC, Landreth GE. Regulation of beta-amyloid stimulated proinflammatory responses by peroxisome proliferator-activated receptor alpha. *Neurochem Int*. 2001; 39(5-6): 449-57.

83. Luth HJ, Munch G, Arendt T. Aberrant expression of NOS isoforms in Alzheimer's disease is structurally related to nitrotyrosine formation. *Brain Res*. 2002; 953(1-2): 135-43.

84. McGeer PL, Rogers J, McGeer EG. Inflammation, anti-inflammatory agents and Alzheimer disease: the last 12 years. *J Alzheimers Dis*. 2006; 9(3 Suppl): 271-6.

85. Bruce-Keller AJ, Keeling JL, Keller JN, Huang FF, Camondola S, Mattson MP. Antiinflammatory effects of estrogen on microglial activation. *Endocrinology*. 2000; 141(10): 3646-56.

86. Wilms H, Zecca L, Rosenstiel P, Sievers J, Deuschl G, Lucius R. Inflammation in Parkinson's diseases and other neurodegenerative diseases: cause and therapeutic implications. *Curr Pharm Des*. 2007; 13(18): 1925-8.

87. Dehay B, Bourdenx M, Gorry P, Przedborski S, Vila M, Hunot S, Singleton A, Olanow CW, Merchant KM, Bezard E, Petsko GA, Meissner WG. Targeting alpha-synuclein for treatment of Parkinson's disease: mechanistic and therapeutic considerations. *Lancet Neurol*. 2015; 14(8): 855-66.

88. Anitua E, Pascual C, Perez-Gonzalez R, Orive G, Carro E. Intranasal PRGF-Endoret enhances neuronal survival and attenuates NF-kappaB-dependent inflammation process in a mouse model of Parkinson's disease. *J Control Release*. 2015; 203170-80.

89. Teismann P, Schulz JB. Cellular pathology of Parkinson's disease: astrocytes, microglia and inflammation. *Cell Tissue Res*. 2004; 318(1): 149-61.

90. Wilms H, Rosenstiel P, Sievers J, Deuschl G, Zecca L, Lucius R. Activation of microglia by human neuromelanin is NF-kappaB dependent and involves p38 mitogen-activated protein kinase: implications for Parkinson's disease. *FASEB J*. 2003; 17(3): 500-2.

91. Zecca L, Wilms H, Geick S, Claasen JH, Brandenburg LO, Holzknecht C, Panizza ML, Zucca FA, Deuschl G, Sievers J, Lucius R. Human neuromelanin induces neuroinflammation and neurodegeneration in the rat substantia nigra: implications for Parkinson's disease. *Acta Neuropathol*. 2008; 116(1): 47-55.

92. Korach KS. Insights from the study of animals lacking functional estrogen receptor. *Science*. 1994; 266(5190): 1524-7.

93. Windler E, Zyriax BC, Eidenmuller B, Boeing H. Hormone replacement therapy and risk for coronary heart disease. Data from the CORA-study--a case-control study on women with incident coronary heart disease. *Maturitas*. 2007; 57(3): 239-46.

94. Castello-Porcar AM, Martinez-Jabaloyas JM. Testosterone/estradiol ratio, is it useful in the diagnosis of erectile dysfunction and low sexual desire? *Aging Male*. 20161-5.

95. Brzozowski AM, Pike AC, Dauter Z, Hubbard RE, Bonn T, Engstrom O, Ohman L, Greene GL, Gustafsson JA, Carlquist M. Molecular basis of agonism and antagonism in the oestrogen receptor. *Nature*. 1997; 389(6652): 753-8.

96. Welsh AW, Lannin DR, Young GS, Sherman ME, Figueroa JD, Henry NL, Ryden L, Kim C, Love RR, Schiff R, Rimm DL. Cytoplasmic estrogen receptor in breast cancer. *Clin Cancer Res*. 2012; 18(1): 118-26.

97. Htun H, Holth LT, Walker D, Davie JR, Hager GL. Direct visualization of the human estrogen receptor alpha reveals a role for ligand in the nuclear distribution of the receptor. *Mol Biol Cell*. 1999; 10(2): 471-86.

98. Govind AP, Thampan RV. Membrane associated estrogen receptors and related proteins: localization at the plasma membrane and the endoplasmic reticulum. *Mol Cell Biochem*. 2003; 253(1-2): 233-40.

99. Lieberman BA. The estrogen receptor activity cycle: dependence on multiple protein-protein interactions. *Crit Rev Eukaryot Gene Expr*. 1997; 7(1-2): 43-59.

100. Diep CH, Ahrendt H, Lange CA. Progesterone induces progesterone receptor gene (PGR) expression via rapid activation of protein kinase pathways required for cooperative estrogen receptor alpha (ER) and progesterone receptor (PR) genomic action at ER/PR target genes. *Steroids*. 2016; 11448-58.

101. Borahay MA, Asoglu MR, Mas A, Adam S, Kilic GS, Al-Hendy A. Estrogen Receptors and Signaling in Fibroids: Role in Pathobiology and Therapeutic Implications. *Reprod Sci*. 2016.

102. Tabarestani S, Motallebi M, Akbari ME. Are Estrogen Receptor Genomic Aberrations Predictive of Hormone Therapy Response in Breast Cancer? *Iran J Cancer Prev*. 2016; 9(4): e6565.

103. Szego CM, Davis JS. Adenosine 3',5'-monophosphate in rat uterus: acute elevation by estrogen. *Proc Natl Acad Sci U S A*. 1967; 58(4): 1711-8.

104. Nakamura I, Kusakabe M, Swanson P, Young G. Regulation of sex steroid production and mRNAs encoding gonadotropin receptors and steroidogenic proteins by gonadotropins, cyclic AMP and insulin-like growth factor-I in ovarian follicles of rainbow trout (Oncorhynchus mykiss) at two stages of vitellogenesis. *Comp Biochem Physiol A Mol Integr Physiol*. 2016; 201132-40.

105. Audy MC, Vacher P, Duly B. 17 beta-estradiol stimulates a rapid Ca2+ influx in LNCaP human prostate cancer cells. *Eur J Endocrinol*. 1996; 135(3): 367-73.

106. Sharma G, Prossnitz ER. GPER/GPR30 Knockout Mice: Effects of GPER on Metabolism. *Methods Mol Biol*. 2016; 1366489-502.

107. Gaudet HM, Cheng SB, Christensen EM, Filardo EJ. The G-protein coupled estrogen receptor, GPER: The inside and inside-out story. *Mol Cell Endocrinol*. 2015; 418 Pt 3207-19.

108. Cheng SB, Graeber CT, Quinn JA, Filardo EJ. Retrograde transport of the transmembrane estrogen receptor, G-protein-coupled-receptor-30 (GPR30/GPER) from the plasma membrane towards the nucleus. *Steroids*. 2011; 76(9): 892-6.

109. Pupo M, Maggiolini M, Musti AM. GPER Mediates Non-Genomic Effects of Estrogen. *Methods Mol Biol*. 2016; 1366471-88.

110. Prossnitz ER, Barton M. The G-protein-coupled estrogen receptor GPER in health and disease. *Nat Rev Endocrinol*. 2011; 7(12): 715-26.

111. Lei B, Peng W, Xu G, Wu M, Wen Y, Xu J, Yu Z, Wang Y. Activation of G protein-coupled receptor 30 by thiodiphenol promotes proliferation of estrogen receptor alpha-positive breast cancer cells. *Chemosphere*. 2016; 169204-11.

112. Prossnitz ER, Barton M. Estrogen biology: new insights into GPER function and clinical opportunities. *Mol Cell Endocrinol*. 2014; 389(1-2): 71-83.

113. Prossnitz ER, Arterburn JB. International Union of Basic and Clinical Pharmacology. XCVII. G Protein-Coupled Estrogen Receptor and Its Pharmacologic Modulators. *Pharmacol Rev*. 2015; 67(3): 505-40.

114. Huang W, Chen Y, Liu Y, Zhang Q, Yu Z, Mou L, Wu H, Zhao L, Long T, Qin D, Gui Y. Roles of

ERbeta and GPR30 in Proliferative Response of Human Bladder Cancer Cell to Estrogen. *Biomed Res Int*. 2015; 2015251780.

115. Catalano S, Giordano C, Panza S, Chemi F, Bonofiglio D, Lanzino M, Rizza P, Romeo F, Fuqua SA, Maggiolini M, Ando S, Barone I. Tamoxifen through GPER upregulates aromatase expression: a novel mechanism sustaining tamoxifen-resistant breast cancer cell growth. *Breast Cancer Res Treat*. 2014; 146(2): 273-85.

116. De Marinis E, Acaz-Fonseca E, Arevalo MA, Ascenzi P, Fiocchetti M, Marino M, Garcia-Segura LM. 17beta-Oestradiol anti-inflammatory effects in primary astrocytes require oestrogen receptor beta-mediated neuroglobin up-regulation. *Journal of neuroendocrinology*. 2013; 25(3): 260-70.

117. Pedersen AL, Brownrout JL, Saldanha CJ. Neuroinflammation and neurosteroidogenesis: Reciprocal modulation during injury to the adult zebra finch brain. *Physiol Behav*. 2018; 18751-6.

118. Arevalo MA, Diz-Chaves Y, Santos-Galindo M, Bellini MJ, Garcia-Segura LM. Selective oestrogen receptor modulators decrease the inflammatory response of glial cells. *Journal of neuroendocrinology*. 2012; 24(1): 183-90.

119. Baez-Jurado E, Rincon-Benavides MA, Hidalgo-Lanussa O, Guio-Vega G, Ashraf GM, Sahebkar A, Echeverria V, Garcia-Segura LM, Barreto GE. Molecular mechanisms involved in the protective actions of Selective Estrogen Receptor Modulators in brain cells. *Front Neuroendocrinol*. 2019; 5244-64.

120. Vesga-Jimenez DJ, Hidalgo-Lanussa O, Baez-Jurado E, Echeverria V, Ashraf GM, Sahebkar A, Barreto GE. Raloxifene attenuates oxidative stress and preserves mitochondrial function in astrocytic cells upon glucose deprivation. *J Cell Physiol*. 2019; 234(3): 2051-7.

121. Straub RH. The complex role of estrogens in inflammation. *Endocr Rev*. 2007; 28(5): 521-74.

122. Rider V, Li X, Peterson G, Dawson J, Kimler BF, Abdou NI. Differential expression of estrogen receptors in women with systemic lupus erythematosus. *J Rheumatol*. 2006; 33(6): 1093-101.

123. Cerciat M, Unkila M, Garcia-Segura LM, Arevalo MA. Selective estrogen receptor modulators decrease the production of interleukin-6 and interferon-gamma-inducible protein-10 by astrocytes exposed to inflammatory challenge in vitro. *Glia*. 2010; 58(1): 93-102.

124. Suzuki S, Brown CM, Wise PM. Neuroprotective effects of estrogens following ischemic stroke. *Front Neuroendocrinol*. 2009; 30(2): 201-11.

125. Barreto G, White RE, Ouyang Y, Xu L, Giffard RG. Astrocytes: targets for neuroprotection in stroke. *Cent Nerv Syst Agents Med Chem*. 2011; 11(2): 164-73.

126. Lanussa OH, Avila-Rodriguez M, Garcia-Segura LM, Gonzalez J, Echeverria V, Aliev G, Barreto GE. Microglial dependent protective effects of neuroactive steroids. *CNS Neurol Disord Drug Targets*. 2016; 15(2): 242-9.

127. Choi SS, Lee HJ, Lim I, Satoh J, Kim SU. Human astrocytes: secretome profiles of cytokines and chemokines. *PLoS One*. 2014; 9(4): e92325.

128. Liu CY, Yang Y, Ju WN, Wang X, Zhang HL. Emerging Roles of Astrocytes in Neuro-Vascular Unit and the Tripartite Synapse With Emphasis on Reactive Gliosis in the Context of Alzheimer's Disease. *Front Cell Neurosci*. 2018; 12193.

129. De Luca C, Colangelo AM, Alberghina L, Papa M. Neuro-Immune Hemostasis: Homeostasis and Diseases in the Central Nervous System. *Front Cell Neurosci*. 2018; 12459.

130. De Marinis E, Fiocchetti M, Acconcia F, Ascenzi P, Marino M. Neuroglobin upregulation induced by 17beta-estradiol sequesters cytocrome c in the mitochondria preventing H2O2-induced apoptosis of neuroblastoma cells. *Cell Death Dis*. 2013; 4e508.

131. Azcoitia I, Arevalo MA, De Nicola AF, Garcia-Segura LM. Neuroprotective actions of estradiol revisited. *Trends Endocrinol Metab*. 2011; 22(12): 467-73.

132. Wang MJ, Huang HM, Chen HL, Kuo JS, Jeng KC. Dehydroepiandrosterone inhibits lipopolysaccharide-induced nitric oxide production in BV-2 microglia. *J Neurochem*. 2001; 77(3): 830-8.

133. Barger SW, Chavis JA, Drew PD. Dehydroepiandrosterone inhibits microglial nitric oxide production in a stimulus-specific manner. *J Neurosci Res*. 2000; 62(4): 503-9.

134. Fujita H, Tanaka J, Toku K, Tateishi N, Suzuki Y, Matsuda S, Sakanaka M, Maeda N. Effects of GM-CSF and ordinary supplements on the ramification of microglia in culture: a morphometrical study. *Glia*. 1996; 18(4): 269-81.

135. Ganter S, Northoff H, Mannel D, Gebicke-Harter PJ. Growth control of cultured microglia. *J Neurosci Res*. 1992; 33(2): 218-30.

136. Barreto G, Santos-Galindo M, Diz-Chaves Y, Pernia O, Carrero P, Azcoitia I, Garcia-Segura LM. Selective estrogen receptor modulators decrease reactive astrogliosis in the injured brain: effects of aging and prolonged depletion of ovarian hormones. *Endocrinology*. 2009; 150(11): 5010-5.

137. Perry VH, Nicoll JA, Holmes C. Microglia in neurodegenerative disease. *Nat Rev Neurol*. 2010; 6(4): 193-201.

138. Schumacher M, Weill-Engerer S, Liere P, Robert F, Franklin RJ, Garcia-Segura LM, Lambert JJ, Mayo W, Melcangi RC, Parducz A, Suter U, Carelli C, Baulieu EE, Akwa Y. Steroid hormones and neurosteroids in normal and pathological aging of the nervous system. *Prog Neurobiol*. 2003; 71(1): 3-29.

139. Tapia-Gonzalez S, Carrero P, Pernia O, Garcia-Segura LM, Diz-Chaves Y. Selective oestrogen receptor (ER) modulators reduce microglia reactivity in vivo after peripheral inflammation: potential role of microglial ERs. *J Endocrinol*. 2008; 198(1): 219-30.

140. Barreto G, Veiga S, Azcoitia I, Garcia-Segura LM, Garcia-Ovejero D. Testosterone decreases reactive astroglia and reactive microglia after brain injury in male rats: role of its metabolites, oestradiol and dihydrotestosterone. *Eur J Neurosci*. 2007; 25(10): 3039-46.

141. Stein DG. Progesterone exerts neuroprotective effects after brain injury. *Brain Res Rev*. 2008; 57(2): 386-97.

142. Garcia-Segura LM, Naftolin F, Hutchison JB, Azcoitia I, Chowen JA. Role of astroglia in estrogen regulation of synaptic plasticity and brain repair. *J Neurobiol*. 1999; 40(4): 574-84.

143. Petrone AB, Simpkins JW, Barr TL. 17beta-estradiol and inflammation: implications for ischemic stroke. *Aging Dis*. 2014; 5(5): 340-5.

144. Wu A, Shi Z, Martin S, Vincent A, Heilbronn L, Wittert G. Age-related changes in estradiol and longitudinal associations with fat mass in men. *PLoS One*. 2018; 13(8): e0201912.

145. Brown LM, Clegg DJ. Central effects of estradiol in the regulation of food intake, body weight, and adiposity. *J Steroid Biochem Mol Biol*. 2010; 122(1-3): 65-73.

146. Qian SW, Liu Y, Wang J, Nie JC, Wu MY, Tang Y, Zhao YX, Li X, Huang HY, Guo L, Liu XS, Xu CJ, Tang QQ. BMP4 Cross-talks With Estrogen/ERalpha Signaling to Regulate Adiposity and Glucose Metabolism in Females. *EBioMedicine*. 2016; 1191-100.

147. Engler-Chiurazzi EB, Brown CM, Povroznik JM, Simpkins JW. Estrogens as neuroprotectants: Estrogenic actions in the context of cognitive aging and brain injury. *Prog Neurobiol*. 2016.

148. Yasrebi A, Rivera JA, Krumm EA, Yang JA, Roepke TA. Activation of Estrogen Response Element-independent ERalpha signaling protects female mice from diet-induced obesity. *Endocrinology*. 2016en20161535.

149. Ponnusamy S, Tran QT, Harvey I, Smallwood HS, Thiyagarajan T, Banerjee S, Johnson DL, Dalton JT, Sullivan RD, Miller DD, Bridges D, Narayanan R. Pharmacologic activation of estrogen receptor beta increases mitochondrial function, energy expenditure, and brown adipose tissue. *FASEB J*. 2016.

150. Shahed A, Simmons JJ, Featherstone SL, Young KA. Matrix metalloproteinase inhibition influences aspects of photoperiod stimulated ovarian recrudescence in Siberian hamsters. *Gen Comp Endocrinol*. 2015; 21646-53.

151. Wang ZF, Pan ZY, Xu CS, Li ZQ. Activation of G-protein coupled estrogen receptor 1 improves early-onset cognitive impairment via PI3K/Akt pathway in rats with traumatic brain injury. *Biochem Biophys Res Commun*. 2016.

152. Said MM, Bosland MC. The anti-inflammatory effect of montelukast, a cysteinyl leukotriene receptor-1 antagonist, against estradiol-induced nonbacterial inflammation in the rat prostate. *Naunyn Schmiedebergs Arch Pharmacol*. 2016.

153. Deshpande R, Khalili H, Pergolizzi RG, Michael SD, Chang MD. Estradiol down-regulates LPS-induced cytokine production and NFkB activation in murine macrophages. *Am J Reprod Immunol*. 1997; 38(1): 46-54.

154. Mak P, Li J, Samanta S, Mercurio AM. ERbeta regulation of NF-kB activation in prostate cancer is mediated by HIF-1. *Oncotarget*. 2015; 6(37): 40247-54.

155. Ascenzi P, di Masi A, Leboffe L, Fiocchetti M, Nuzzo MT, Brunori M, Marino M. Neuroglobin: From structure to function in health and disease. *Mol Aspects Med*. 2016.

156. Zhang LN, Hu SB, Deng SY, Chen CX, Wu L, Peng QY, Huang L, Ai YH. [Influences of DNA methylation upon neuroglobin sustained expression in oxygen- glucose deprivation model]. *Zhonghua Yi Xue Za Zhi*. 2016; 96(39): 3164-9.

157. Baez E, Echeverria V, Cabezas R, Avila-Rodriguez M, Garcia-Segura LM, Barreto GE. Protection by Neuroglobin Expression in Brain Pathologies. *Front Neurol*. 2016; 7146.

158. Fan R, Yu T, Lin JL, Ren GD, Li Y, Liao XX, Huang ZT, Jiang CH. Remote ischemic preconditioning improves post resuscitation cerebral function via overexpressing neuroglobin after

cardiac arrest in rats. *Brain Res*. 2016; 1648(Pt A): 345-55.

159. Amri F, Ghouili I, Amri M, Carrier A, Masmoudi-Kouki O. Neuroglobin protects astroglial cells from hydrogen peroxide-induced oxidative stress and apoptotic cell death. *J Neurochem*. 2016.

160. Toro-Urrego N, Garcia-Segura LM, Echeverria V, Barreto GE. Testosterone Protects Mitochondrial Function and Regulates Neuroglobin Expression in Astrocytic Cells Exposed to Glucose Deprivation. *Front Aging Neurosci*. 2016; 8152.

161. Brunori M, Vallone B. Neuroglobin, seven years after. *Cell Mol Life Sci*. 2007; 64(10): 1259-68.

162. De Marinis E, Ascenzi P, Pellegrini M, Galluzzo P, Bulzomi P, Arevalo MA, Garcia-Segura LM, Marino M. 17beta-estradiol--a new modulator of neuroglobin levels in neurons: role in neuroprotection against H(2)O(2)-induced toxicity. *Neurosignals*. 2010; 18(4): 223-35.

163. Trent JT, 3rd, Watts RA, Hargrove MS. Human neuroglobin, a hexacoordinate hemoglobin that reversibly binds oxygen. *J Biol Chem*. 2001; 276(32): 30106-10.

164. Yu ZL, Qiu S, Chen XC, Dai ZH, Huang YC, Li YN, Cai RH, Lei HT, Gu HY. Neuroglobin - a potential biological marker of retinal damage induced by LED light. *Neuroscience*. 2014; 270158-67.

165. Hua S, Antao ST, Corbett A, Witting PK. The significance of neuroglobin in the brain. *Curr Med Chem*. 2010; 17(2): 160-72.

166. DellaValle B, Hempel C, Kurtzhals JA, Penkowa M. In vivo expression of neuroglobin in reactive astrocytes during neuropathology in murine models of traumatic brain injury, cerebral malaria, and autoimmune encephalitis. *Glia*. 2010; 58(10): 1220-7.

167. Cabezas R, Baez-Jurado E, Hidalgo-Lanussa O, Echeverria V, Ashrad GM, Sahebkar A, Barreto GE. Growth Factors and Neuroglobin in Astrocyte Protection Against Neurodegeneration and Oxidative Stress. *Mol Neurobiol*. 2018.

168. Brittain T, Skommer J, Henty K, Birch N, Raychaudhuri S. A role for human neuroglobin in apoptosis. *IUBMB Life*. 2010; 62(12): 878-85.

169. Kloosterboer HJ. Tissue-selective effects of tibolone on the breast. *Maturitas*. 2004; 49(1): S5-S15.

170. Reed MJ, Kloosterboer HJ. Tibolone: a selective tissue estrogenic activity regulator (STEAR). *Maturitas*. 2004; 48 Suppl 1S4-6.

171. Steckelbroeck S, Jin Y, Oyesanmi B, Kloosterboer HJ, Penning TM. Tibolone is metabolized by the 3alpha/3beta-hydroxysteroid dehydrogenase activities of the four human isozymes of the aldo-keto reductase 1C subfamily: inversion of stereospecificity with a delta5(10)-3-ketosteroid. *Mol Pharmacol*. 2004; 66(6): 1702-11.

172. Radowicki S, Arsoba J, Dubrawski W. [Tibolone (Livial) in the treatment of disorders of the postmenopausal period (preliminary report)]. *Ginekol Pol*. 1988; 59(11): 705-8.

173. de Gooyer ME, Kleyn GT, Smits KC, Ederveen AG, Verheul HA, Kloosterboer HJ. Tibolone: a compound with tissue specific inhibitory effects on sulfatase. *Mol Cell Endocrinol*. 2001; 183(1-2): 55-62.

174. Colombo D, Ferraboschi P, Franchini L, Nishino H, Takayasu J, Tokuda H. Anti-tumor-Promoting activity of tibolone and its metabolites. *Arzneimittelforschung*. 2008; 58(2): 86-90.

175. Carswell HV, Macrae IM, Gallagher L, Harrop E, Horsburgh KJ. Neuroprotection by a selective estrogen receptor beta agonist in a mouse model of global ischemia. *Am J Physiol Heart Circ Physiol*. 2004; 287(4): H1501-4.

176. Tiwari-Woodruff S, Morales LB, Lee R, Voskuhl RR. Differential neuroprotective and antiinflammatory effects of estrogen receptor (ER)alpha and ERbeta ligand treatment. *Proc Natl Acad Sci U S A*. 2007; 104(37): 14813-8.

177. Spence RD, Voskuhl RR. Neuroprotective effects of estrogens and androgens in CNS inflammation and neurodegeneration. *Front Neuroendocrinol*. 2012; 33(1): 105-15.

178. Sinchak K, Mills RH, Tao L, LaPolt P, Lu JK, Micevych P. Estrogen induces de novo progesterone synthesis in astrocytes. *Dev Neurosci*. 2003; 25(5): 343-8.

179. Herzog R, Zendedel A, Lammerding L, Beyer C, Slowik A. Impact of 17beta-estradiol and progesterone on inflammatory and apoptotic microRNA expression after ischemia in a rat model. *J Steroid Biochem Mol Biol*. 2016.

180. Yousuf S, Atif F, Sayeed I, Wang J, Stein DG. Neuroprotection by progesterone after transient cerebral ischemia in stroke-prone spontaneously hypertensive rats. *Horm Behav*. 2016; 8429-40.

181. Ng YW, Say YH. Palmitic acid induces neurotoxicity and gliatoxicity in SH-SY5Y human neuroblastoma and T98G human glioblastoma cells. *PeerJ*. 2018; 6e4696.

182. Crespo-Castrillo A, Yanguas-Casas N, Arevalo MA, Azcoitia I, Barreto GE, Garcia-Segura LM. The Synthetic Steroid Tibolone Decreases Reactive Gliosis and Neuronal Death in the Cerebral Cortex of Female Mice After a Stab Wound Injury. *Mol Neurobiol*. 2018.

183. Morselli E, Fuente-Martin E, Finan B, Kim M, Frank A, Garcia-Caceres C, Navas CR, Gordillo R, Neinast M, Kalainayakan SP, Li DL, Gao Y, Yi CX, Hahner L, Palmer BF, Tschop MH, Clegg DJ. Hypothalamic PGC-1alpha protects against high-fat diet exposure by regulating ERalpha. *Cell Rep*. 2014; 9(2): 633-45.