Université de Montréal

# The role of GPR120 in diet induced obesity, mood dysregulation, and microglial function

Par Zahra Dashtehei Pour

Département de Nutrition

Faculté de Médecine

Mémoire présenté à la Faculté de Médecine

en vue de l'obtention du grade de Maîtrise ès sciences (M.Sc.) en Nutrition

Décembre, 2020

Université de Montréal

Faculté des études supérieures et postdoctorales

Ce mémoire est intitulé :

**Le rôle de GPR120 dans l'obésité induite par l'alimentation, les troubles de l'humeur et la fonction microgliale**

Présenté par:

Zahra Dashtehei Pour

a été évaluée par un jury composé des personnes suivantes :

Dr. Geneviève Mailhot, PhD,

Président-Rapporteur

Dr. Matthieu Ruiz, PhD,

Membre du jury

Dr. Stephanie Fulton, PhD,

Directrice de recherche

**Résumé**

L'obésité est un facteur de risque majeur pour le développement de maladies psychiatriques, telles que le trouble dépressif majeur et la schizophrénie. Une consommation excessive de graisses saturées est bien connue pour provoquer non seulement des troubles métaboliques, mais également des comportements anormaux chez les modèles animaux et les humains. Les acides gras saturés augmentent l'inflammation dans divers tissus. Plusieurs études ont démontré que l'activation de la microglie en tant qu'acteur central de la neuroinflammation joue un rôle crucial dans l'anxiété liée à l'inflammation et les comportements dépressifs dans les cas d'obésité. En outre, il existe de plus en plus de preuves démontrant l'effet bénéfique de la supplémentation en acides gras polyinsaturés n-3 (AGPI n-3) sur les comportements cognitifs, anxieux et dépressifs. Nous avons précédemment montré que la supplémentation en AGPI n-3 via l'administration d'huile de poisson (FO) a des effets anxiolytiques sur les souris rendues obèses par une diète riche en gras saturés (SHFD). De plus, l'activation du récepteur couplé aux protéines G 120 (GPR120), un récepteur des AGPI n-3, dans le cerveau, attenue l'anxiété et les comportements dépressifs induits par la SHFD. Cependant, les mécanismes par lesquels GPR120 régule les changements induits par la SHFD dans les comportements liés à l'humeur ne sont toujours pas compris. Dans la présente étude, nous avons étudié le rôle du GPR120 dans les troubles anxieux et dépressifs reliés à la neuroinflammation. Parmi plusieurs types de cellules neurales, la microglie exprime fortement GPR120. Un agoniste de GPR120 (Compound A; CpdA) réduit la production et la libération de cytokines inflammatoires (IL-1B, IL-6, MCP1 et TNF-α) induites par les lipopolysaccharides (LPS) dans la microglie en culture. D'autre part, l'administration centrale de CpdA par injection intracérébroventriculaire (ICV) améliore le comportement anxieux et de malaise suite à l'injection de LPS systémique in vivo. De plus, l'acide eicosapentaénoique (EPA) et l'acide docosahexaénoique (DHA) sont des AGPI n-3 et des agonistes naturels du GPR120. L'EPA et le DHA suppriment l'inflammation dans un modèle de culture de microglies primaires, telle qu'évaluée par l'expression et la sécrétion de cytokines. Ces résultats suggèrent que l'activation du GPR120 contribue à l'amélioration de l'anxiété et des comportements de type dépressif liés à l'inflammation grâce à la régulation de la microglie.

**Mots clés:** acides gras oméga-3, obésité, neuroinflammation, GPR120, comportements anxiodépressifs

**Abstract**

Obesity is a major risk factor for the development of psychiatric diseases, such as major depressive disorder and anxiety. Excess intake of saturated fat is well known to cause not only metabolic diseases, but also abnormal behavior in humans and animal models. Saturated fatty acids enhance peripheral and central inflammation. Several studies have demonstrated that microglia activation as a central player in neuroinflammation plays a crucial role in inflammation-related anxiety and depressive-like behaviors in the case of obesity. Also, there is increasing evidence to demonstrate the beneficial effect of n-3 PUFAs supplementation on cognitive, anxiety and depressive-like behaviors. Previously, we reported that the n-3 PUFAs supplementation by the administration of fish oil (FO) has demonstrated anxiolytic effects on saturated high fat diet (SHFD)-induced obese mice and that, the activation of G-protein coupled receptor 120 (GPR120), a lipid sensor for n-3 poly-unsaturated fatty acids (n-3 PUFAs), in the brain rescued SHFD-induced anxiety and depressive-like behaviors. However, it is still unclear how GPR120 regulates SHFD-induced changes in mood-related behaviors. In the present study, we focused on the role of GPR120 on neuroinflammation in inflammation-related anxiety and depressive-like behaviors. Amongst the several types of brain cells, microglia demonstrated a high expression of GPR120. Compound A (CpdA), a selective agonist of GPR120, reduced lipopolysaccharide (LPS)-induced inflammatory cytokine (IL-1B, IL-6, MCP1, and TNF-a) expression and release in cultured microglia. Additionally, central administration of cpdA via intracerebroventricular (ICV) injection ameliorated neuroinflammation and systemic LPS injection-induced anxiety-like and sickness behavior in vivo. Furthermore, n-3 PUFAs eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) as natural agonists of GPR120 suppressed inflammation in primary cultured microglia as assessed by cytokine expression. These results suggest that GPR120 activation contributes to the amelioration of inflammation-related anxiety and depressive-like behaviors through the regulation of microglia.

**Keywords:** Omega-3 fatty acids, obesity, neuroinflammation, GPR120, anxiodepressive behaviors

**Table of Contents**

**The role of GPR120 in diet induced obesity, mood dysregulation and microglial function**

Résumé ..... i

Abstract ..... iii

Table of Contents ..... iv

List of figures..... viii

List of abbreviations ..... ix

Acknowledgments..... xii

**Chapter 1: INTROUDUCTION**

**1. Obesity and mood disorders: from pathophysiology to biological mechanisms ..... 1**

1.1. Epidemiology and pathophysiology of obesity ..... 1

1.2. Relationship between obesity, metabolic and mood disorders ..... 3

1.3. Biological mechanisms influencing obesity, metabolic disorder and depression.....5

1.3.1. systemic/chronic low-grade inflammation..... 6

1.3.2. Adipose tissue and metabolic dysregulation..... 7

1.3.3. Hypothalamic-pituitary-adrenal (HPA) axis dysfunction..... 8

1.3.4. Gut-brain axis dysfunction in both obesity and depression ..... 9

**2. Neuroinflammation, a central phenomenon in food and mood dysregulation.....** 10

2.1. Neuroinflammation definition..... 10

2.2. Diet-induced obesity augmented neuroinflammation and microglial activation..... 11

2.3. Obesity, nucleus accumbens dysfunction and depression…….. 12

**3. Microglia a central player in neuroinflammation…….. 13**

3.1. The overview of microglia origin…….. 13

3.2. Microglia phenotypes (resting and activated forms) …….. 14

3.3. Microglia play active roles in obesity and depression…….. 16

3.4. Inflammatory mechanisms in microglia…… 17

3.4.1. TLR4/Nuclear Factor-κB (NFκB) …….. 17

3.4.2. NLRP3 inflammasome and their correlation with obesity and depression…….. 18

**4. Types of dietary fat and their effect on the obesity - mood axis …….. 20**

4.1. Unsaturated fatty acids (UFAs) definition, biosynthesis and sources…….. 21

4.2. Beneficial effects of poly unsaturated fatty acids on anxiety and depression…….. 23

4.3. Mechanisms underlying the protective effect of n-3 fatty acids on inflammation…… 25

**5. Free fatty acid G protein couple receptors for long chain unsaturated fatty acids…… 25**

5.1. Overview of the free fatty acid receptors (FFARs) family …….. 25

5.2. Long-chain fatty acid receptors (GPR-40 and GPR120) …… 26

5.2.1. G protein-coupled receptor-120 (GPR120) …… 27

5.3. GPR120 biological and physiological functions…… 29

5.3.1. Functions of GPR120 in insulin sensitization …….. 29

5.3.2. Functions of GPR120 in energy homeostasis, appetite control, and food intake…. 30

5.3.3. Functions of GPR120 on inflammation responses …… 31

5.3.3.1 GPR120-Mediate anti-inflammatory mechanisms…….. 32

5.3.3.1.1. Inhibition of TLR4/ NFκB pathway…….. 32

5.3.3.1.2. Inhibition of NLRP3 inflammasome…… 33

**Chapter 2: BACKGROUND AND HYPOTHESIS…….. 35**

2.1. Background ……. 35

2.2. Hypothesis…… 36

2.3. Contribution to the project and article…… 40

**Chapter 3: RESULTS**

**3.1. Article (unpublished) …… 38**

Microglial GPR120 activation protects against neuroinflammation and associated behavioral alteration.

Abstract …….. 40

Introduction …… 42

Methods …… 43

Results …… 50

Discussion …… 57

Conclusion …… 60

References …… 61

**Supplementary Figure 1** …… 67

**Appendix1**. Microglia GPR120 and GPR-40 mRNA expression ……68

**Appendix2.** Microglial cell culture isolation and treatment flow chart (in vitro)……69

**Appendix3.** Published Article; Fish oil supplementation alleviates metabolic and anxiodepressive effects of diet-induced obesity and associated changes in brain lipid composition in mice……70

**Chapter 4: DISCUSSION and CONCLUSION**

4.1. Discussion …… 71

4.2. Limitation and future perspectives …… 81

4.3. Conclusion …… 83

**Chapter 5: REFERENCES** …… 84

**List of figures**

***Figure 1.*** *Multiple factors responsible for obesity [17]…….. 2*

***Figure 2.*** *Biological mechanisms linking depression and obesity [53]…….. 6*

***Figure 3.*** *The diagram of microglial origin, CNs colonization microglial progenitor and homeostasis during brain development in mouse [154]…… 14*

***Figure 4.*** *Different microglial phenotypes related to different functional states of microglia and their activating factors and released molecules[166]……16*

***Figure 5.*** *Unsaturated fatty acids and eicosanoids synthesis in mammals by enzymatic desaturase and elongase [241] …… 23*

***Figure 6.*** *Amino acid diagram of GPR120 in humans [295] …… 28*

***Figure 7.*** *Mechanism of anti-inflammation upon GPR120 activation by omega-3 fatty acids in macrophages [321] …… 33*

**List of abbreviations**

**AA:** Arachidonic acid [22:4 (n-6)]

**AgRP:** Agouti-related peptide

**Akt or PKB:** Proteine kinase B

**ALA:** α-linolenic acid [18:4(n-3)]

**ANOVA:** Analysis of variance

**ApoB:** Apolipoproteine B-100

**ARC:** Arcuate nucleus

**Arg-1:** Arginase 1

**ATB:** Antibiotic

**BBB:** Blood-brain barrier

**BDNF:** Brain-derived neurotrophic factor

**BMI:** Body mass index

**CCR2:** CC chemokine receptor 2 CD11b

**CNS:** Central nervous system

**COX2:** Cyclooxygenase 2

**CpdA:** Compound A (GPR120 specific synthetic agonist)

**CRP:** C-reactive protein

**CX3CR1:** Chemokine (C-X3-C motif) receptor1

**DGLA:** Dihomo-γ-linolenic acid

**DHA:** Docosahexaenoic acid [22:6(n-3)]

**DMEM:** Dulbecco's modified Eagle's medium

**DPA:** Docosapentaenoic acid [22:4(n-6)]

**ELISA:** Enzyme-linked immunosorbent assay

**EPA:** eicosapentaenoic acid [20:5(n-3)]

**EPM:** Elevated-plus maze

**ERK 1/2:** Extracellular signal-regulated kinase 1/2

**EYFP:** Enhanced yellow fluorescent protein

**FBS:** Fetal bovine serum

**FFAR:** Free fatty acid receptor

**Fizz-1:** Resistin-like molecule alpha1

**FST:** Forced swim test

**GLP-1:** Glucagon-like peptide-1

**GLUT4:** Glucose transporter type 4

**GPR120 KO:** G-protein coupled receptor 120 knockout.

**GPR120:** G-protein coupled receptor 120

**GPR40:** G-protein coupled receptor 40

**HFD + FO:** High fat diet + fish oil

**HFD:** High fat diet

**HPA:** Hypothalamic-pituitary-adrenal

**ICV:** Intracerebroventriculare

**IFγ:** Interferon gamma

**IKK:** IκB kinase.

**IL-10:** Interleukine-10

**IL-1β:** Interleukine-1 beta

**IL-6:** Interleukine-6

**iNOS:** Nitric oxide synthase

**JNK:** c-Jun N-terminal kinases

**LA :** Linoleic acid [18:4(n-6)]

**LPS:** Lipopolysaccharide

**MCP-1:** monocyte chemoatractant protein 1

**MKK4:** Mitogen-activated protein kinase 4

**NAc:** Nucleus acumbens

**NFκB:** Nuclear factor kappa-light-chain-enhancer of activated B cells.

**NPY:** Neuropeptide Y

**OFT:** Open field test

**P38 MAPK:** p38 mitogen-activated protein kinases

**PBS:** Phosphate buffered saline

**PFC:** Prefrontal cortex

**PKC- θ:** Protein kinase C-theta

**POMC:** Pro-opiomelanocortin

**PPAR-γ:** Peroxisome proliferator-activated receptor gamma

**PVT:** Paraventricular thalamic nucleus

**qPCR:** Quantitative polymerase chain reaction

**ROS:** Reactive oxygen species

**RT-PCR:** Real time polymerase chain reaction

**SHFD:** Saturated high fat diet

**SIRT1:** Sirtuine 1

**SNc:** Substantia nigra pars compacta

**SOCS3:** Suppressor of cytokine signaling 3

**TAB1:** TGF-β activated kinase 1

**TAK1:** Transforming growth factor beta-activated kinase 1

**TGF-β:** Transforming growth factor beta

**TLR-4:** Toll-like receptor 4

**TNFR:** Tumor necrosis factor receptor

**TNF-α:** Tumor necrosis factor alpha

**VTA:** Ventral tegmental area

**W-3 or n-3:** Omega-3 fatty acid

**WAT:** White adipose tissue

**WT:** Wild-type

**YM1:** chitinase-likeprotein-1

*My lovely mother and father Simin and Shahrokh and best sisters*

*(Zohreh, Bahareh and Sharareh)*

***Acknowledgments***

First of all, I would like to appreciate my director Dr. Stephanie Fulton for her exceptional support and motivation not only in my master's research but also in my life as a big advisor while always encouraging me to do my best, believing in my abilities as well as for providing me with the best training opportunities and every chance for success.

In every society, the researchers and their findings serve an important part in the development and progress. Unfortunately, my thesis writing has been synchronized with the new challenge that whole the world is faced with, the corona pandemic. In these hard circumstances for everyone, the important role and responsibility of the researchers in the field of biology to find solutions and rescue human lives in as short time as possible is bolded more than ever. So, as a small player in this field I have become more aware of these efforts' impact on human life. Therefore, I feel more responsibility than before in this context. I would like to deeply thank all people who work non-stop on finding a way to solve this problem and I hope all can comes back to happiness and we can hug each other without any worries.

I would also like to greatly thank other nice collaborator in Dr. Stephanie Fulton's and Dr. Thierry Alquier's lab team; Demetra Rodaros, Diane Bairamian, Genevieve Demers, Khalil Bouyakdan, Cecile Hryhorczuk, Romane Manceau, and the others that I have been fortunate to work with. Without their kindness and patient guidance, assistance and contributions, I could not finish this way.

I would especially like to thank David Lau and Danie Majeur for being always available to help, proofreading and checking the language of my thesis. Also, I would especially grateful to my dear colleagues, Arturo Israel Machuca Parra and Shingo Nakajima for being available for experimental guidance and scientific discussions.

Finally, I would like to thank my lovely parents as the biggest fortunate and chance that I have in my life for their unconditional support, encouragement and all things they teach me and I especially would like to thank my amazing sisters for their kind support and consideration during all stages of my life.

# Chapter 1: INTROUDUCTION

## 1. Obesity and mood disorders: from pathophysiology to biological mechanisms

### 1.1. Epidemiology and Pathophysiology of obesity

Obesity is a Latin word that is derived from Obēsus in meaning of fat, plump or stout. Over recent decades, multiple health challenges linked to overweight and obesity present one of the main global health threats. Overweight and obesity are characterized as abnormal or excessive fat accumulation that can have adverse consequences on an individual's health (WHO, 2018). Obesity is known as a relapsing, progressive disease involving chronic low-grade inflammation [1] which is a predisposing factor for numerous pathological conditions and chronic diseases such as type 2 diabetes mellitus (T2D) [2], cardiovascular disease (CVD), musculoskeletal disorders, several types of cancers (e.g. breast, endometrium, liver, pancreas, prostate and kidney) [2, 3], and mental disorders such as dementia [4], depression, and anxiety [5-10].

According to the World Health Organization's latest data (WHO, 2018), the prevalence of obesity has nearly tripled since 1975 worldwide. In 2016, approximately half of the adults (18 years and older) were obese or overweight, with over 650 million adults and 340 million children and adolescents having obesity [11]. Based on the predictions of the Kelly et al. study, if current trends continue, around 57.8% of the world population will be overweight or obese by 2030 [12]. Like many countries, Canada is no exception in this situation. According to a publication by the Public Health Agency of Canada, the prevalence of obesity in Canada has significantly increased over the past three decades. In 2018, around 63.1 percent of Canadian adults (18 and older) had excess weight (26.8% obese and 36.3% overweight). Several studies predict continued increases in obesity rates by country. For example, by two estimated reports, if the current trends continue among adults, the prevalence of obesity will rise to more than one-third of Canadian adults, by the year 2031 [13] and 42% of the adult population in United States by 2030 [14]. Taken together, these data point demonstrated an alarming trend of rising global obesity rates.

In humans, the simple and surrogate marker for diagnosis and classification of overweight and obesity is Body Mass Index (BMI). BMI is defined as a person's weight in kilograms divided by the square of height in meters ($kg/m^2$). The categories of the range of BMI include normal BMI (defined as the range 18.5 to 24.9 $kg/m^2$), overweight BMI (BMI ≥ 25 $kg/m^2$), obese (BMI ≥ 30 $kg/m^2$), and severe obesity (BMI ≥ 40 $kg/m^2$), for both sexes in adults 18 years of age and older. The average BMI ranges in adults from 1975 to 2016 were 22.1 to 24.8 $kg/m^2$ in women and 21.7 to 24.5 $kg/m^2$ in men [15]. Although BMI guidelines are a useful tool for screening obesity, they have some limitations. For example, distinguishing lean muscle from fat mass or differences in fat distributions (visceral or subcutaneous) is not possible using BMI. Furthermore, individual body composition, which is a main factor to determining the risk of cardio-metabolic disease, varies with age, sex, and ethnicity, but BMI calculation is independent from these three factors [16]. Despite the low accuracy and sensitivity of BMI, it still remains a beneficial measurement for population health trends tracking obesity health risk and prevalence in epidemiological studies [6].

The etiology of obesity is highly complex. It can be related to the interaction between three groups of factors, including biological, environmental, and behavioral (Figure 1) [17].

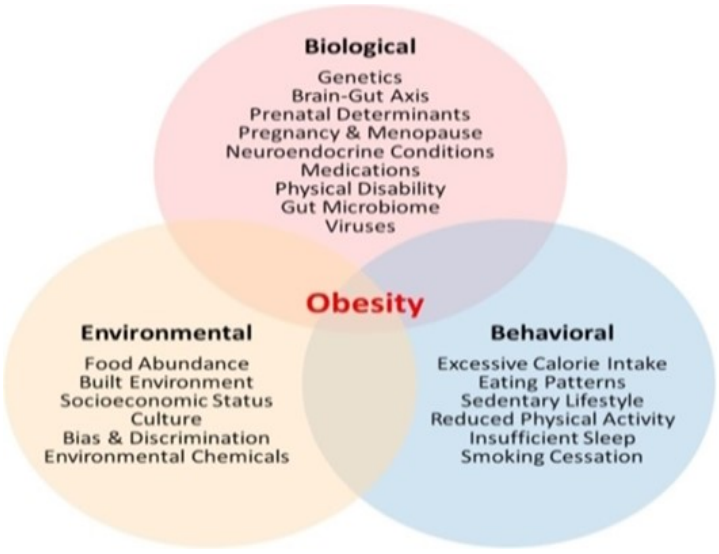



**Figure 1.** Multiple factors responsible for obesity. Copied from Hoda C. Kadouh et al., 2017 [17]

In the past few decades, the global genetic basis has not fundamentally changed, but eating behavior and sedentary lifestyle have become more popular than ever before in worldwide. Dietary habits rapidly changing over the past few decades may be a key factor leading to growing rates of obesity predisposition and changes to whole-body physiology [18]. In particular, "Western diet" is a nutritional pattern including increased consumption of high-calorie content, such as high-fat and cholesterol especially high levels of long-chain saturated fatty acids (LC-SFAs), which include myristate, palmitate, and stearate. Numerous costly diseases and disorders that can reduce quality of life and increase mortality are associated with obesity. According to the continued increases of obesity rates in many countries and its associated disorders, the development of new treatments for obesity seem imperative.

### 1.2. Relationship between obesity, metabolic and mood disorders

Over recent decades, mental health disorder prevalence is greater than 20% within the population and is considered a rising threat in global health [19, 20]. Among mental disorders, depression and anxiety are most common [21]. According to a WHO report in 2016, depression is highly prevalent in all age groups of people in the world [22]. More than 300 million individuals across the world have been diagnosed with depression, equivalent to 4.4% of the world's population [23]. Of note, women are twice as likely as men to experience depression [24]. According to the Global Burden of Disease Study (GBD), which includes the evaluation of 195 countries between 1990 -2017, depressive disorder prevalence has increased in many age groups and communities, and was one of the top three leading causes of disability in 2017 [25]. In Canada, depression is a fairly frequent disease with a 11.2% lifetime prevalence rate [26]. According to the Mental Health Commission of Canada report in 2013, the total number of at-risk individuals for developing depression was around 3.2 million Canadians in the age category of 12 to 19 years.

Depressive disorders are characterized by a set of psychological conditions such as tiredness and loss of interest or pleasure, feelings of guilt, worthlessness, cognitive or somatic disturbance, including changes in sleep or appetite that can affect a person's mood. The three

most common types of depression include: (1) major depressive disorder (MDD), which can be categorized as mild, moderate, and severe; (2) dysthymia (chronic form of mild depression); and (3) bipolar disorder [27].

Anxiety disorders are another common class of mood disorders which is marked by excessive fear or persistent worrying, often in response to specific objects or without acceptable reason [28]. There are several types of anxiety disorders such as generalized anxiety disorder, panic disorder, and social anxiety disorder, among others [29]. Anxiety disorders are considered the sixth leading cause of disability worldwide in terms of years lived with disability worldwide [30, 31]. The diagnosis of anxiety disorders are 1.5 to 2 times greater in women than men [32]. Furthermore, depression is the most common disorder comorbid with anxiety disorders among both sexes [33, 34].

The available data suggest a strong link between obesity and mood disorders, especially depression and anxiety in a bidirectional manner [5, 35-40], even from the early stages of life [41]. For example, in a systematic review of population-based samples by Fait et al, 80% of the studies reported a significant association of obesity-to-depression pathways and 53% for depression-to-obesity [42]. This suggests that weight gain and obesity may promote anxiety and depression, and that anxiety and depression may also frequently precede the development of obesity and eating disorders.

On the other hand, Metabolic syndrome (MetS) is defined as a combination of abdominal obesity and metabolic abnormalities, including raised fasting plasma glucose, elevated blood pressure, and altered lipid metabolism that presents a major risk factor for metabolic and CVD [43]. Both obesity and T2D as important criteria of syndrome metabolic are growing public health problems [12, 44]. According to a statistics Canada report from 2018, the prevalence of T2D was higher among obese Canadians (13.4%) compared to normal weight (2.9%). Many line of evidence illustrate obesity (increases in BMI and/or waist circumstances) are considered a key player in metabolic dysregulation and coronary disease [12, 44-47]. Furthermore, MetS was shown to be associated with an increased prevalence of depression [48], and a growing body of evidence supports the link between obesity and metabolic syndrome risk

factors with depression [49-51]. In this regard, it was demonstrated in two large nationally representative samples of United States adults that both obesity and MetS (independent of each other) showed significant associations with depressive symptoms, with both conditions present together having a synergistic association with depression [43, 52]. All of these results support the strong linkage between obesity and metabolic syndrome with depression.

### 1.3. Biological mechanisms influencing obesity, metabolic disorder and depression

Depression and obesity are multifactorial and complex, resulting from the interaction between biological, environmental, and behavioral factors [53]. These include genetics, chronic stress, unhealthy diet, and sedentary lifestyle which contribute to immune-metabolic dysregulations and increases in peripheral and central inflammation [53].

Genetic factors may contribute to the development of both depression and obesity [54, 55]. Transcriptomic data analysis showed that genes near BMI-associated loci are highly expressed in the hypothalamus and pituitary gland, which are key sites of central appetite regulation and energy homeostasis, as well as the hippocampus and limbic system, which are brain regions involved in memory, learning and mood regulation [56].

Dietary habits and the composition of dietary patterns are the most critical modifiable risk factor for obesity and mental health problems, a notion supported by a large body of evidence from several epidemiological studies [57, 58]. Unhealthy dietary patterns have been associated with more metabolic and psychiatric symptoms [59, 60]. A study on 1634 adults from 9-year follow-up assessment by Gibson-Smith et al., showed that subjects with depressive and anxiety disorders had the lowest diet quality score (poor diet) compared to healthy controls, suggesting a link between poorer diet quality and greater severity of mood disorder symptoms [61]. This evidence has supported the linkage between dietary habit and mood regulation.

In general, depression and obesity are associated with metabolic alterations, including increased activation of the hypothalamo-pituitary-adrenal (HPA) axis, hyperinsulinemia, hyperleptinemia, and systemic low-grade inflammation, which may be driven by alterations in

gut and adipose tissues (Figure 2) [53, 62]. More detailed discussions of each relative biological mechanism pathway and the role they play in obesity and depression are below.

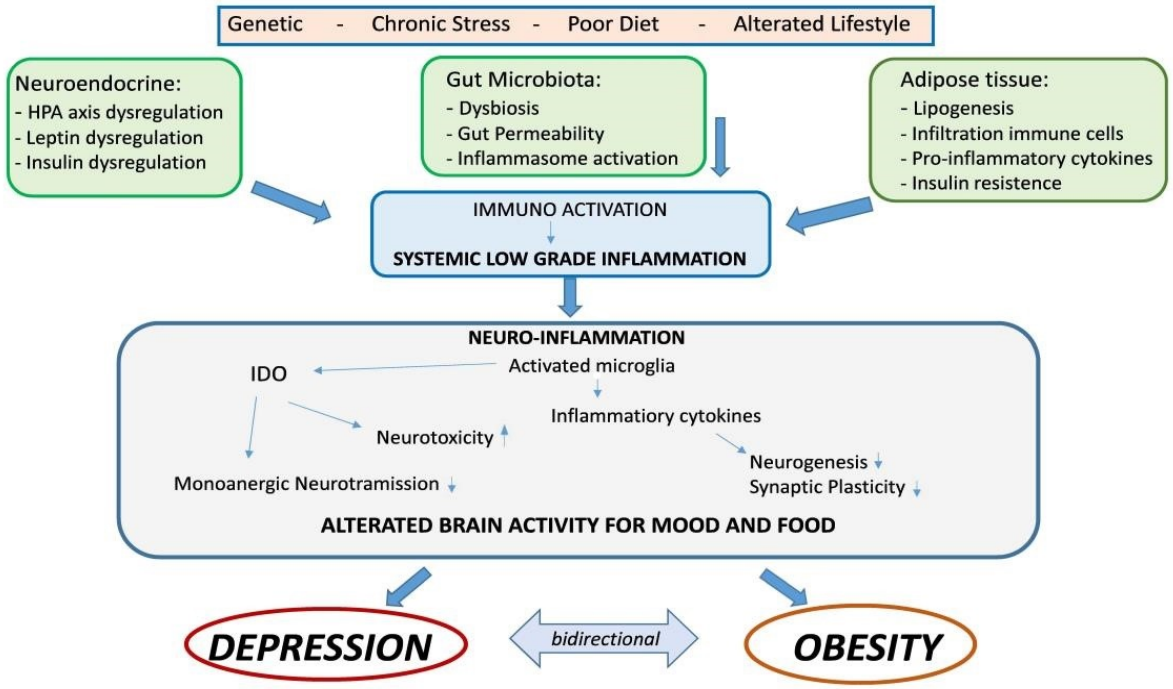



**Figure 2.** Biological mechanisms linking depression and obesity copied from Milano et al., 2020 [53]

### 1.3.1. Systemic/chronic low-grade inflammation

Low-grade chronic inflammation is characterized by elevated circulating levels of inflammatory cytokines, an increase in macrophage infiltration in peripheral tissues, as well as increased circulating levels of C-reactive protein (CRP) (3 mg/L - 10 mg/L) [63-65]. Low-grade systemic inflammation is a common and important feature that may be present in a wide range of chronic conditions including obesity, metabolic syndrome, diabetes mellitus, cardiovascular disease [64, 65] and depression/anxiety disorders [34, 66, 67].

The growing evidence of the associations between obesity, inflammation and depression is robust [68]. A recent UK Biobank large-scale study showed that the consumption of carbohydrate, fat, and saturated fat was significantly higher in people with MDD than in the

healthy control group, and additionally, that levels of dietary inflammation were significantly increased in people with MDD [66]. Furthermore, in many studies, both anxiety and depression present immune dysregulation, raised serum inflammatory marker levels and increased chronic inflammation [69-72].

Long-term HFD feeding is known to produce systemic, chronic inflammation in animals and humans [73, 74]. Obesity in individuals [75] and animal models of obesity [76, 77] are closely associated with increased inflammatory cytokines including interleukin (IL)-1β, tumor necrosis factor (TNF)-α, and interleukin (IL)-6 in different organs, such as liver, adipose tissue, skeletal muscle, pancreatic islets, and brain [78]. The CNS can be affected by peripheral inflammation through increased blood-brain barrier (BBB) permeability [53, 79], which leads to neuroinflammation, considered a hallmark feature of depression/anxiety disorders. Together, these findings suggest obesity-related inflammation may have important impacts on the CNS that could provide a mechanistic basis for obesity-associated cognitive impairments.

### 1.3.2. Adipose tissue and metabolic dysregulation

Obesity is characterized by an enlargement of white adipose tissue (WAT) [80]. White adipocytes not only are a main source of fat storage but are also considered an endocrine organ for their capacity to secrete adipokines [63]. Diet induced obesity (DIO) by accumulation of lipids in adipose tissue triggers an inflammatory response in adipocytes [81-83]. Also, HFD-induced obesity is associated with increased infiltration and accumulation of M1-like adipose tissue macrophages (the active forms of macrophages) [84], that results in an increased secretion of several inflammatory cytokines [85]. For this reason, WAT is considered as the key site of beginning low-grade systemic inflammation [80]. The elevated of pro-inflammatory cytokines from adipose tissue can lead to different metabolic dysregulation, including increased insulin resistance, which causes increased hepatic glucose production and reduced glucose uptake in muscle and adipose tissue, as well as increased β-cell dysfunction and the synthesis of acute phase proteins (e.g. CRP) from hepatocytes [81-83, 86]. The elevated circulating levels of CRP as a systemic inflammation marker has a strong association with obesity [87]. Consistently,

our previous study has shown that there is a significant increase in the plasma levels of CRP in HFD mice [88]. Similarly, plasma CRP levels are significantly elevated in patients with MDD, which suggests that this factor can serve as a pro-inflammatory biomarker in patients with MDD [89]. According to these results it seems that DIO is associated with adipocytes and macrophage activation to induce inflammation and altered regulation of endocrine hormones, which can lead to neuroinflammation, metabolic dysregulation and inflammatory related diseases.

### 1.3.3. Hypothalamic-Pituitary-Adrenal (HPA) axis dysfunction

There is steady communication between the immune and neuroendocrine systems [83]. One of the connecting mechanisms between depression and obesity is hyper-activation of the hypothalamic-pituitary-adrenal (HPA) axis [84-86]. HPA activation causes the release of glucocorticoids (e.g. cortisol) from the adrenal glands into the circulation to affect inflammatory, cardiovascular, metabolic and behavioral processes [87]. Thus, hyper activation of this axis leads to chronic increases in circulatory glucocorticoids (hypercortisolism) [90]. Numerous studies have demonstrated that pro-inflammatory cytokines, such as IL-1β, IL-6, TNF-α, and IFN-α can activate the HPA axis [91, 92]. In addition, DIO in mice aggravates HPA axis activation in response to immune threat, along with increased neuroinflammation, resulting in depressive-like behavior [91, 92]. In particular, in obesity and depression, chronic inflammation may reduce the sensitivity of the cortisol-binding receptor, which reduces the negative feedback response to cortisol, and thereby contributes to HPA axis dysfunction [93, 94].

Also, different studies have shown that long term exposure to high cortisol leads to neuronal damage and death in the limbic regions (hippocampus and amygdalae) associated with stress and mood [95-97]. Moreover, HPA axis dysfunction reduces the synthesis of neurotransmitters such as serotonin (5-hydroxytriptamine, 5-HT), and norepinephrine (NE) which play role to regulate anxiety and mood [98]. In addition, HPA axis dysfunction may also be linked with reduced brain-derived neurotrophic factor (BDNF) expression, which acts as a regulator of neurogenesis, neural apoptosis [99] and neuroplasticity [100] and contributes to mood regulation, particularly under inflammatory conditions [101]. HPA axis hyperactivity and

glucocorticoids have recently been shown to sensitize microglia to immune challenges and induce the overproduction of brain pro-inflammatory cytokines in animal models of obesity [92, 102], this phenomenon is also consistently observed in subjects with depressive disorders [103]. Taken together, these findings highlight DIO-related HPA axis hyper-activation may elevate microglial activity and neuroinflammation, as well as reduce synthesis of neurotransmitters associated with mood, which together may exacerbate mood dysregulation.

### 1.3.4. Gut-brain axis dysfunction in both obesity and depression

There is a complex bidirectional communication network between the brain and the intestine. In the last years, increasing evidence suggests that obesity involves alterations of the permeability of the intestinal barrier [104], intestinal microbiota strains composition and their metabolites (e.g. GLP-1) [105]. Preliminary studies in animal models show that gut microbiota plays a role in depression and anxiety [103, 104]. Diet has been shown to be one of the most critical factors modulating gut microbiota composition in the short and long term, and plays a key role in the central regulation of food intake and behavior in the brain [105]. Consistent with this, unhealthy diets have been shown to increase the susceptibility of depression via dysbiosis of the gut microbiota [106]. For instance, western diets that contain high amounts of SFA alter the balance of different microbiota species [107, 108]. This leads to elevated intestinal permeability, which in turn will result in increased passage of bacterial endotoxin molecules such as lipopolysaccharides (LPS) into the bloodstream [109]. Gut-derived LPS may initiate an inflammatory cascade in adipocytes and the liver which leads to enhanced peripheral inflammatory tone [110]. In this regard, studies in mice and healthy human individuals have demonstrated that HFD feeding for 4 weeks increased plasma LPS levels by three fold [111, 112]. Many lines of research have supported the important role of increased plasma levels of LPS in influencing systemic immunity in the pathophysiology of diet-induced obesity and metabolic disorders (e.g. T2D) in humans and mice, as well as in patients with anxiety and depressive symptoms [113-116].

In addition, the microbiome-gut–brain axis has been shown to modulate microglia proliferation and maturation. Also, gut microbiota dysbiosis via chronic activation of microglia in the CNS can result in neuroinflammation [117], which potentially contributes to the behavioral phenotype of high anxiety-like behavior in rats. In this context, Erny et al. demonstrated the role of gut microbiota in depression through modulation of microglial functions which may link microglia and associated neural circuitry as a potential factor in the onset of depression [118].

Overall, these results suggest that high fat diet impacts on gut microbiota strains and their metabolites may play a role in elevating the microglial activity and neuroinflammation in the brain with relevance to the onset of depression and mood disorders [117, 118]. However, further research is required to establish the mechanisms connecting high fat diets to gastrointestinal microbiota dysbiosis and resulting microglial activity, neuroinflammation induction and behavioral alterations.

## 2. Neuroinflammation, a central phenomenon in mood dysregulation

### 2.1. Neuroinflammation definition

Neuroinflammation is the set of inflammatory responses originating in CNS (the brain and spinal cord) that are essential in maintaining and restoring homeostasis [119, 120]. The activities of endothelial, neuronal, and glial cells within the neurovascular unit play an important role in the regulation of neuroinflammation via pro- and anti-inflammatory mechanisms [119]. Neuroinflammation is considered as a two-sided sword with a dual role including neuroprotective and neurotoxic activity. Neural inflammation in the short term is considered neuroprotective and may have beneficial effects. Whereas, chronic neuroinflammation shifts the brain microenvironment towards a pro-inflammatory state that can be neurotoxic [119, 121], and it may lead to behavioral alterations such as sickness behavior [122].

Neuroinflammation stands out as a crucial component of alterations of brain function and a hallmark feature of cognitive and emotional disorders including Alzheimer's disease [123] and

depression [124]. Many factors can initiate neuroinflammation in the CNS, such as the ageing process, direct cell injury, obesity, hypertension, depression, diabetes, tumors, infections, and drugs [125]. Among them, peripheral infection and trauma are considered as two major processes that initiate inflammation in the brain.

There are various immune components (cellular and molecular) that contribute to neuro-inflammatory processes. The pro-inflammatory mediators which are in the central nervous system are produced locally by either resident brain cells including endothelial cells, glia or neuronal cells [126]. Brain glial cells include astrocytes, microglia, and oligodendrocytes, which are the most abundant cell types in the CNS and play a crucial role in the brain's immune response to both systemic and central inflammation [127]. Microglia act as a first immune defense in the CNS, playing the most important role in the brain's innate immunity by acting as the primary cellular mediator of neuroinflammation [128]. Therefore, control of microglial function may be effective potential strategy to improve the neuroinflammation and its related diseases.

### 2.2. Diet-induced obesity augmented neuroinflammation and microglial activation

Obesity is linked to peripheral metabolic dysregulation [62] and induces peripheral inflammation that results in sustained production of inflammatory factors with adverse effects on neuronal function [123, 124, 126]. It appears that the type of the diet has an effect on obesity-induced neuro-inflammation. Based on numerous studies, high-fat diet, especially excessive consumption of SFAs, can induce inflammation in brain regions [129-134]. High fat diet increases circulating levels of SFA and elicits peripheral bacterial responses that can disrupt BBB integrity. However, LPS does not (or only to a limited extent) cross the BBB [79] , but it can activate TLR-4 on the surface of brain endothelial and neural cells (e.g. microglial and astrocyte) and up-regulate pro-inflammatory transcription factors, including NF-κB (nuclear factor κB) and AP-1 (activator protein-1), as well as increase the production of pro-inflammatory mediators (cytokines, chemokines, and reactive oxygen species) in the brain [126, 135, 136]. Furthermore, microglial activation and cytokine production can upregulate the infiltration of innate immune

cells, such as monocytes and macrophages, into the CNS and recruitment of adaptive immune components, such as B cells [137]. These collective factors can contribute to neuroinflammation and associated diseases.

### 2.3. Obesity, nucleus accumbens dysfunction and depression

The brain reward circuitry mediates feelings of reward or pleasure. The structural and functional changes in this are associated with reward-related behavioral impairments in motivated and mood disorders [138]. These include the limbic and, cortical networks, as well as structures including hypothalamus, amygdala, prefrontal cortex (PFC), thalamus, striatum, nucleus accumbens (NAc), ventral tegmental area (VTA), and hippocampus [74, 139, 140]. Our previous results found chronic HFD elicits neuroplastic changes in the VTA and striatum, and promotes depressive-like behavior in mice [141]. The VTA and NAc are involved in reward and saliency. There are major connections (dopaminergic, glutamatergic and GABAergic) among VTA and NAc in brain [138]. Furthermore, another study demonstrated that chronic overconsumption of SFAs (palm oil) moderates NAc dopamine signaling [142]. Several evidences highlight the NAc as a major component of the ventral striatum which plays critical functional connection with the VTA, amygdala and hippocampus to mediate all forms of adaptive and pathological motivated behavior [140, 143-145].

NAc activity is significantly enhanced in obesity-susceptible individuals [146]. Also, prolonged chronic inflammation by high-fat diet induced obesity elicited anxio-depressive behavior and NAc molecular adaptations that correlate with the extent of behavioral despair [142]. We previously demonstrate that SHFD increases peripheral inflammation, and multiple pro-inflammatory signs in the NAc, including reactive gliosis, increased expression of cytokines, NFκB transcriptional activity as well as, trigger anxiodepressive behavior [147]. Metabolic inflammation and NFκB-mediated neuroinflammatory responses in the NAc contribute to the expression of anxiodepressive behavior caused by a diet high in saturated fat and sugar [147]. Selective NAc IKKβ inhibition, prevented anxiodepressive behavior via downregulated the

expression of inflammatory markers [147]. In summary, these line of evidences highlight the major implications of the NAc in the response to stress [148] and regulation of DIO elicited anxio-depressive behavior [140, 144, 145]. We chose NAc to analyse the treatment ICV (Intracerebroventricular) injection on regulation of anxio-depressive behavior.

## 3. Microglia a central player in neuroinflammation

### 3.1. Overview of microglia origin

Microglia are immune cells in the brain that are located in both white and gray matter and comprise ~5–20% of brain glial cells [127, 149]. Microglia cells are of mesodermal origin, they are derived from primitive myeloid progenitor cells in the extra-embryonic yolk sac (YS) that is the first site of hematopoiesis. Migration and colonization into neuro epithelium of the brain and the conversion to microglia starts in early embryogenesis, around E9.5. Microglia expands and colonizes the whole CNS. From E13.5, BBB formation begins and the developing brain is protected from fetal liver hematopoiesis. In the developing brain during the embryonic developmental period microglial cells proliferate, migrate, and localize to their destination and differentiate into ramified microglia (Figure 3). Additionally, microglia are long-lived cells and under steady-state conditions, and are predominantly self-maintained without replenishment from hematopoietic progenitors from the embryonic period until adulthood [150-153]. Throughout adulthood, microglia play critical roles in the development and function of the brain [149].

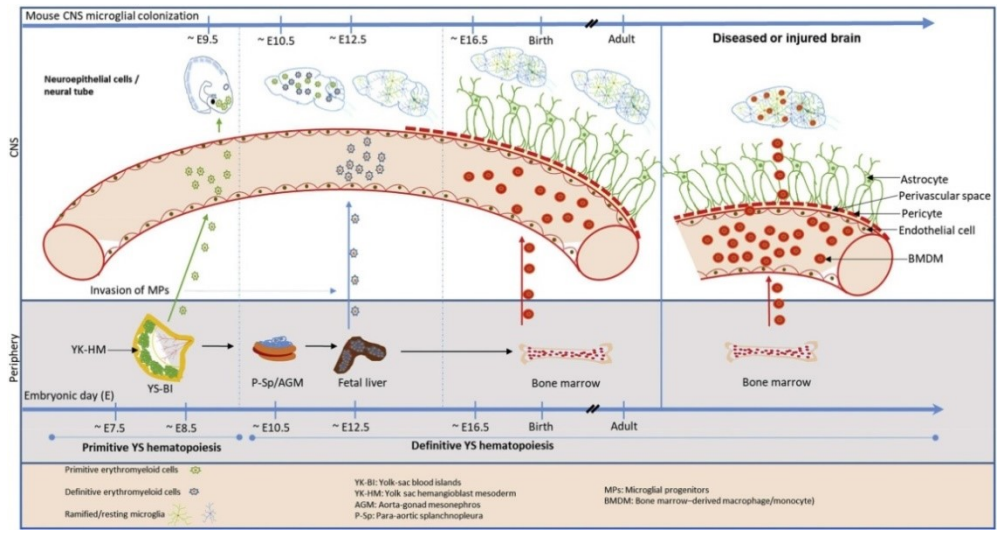



**Figure 3.** The diagram of microglial origin, CNS colonization of microglial progenitors and homeostasis during brain development in mouse, copied from Oluwaseun. F et al.,2020 [154].

In terms of in vitro cell selection, there are various microglia in vitro models that have been developed, including microglia cell lines, stem cell-derived microglia cultures, and primary dissociated cell cultures from mice, rats, macaques and humans [155, 156]. Each of these cell lines has some advantages and limitations. In the present study, we chose primary microglia cells from neonatal mouse brains (P1-P2) because primary microglia are easy to maintain, as well as their availability is greater in early age due to their unrestricted proliferative capacity. Therefore, these factors allow for better cell culture models of development to obtain a better understanding of microglia biology and post-mortem CNS tissue under more controlled conditions [155].

### 3.2. Microglia phenotypes (resting and activated forms)

Microglia consistently monitor the brain microenvironment and detect endogenous and exogenous stimuli which can activate microglia and stimulate the release of anti- or pro-inflammatory molecules [127]. Under physiological conditions, microglia usually are the first cell population involved in the regulation of CNS homeostasis through different pathways, such as phagocytosis of pathogens and cell debris, regulation of neurotransmitter recycling, neuronal

synaptic activity, and immune responses [157]. In the early stages of microglia activation, microglia help to remove pathogens and cell debris, thus facilitating tissue repair [158]. Microglial over-activation leads to de novo production of pro-inflammatory mediators, cytokines, and chemokines [109, 159].

To date, there are two main cell states to describe microglial polarization, which include: the resting and activated forms. Normally, resting microglia (M0) exists in a ramified state, which is characterized by long, ramified processes with comparatively small cell bodies [160]. Resting microglia mostly maintain a steady state of the brain's environment under a physiological state [151]. Microglia can be activated by various types of stimuli and dynamically alter their morphology and gene expression, allowing them to release a variety of inflammatory mediators to serve both pro-inflammatory and anti-inflammatory functions [161].

During the course of inflammation or injury, activated microglia and their phenotype can switch to two different molecular and morphological profiles that are classically described for Microglia: M1 and M2. Microglia cells when exposed to pro-inflammatory stimuli such as LPS, interferon gamma (IFNγ) and TNF-α produce inflammatory cytokines, such as IL-1α/β, IL-6, TNF-α, and IL-12, and chemokines, like monocyte chemoattractant protein-1 (MCP-1) and IFN- γ, resulting in production of active oxygen/nitrogen species (ROS/RNS), neurotoxic properties and express cell surface markers. These pro-inflammatory stimuli have been associated with morphological alterations from a ramified (M0) to an amoeboid or "M1" phenotype, which are characterized by a rounded morphology with no or few processes [160, 162-164]. This phenotype has strongly been associated with neuroinflammation, microglial apoptosis, and inhibited microglial phagocytic activity which can contributes to neuronal dysfunction, pathogenesis of neuroinflammatory and neurodegenerative diseases [165, 166].

In contrast, the M2 phenotype occurs to resolve inflammation and increase phagocytic activity which is essential for the clearance of senescent cells and debris elimination of the M1 phenotype in microglia [166, 167]. This anti-inflammatory phenotype is activated in response to anti-inflammatory stimuli such as IL-4, IL-13, Il-10 and transforming growth factor β (TGF-β)

results in expression of M2 polarized cells anti-inflammatory molecules such as Fizz-1, arginase-1(Arg-1), and Ym1 [164, 168, 169]. M2 phenotype is involved in resolution of inflammation and restoring of homeostasis [166]. All these morphological profiles have the potential to transform into each other according to their received stimuli [166]. The different microglial phenotypes and their activating factors and released molecules illustrated in Figure 4.

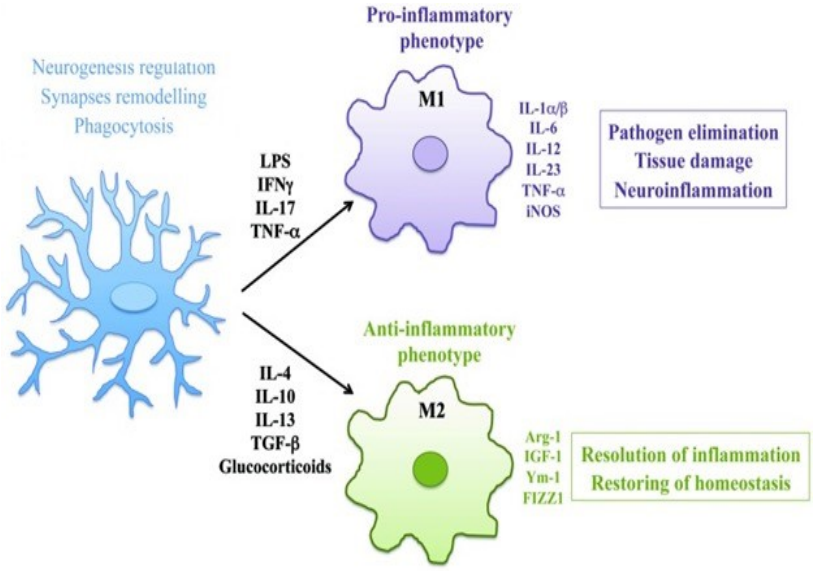



**Figure 4.** Different microglial phenotypes related to different functional states of microglia and their activating factors and released molecules, copied from Salvi et al.,2017 [166]

### 3.3. Microglia play active role in obesity and depression

There is increasing evidence to demonstrate the crucial role of microglial activation with neuroinflammation and depression [170]. Chronic HFD consumption in mice has been demonstrated to elicit obesity and increase microglial activation [171, 172]. Moreover, an in vitro study on microglial cultures (BV-2 immortalized cell) demonstrated that SFA (palmitic acid) activated microglia and initiated pro-inflammatory mediator production by stimulation of the TLR4/NF-κB pathway [173]. Sustained stimulation of microglia promotes the synthesis and

secretion of a large number of inflammatory factors which lead to the recruitment of peripheral myeloid cells to the neural tissue, the suppression of astrocytic functions, and promotion of neural death [162, 163]. HFD-fed mice demonstrated more TNF-α release from microglia in comparison to the control group [171]. Also, TNF-*α* stimulation increases microglial phagocytic activity [174]. Further, Camara et al. demonstrated that TNF-α antagonists (Etanercept) inhibited microglial TNF-α expression and reduced hippocampal microglia numbers and brain inflammation, in addition to decreasing depression- and anxiety-like behavior in C57BL/6 mice [175]. On the other hand, over expression of microglia in the anterior cingulate cortex, hippocampus and PFC associated with the onset of depression through neuroinflammation in patients with depression (postmortem studies) and in a rat model of depression [176, 177]. In accordance with this, minocycline, an inhibitor of microglia, was shown to reduce microglial number and activation in the prefrontal cortex, while reversing sickness and depressive-like behaviors [110, 178]. Conversely, pharmacological antidepressant treatment inhibited microglial TNF-α production in murine microglial cell lines [179]. It seems that control of microglial activity may be involved in the propagation of neuroinflammation as an essential factor in the etiology of depression [180]. The combinations of these results clearly suggest microglia targeted therapies for depression and regulation of microglia activation in a controlled manner could be of interest for preventive or therapeutic strategies in CNS inflammatory diseases. For instance, anti-inflammatory agents have been proposed for depression treatment [181].

### 3.4. Inflammatory mechanisms in microglia

Neuroinflammation in the CNS can be mediated by many of factors including exogenous pathogen-associated molecular patterns (PAMPs), and/or endogenous damage-associated molecular patterns (DAMPs), which are recognised by innate immune receptors expressed on the surface of microglia [182]. The other receptors responsible for pro/anti-inflammatory cytokine and chemokines responses are cytokine receptors, including IL-1R, TNFR, IL-10R, and TGFR [174, 183]. FFAs are involvement in the activation and production of inflammatory

cytokines includes action at pattern-recognition receptors (PRRs), such as toll-like receptors (TLRs), especially TLR4.

### 3.4.1. TLR4/Nuclear Factor-κB (NF-κB) signaling pathway

TLR4 is widely expressed in different organisms and CNS cells [184]. This receptor has been suggested to serve as a mediator of neuropathological conditions such as depression [125, 185]. Some evidence has shown that this receptor is predominantly expressed on microglia to control the induction of inflammatory signaling in the brain [185, 186]. Also, microglial cytokine production through TLR4-dependent signaling is regarded as a mediator of central immune signaling in LPS-induced sickness behavior [187]. Furthermore, the review on both in vitro and in vivo studies by Zhou et al., demonstrated that TLR4 is clearly implicated in SFA-induced inflammation [188]. The absence of TLR4 function protects against obesity induced by SHFD (palmitic acid and lard) in mice [189].

LPS is an endotoxin component derived from gram-negative bacterial cell walls, and serves as a marker of TLR4. In obesity, SFAs [128, 173] and LPS are able to increase microglial TLR4 activation followed by phosphorylation of MAPKs (JNK, ERK and p38) and p65-NF-κB which enhance inflammatory mediators contributing to the damage or death of neurons [128, 173, 190-194]. In this regard, Seong and colleagues demonstrated LPS-impaired adult hippocampal neurogenesis through promotion of the microglial cells TLR4-NF-κB signaling pathway following LPS injection in mice [195]. In this context, Alshamari et al. showed that anxiety-like behavior is associated with systemic inflammation being increasing (via increased TNF-α/TLR-4 /NF-κB/IL-17A signaling) in *db/db* mice [196]. Together, these results suggest that TLR4 and its downstream signaling pathway play one of the most important roles in DIO-induced peripheral and central inflammation and that their blockage might be considered as a therapeutic strategy for impeding the progression of neuroinflammation-mediated disease.

### 3.4.2. NLRP3 inflammasome signaling pathway

Nod-like receptor protein 3 (NLRP3) inflammasome is a cytoplasmic intracellular multiprotein complex that is mainly composed of three units: a receptor protein (NLRP3), an adaptor protein (ASC), and an effector protein (caspase 1) with crucial roles in innate and adaptive immunity. The NLRP3 inflammasome is considered the main regulator of the production of IL-1β pro-inflammatory cytokines, which are involved in many obesity-associated diseases, such as type 2 diabetes [197], atherosclerosis [198], and depression [199-201]. Also, activation of the NLRP3 inflammasome by diverse metabolic stimuli can cause overexpression of the IL-1β bioactive form as a potent pro-inflammatory cytokine that may impair metabolic and immune function in different cells [202].

A systematic review revealed that in obese individuals the expression of NLRP3 and IL-1β is elevated in the subcutaneous and visceral fat deposits [203]. In addition, gene expression of IL-1β, caspase-1 and NLRP3 are increased in adipose tissues of mice consuming a HFD enriched with palmitic acid [197] and obese individuals with a higher ratio of visceral fat [204]. Moreover, blockade of NLRP3 expression reduces adipose tissue inflammation [205].

Furthermore, evidence from studies suggests that NLRP3 dysfunction is a common feature of chronic inflammatory diseases, such as depression [201, 206, 207]. Supporting this notion, NLRP3 inflammasome activation is not only observed in animal model of depression [208], but also in depressive patients [209].

In the brain, LPS elicits mature IL-1β NLRP3-dependent levels [210]. Microglia is the main source of functional NLRP3 inflammasome formation and IL-1β secretion by LPS activation (in vivo and in vitro) between the glial cells of the mouse brain [211-213]. In a study of He et al, the NLRP3 inflammasome was shown to have a mediating role in microglia inflammatory processes [214]. In this regard, Butler et al, showed that short-term (3-day) consumption of a SHFD increased gene expression of microglial priming markers, including, CD11b, MHCII and NLRP3, as well as the pro-inflammatory marker IL-1β in both hippocampus and amygdala-derived microglia [215]. Microglial-specific activation of NLRP3 plays an important role in neuro-inflammatory conditions in the brain [212]. Also, Zhao et al. experiment reported that NLRP3-IL-1β-IL-1R1

signaling does not participate in the initiation of acute neuroinflammation, but rather the critical role of NLPR3-generated IL-1β is to gate the transition of acute to chronic peripheral LPS-elicited neuroinflammation [210]. Thus, according to these results, microglial-dependent inflammasome activation seems to have a role in the brain, especially in neuro-inflammatory conditions and in the context of mood disorders, however, more studies are needed to determine if this pathway is responsible for obesity induced neuroinflammation and microglial function.

## 4. Types of dietary fat and their effect on the obesity - mood axis

As the result of dietary habits alteration, the total fat content, SFAs composition, and the balance between n-3 and n-6 PUFAs has been disturbed. In particular the ratio of n-6 to n-3 PUFAs has increased to around 16:1 and even greater [59, 216] which can promote the pathogenesis of many diseases, including obesity, metabolic syndrome and mood disorder [61, 217]. Fatty acids (FAs) are simplest lipids that turn into other more complex lipid components, which are associated with various roles, including biological membrane structures by phospholipid synthesis, metabolic function by storage as triglycerides, degradation by β-oxidation, enzymatic activity modulation, and substrates for cytokine synthesis [218].

The effects of different FAs on innate immunity are quite complex depending on the number of carbons, degree of saturation, and location of the double bond in the hydrocarbon chain. Saturated fatty acids (SFAs) containing no double bonds in their carbon backbone, include lauric acid (C12:0), myristic acid (C14:0), palmitic acid (C16:0) and stearic (C18:0), and others [219]. In particular, “Western diet” is a nutritional pattern including increased consumption of high-calorie content, including high-fat and cholesterol, and especially high levels of long-chain saturated fatty acids (LC-SFAs), such as palm oil is enriched in palmitate [59, 216, 220].

In contrast, “Mediterranean diet” is characterized by high consumption of healthy components of fat like seafood (rich in n-3 fatty acids), olive oil (source of n-9, especially oleic acid), polyphenols, some vitamins, minerals and dietary fibers. The Mediterranean diet may help to

moderate the risk of chronic diseases like cardiovascular disease, metabolic disease, diabetes, and cancer [221, 222]. According to the recent cross-sectional study from the National Health and Nutrition Examination Survey (NHANES) 2009–2016 results, n-3 and n-6 PUFA intake was inversely associated with the risk of depressive symptoms, while a higher n-6/n-3 ratio was positively associated with the risk of depressive symptoms [223]. In addition, the finding from a meta-analysis of double-blind randomized placebo-controlled trials supports an overall beneficial effect of n-3 PUFA on the improvement of depression symptoms [224]. Moreover, our previous study demonstrated that FO supplementation, as a source of n-3 PUFAs, alleviates metabolic and mood disturbances associated with excess fat intake and obesity in male mice [88]. Together, these studies demonstrate that n-3 PUFAs have a protective effect against mood disturbances.

### 4.1. Unsaturated fatty acids (UFAs): Definition, biosynthesis and sources

A subgroup of fatty acids that is known for its health benefits are unsaturated fatty acids, which includes monounsaturated fatty acids (MUFAs) with one double bond, and PUFA with two or more double bonds. Examples of MUFAs include oleic acid (C18:1, n-9), with 18 carbon atoms as the major MUFAs characteristic of the Mediterranean diet and that is enriched in olive oil [222, 225]. PUFAs are biologically active fatty acids involved in many bodily functions. Linoleic acid (LA) and alpha-linolenic acid (ALA) with 18 carbon atoms are two essential dietary PUFA in humans because the body cannot synthesize them due to lack of enzymes able to introduce double bonds before the 9th carbon from the methyl end of a fatty acid, thus they must be obtained from the diet. These essential fatty acids are found predominantly in fish, seeds, nuts, and vegetable oils such as walnuts, flaxseed, canola oil, soybean, corn oil and rapeseed oil [226].

LA (C18:2 n-6) is considered a precursor of the long-chain n-6 PUFAs such as dihomo-γ-linolenic acid (DGLA: C20:3 n-6), arachidonic acid (AA: C20:4 n-6), and docosapentaenoic acid (DPA: C22:5 n-6). Indeed, AA as an n-6 fatty acid is the precursor for potent pro-inflammatory eicosanoids such as prostaglandins, leukotrienes, thromboxanes and lipoxin by cycloxygenase

(COX) and lipoxygenase (LOX) enzymes. Thus, high intake of n-6 may further promote oxidative stress, apoptosis, and neuro-inflammatory responses [227].

On the other hand, ALA (C18:3 n-3) is another essential fatty acid that can be converted to long-chain n-3 PUFAs (LC-PUFAs, ≥20 carbon atoms) such as eicosapentaenoic acid (EPA C20:5, and docosahexaenoic acid (DHAC22:6) [228]. DHA and EPA have anti-inflammatory roles in different cell types. In this regard, chronic dietary n-3 PUFA deficiency increases the production and release of IL-6 and TNF into the circulation [229]. Indeed, the derived compounds from DHA and EPA such as resolvins and protectins [230], have shown anti-inflammatory, anti-apoptotic, and antioxidant properties [231]. Thus, n-6 and resolvins and protectins associated with n-3 PUFAs can have opposing effects to modulate inflammatory processes.

Biosynthetic processing of long chain UFAs including DHA, EPA and AA from their main dietary precursors ALA and LA mainly occurs in the endoplasmic reticulum (ER) by elongation and desaturation, followed by *β*-oxidation in the peroxisome (Figure 5) [232, 233]. Since the same desaturation enzymes are used for both ALA and LA classes of fatty acids, there exists competition between them, resulting in competition for the synthesis of pro or anti-inflammatory eicosanoids. Thus, higher concentrations of EPA and DHA than AA tip the eicosanoid balance towards less inflammatory activity [234].

However, EPA and DHA can be formed from ALA in humans, but the biological conversion rate of ALA and LA into DHA, EPA and AA within organisms is low, slow and insufficient [235]. Thus, dietary consumption is gently recommended through main sources such as foods (e.g., fish) and/or dietary supplements (e.g., fish oil) [233, 236-238]. All fish, especially fatty fish such as salmon, sardine, and tuna, are considered excellent dietary sources of EPA and DHA. Also, several EPA and DHA dietary enrichment supplement formulations include oil derived from fish, krill, cod liver and algae [239]. Also, the fatty acid content of marine foods will be affected by factors like fish species, the geographical location and season of fishing, as well as the fish feeding sources and environment [240].

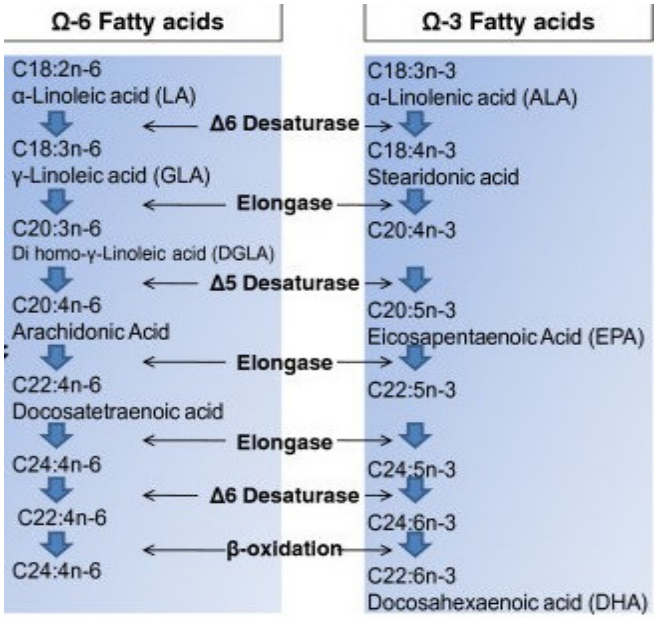



**Figure 5.** Unsaturated fatty acids and eicosanoids synthesis in mammals by enzymatic desaturase and elongase, modified from Boer et al., 2012 [241].

### 4.2. Beneficial effect of poly unsaturated fatty acids on anxiety and depression

According to the many systematic reviews and meta-analyses of observational studies, evidence points towards an inverse association between Mediterranean diet and odds of depression and anxiety disorders [242-246]. A 2016 meta-analysis of 26 studies found that higher fish intake was associated with a 17% lower risk of depression [247]. Also, amongst unsaturated fatty acids, n-3 PUFAs have been reported to be of relevance for the treatment of several mental disorders such as schizophrenia, hyperactivity disorder [248], mood disorders and anxiety disorders [249, 250]. For example, Su et al. revealed that a high dose of 6.6 g/day n-3 PUFAs (4.4 g EPA + 2.2 g DHA per day) was linked to a significant decrease on the score of the 21-item Hamilton Depression Rating Scale (HDRS) [251]. But in another, double-blind, randomized controlled clinical trial by Mischoulon et al, neither oral EPA nor DHA enriched n-3 fatty acids (1 mg/d) showed a superior anti-depressive effect over the placebo in MDD patients

[252]. These different observations may be related to the different consumption dosage between these two trial experiments.

However, the majority of studies support the theory that EPA alone or in combination with DHA may be superior in moderating depression symptoms in comparison to DHA alone or placebo [253, 254]. In this regard, two different studies using single supplementations of DHA didn't observe significant effects on depression and mood disorders, even with high doses of DHA [255, 256], while Nemet et al., reported patients undergoing antidepressant therapy treated with supplementation of single EPA (≤ 2 g/day) demonstrated significant improvement of depressive symptoms [257].

An interesting research report by Rapaport et al., showed that subjects with major depression and a high number of inflammatory biomarkers had a better response to EPA than placebo and a lower response to DHA than placebo [258]. Indeed, our previous study has shown that, FO (rich in EPA and DHA) supplement ameliorates the metabolic and anxiodepressive behavior concurrent with excess pro-inflammatory brain lipids and reduced brain gliosis in SHFD male mice [88]. Further, we could hypothesize that the regulatory effect of n-3 PUFAs on brain inflammation and its associated depression and mood disorders may depend on the more potent anti-inflammatory effects of this fatty acid on microglia function as a main player in the regulation of neuroinflammation and depression symptoms.

### 4.3 Mechanisms underlying the protective effect of n-3 fatty acids on inflammation

To date, the line of evidence has demonstrated that n-3 PUFAs contribute to an improved metabolic profile by various mechanisms in the different organs including adipose tissue, liver, and skeletal muscle [259]. In this regard, n-3 PUFAs have shown their potential in regulating adipocyte function by modulating lipid metabolism (lipolysis and lipogenesis); or by regulating energy expenditure, such as by impacting mitochondrial and peroxisomal fatty acid β-oxidation, and improving inflammation [260, 261].

Several studies confirmed that n-3 fatty acids exert protective effects to reduce inflammation by various mechanisms including activation of peroxisome proliferator-activated receptor gamma (PPARγ) [262], inhibition of TLR-4, initiation of anti-inflammatory signaling through GPR120 (n-3 receptor) [263], inhibition of Nf-kB activation [264], and enzymatic mechanisms, such as the synthesis of specialized pro-resolving lipid mediators (SPMs), including resolvins, protectins, and maresins with anti-inflammatory effects [265, 266]. In the following section, we will describe in details the anti-inflammatory signaling through GPR120 as important mechanisms involved in the control of inflammation via PUFAs.

## 5. Free Fatty Acid Receptors Family for long chain unsaturated fatty acids

### 5.1. Overview of the free fatty acid receptor (FFAR) family

There are different receptors in neural cells to sense and transduce FFAs signals, including Free Fatty Acid Receptors (FFARs), which are plasma membrane receptors belonging to the G-protein-coupled receptor (GPCR) family; the transcription factors Peroxisome Proliferator-Activated Receptors (PPARs), which mediate FAs regulatory functions in the nucleus cytosolic transport, and Fatty Acid-Binding Proteins (FABPs) [218]. Between them, the GPCR family is the most attractive target class for pharmaceuticals with around one third of therapeutic drugs that are used clinically on the market belonging to this family [267]. However, current drugs include just 10% of all GPCRs encoded in the human genome; but remain the most popular target for drug discovery [268]. GPCRs are the largest group of protein cell-surface receptors in humans and other mammalian species with a total of 1265 GPCRs in the human genome [269].

GPCRs are also known as seven transmembrane (7TM) domain receptors because seven membrane-spanning α-helices (TM) domains are a common structural part of all GPCRs, along with an extracellular N-terminus and intracellular C-terminus that are combined with variable extracellular and intracellular elements [267]. GPCRs are generally classified in six classes (A-F) according to their amino acid sequences and similarities in biological function. These classes are defined rhodopsin-like (class A), secretin-like (class B), metabotropic glutamate (class C),

pheromone (class D), cAMP (class E) and frizzled (class F) [270, 271]. Class A is considered a largest group of this receptor family, accounting for about 80% of GPCRs [272]. FFARs are a cell surface receptor family from class A that are activated by endogenous and dietary free-fatty acids [270]. FFAs act as a stimulator or ligand for these receptors depending on their chain length and degree of desaturation [273, 274]. FFARs are expressed in various types of tissues and cells, such as enteroendocrine cells, immune cells, adipocytes and pancreatic β-cells. They play a bio sensory role for different lipid groups and are divided in five subclasses based on the corresponding ligands carbon length (short, medium and long chain fatty acids) including GPR40, GPR41, GPR43, GPR84, and GPR120 [274, 275]. GPR41 (FFAR3) and GPR43 (FFAR2) are activated by short chain fatty acids (C2-C6), GPR84 is activated by medium chain fatty acids (C9-C14), and GPR40 (FFAR1) and GPR120 (FFAR4) are activated by medium and long chain fatty acids (C8- C22)[276].

Natural PUFAs are all dual agonists for GPR120 and GPR-40. GPR120 exhibits high affinity for natural ligands, including SFAs of C14, C16 and C18, with affinities around $EC_{50}$ = 30, 52 and 18 μM, respectively and UFAs (C16–C22) [277, 278]. Based on evidence from studies, GPR120 was demonstrated to be more responsive to n-3 PUFA stimuli in the obese state [279-282]. Nevertheless, it has a greater affinity for PUFAs, particularly n-3 PUFAs, including ALA, DHA, and EPA [277].

### 5.2. Long Chain free Fatty Acid receptors (GPR-40 and GPR120)

GPR120 (FFAR4) and GPR-40 (FFAR1) are categorized as two lipid sensing GPCRs that share similar endogenous ligands. There is limited homology (10% amino acid similarity) between these two receptors [283]. GPR120 and GPR40 gene expression and protein contents were detected in different tissues, such as adipose tissue [279], liver [284], hypothalamus [285], retina [282] and macrophages [279]. The presence of GPR40 and GPR120 has been observed in many cells and tissue types playing roles in metabolic and inflammation regulation [277]. In this regard, Oh et al. showed that GPR40 is weakly expressed in macrophages, while GPR120 is highly expressed in adipocytes and macrophages. Furthermore, Oh et al., and Dátilo et al. in

two different studies on macrophages and retinal cells in obese and type 2 diabetes mouse models demonstrated that GPR120, but not GPR40, can effectively respond to n-3 fatty acids [281, 282]. Also, Li et al. found that GPR120 has been expressed at much higher levels than GPR-40 in the mouse heart, and further that GPR120 activation via n-3 PUFAs demonstrated beneficial cardioprotective actions by mitigating vascular inflammation, thrombus formation, and neointimal hyperplasia [286].

However, there are not many experiments examining the expression of these receptors in central nervous system cells and their effect on the regulation of neuro-inflammatory responses. Our present study provides the first evidence of brain GPR120 expression, which is enriched in cultured human and mouse microglial cells isolated from fetal brains and pups brain respectively, in comparison to other glial cells (astrocytes and neurons) (Chapter2, Article 1). Additionally, our results demonstrated that the mRNA expression level for GPR40 is approximately four times lower compared to GPR120 in primary microglia. The relative gene expression of GPR120 and GPR40 is illustrated in Appendix 1. These results suggest GPR120 may have a more effective role in response to n-3 anti-inflammatory responses than GPR40 in innate immune cells.

### 5.2.1. GPR120 (G protein-coupled receptor-120)

GPR120, also known as free fatty acid receptor 4 (FFAR4), is a plasma seven-transmembrane protein receptor that was first described in 2003 by Fredriksson. The coding region of human GPR120 consists of four exons that are located on chromosome 10q23.33 and three exons for mice [287]. In humans, GPR120 is expressed in a multitude of tissues and cell types, with high expression in the small and large intestine, adipose tissue (both BAT and WAT), inflammatory macrophages, lungs, gastric mucosa, enteroendocrine L cells, endocrine-pancreas, Kupffer cells, and cells of the tongue [260, 288-290]. In the CNS, according to the Genotype-Tissue Expression (GTEx) Project and human protein atlas data report, higher mRNA expression levels of GPR120 are found in the cerebral cortex, hypothalamus, amygdala, basal ganglia, hippocampal formation and spinal cord. Up to now, two protein isoforms for the FFA4 gene have been

reported in humans; FFA4-L (long; 377 amino acids) and FFA4-S (short; 361 amino acids), with distinct signaling functions. Rat and mice have only the short isoform of FFA4 and the sequence homologies of FFA4-S in human with the rat and mouse protein is 85% and 86%, respectively [291]. FFA4-S functions through both Gαq/11 and β-arrestin downstream signal cascades, while FFA4-L functions only through downstream β-arrestin signaling [292, 293]. Thus, both isoforms can activate β-arrestin-2, and recruit the transforming growth factor β-activated kinase 1 (TAK1)-binding protein 1 (TAB1), which inhibits TAK1, leading to anti-inflammatory effects [294].

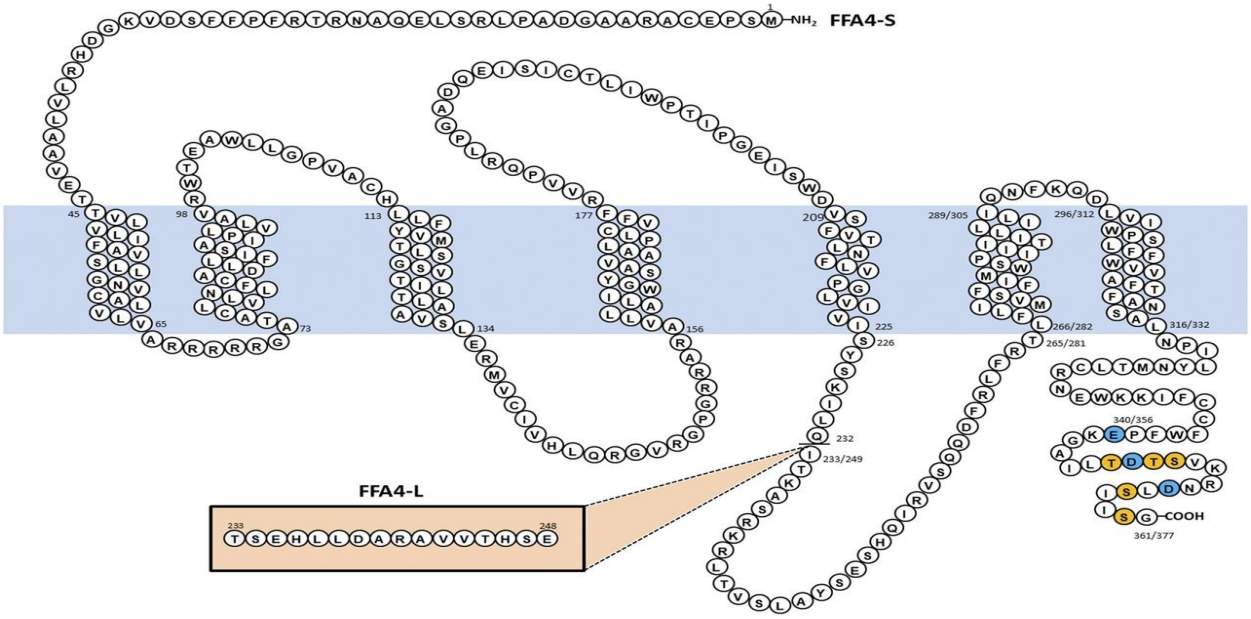



**Figure 6.** Amino acid diagram of GPR120 in humans. GPR120-S amino acid residues relative to initiating methionine. The insert shows the additional 16 amino acid residues to GPR120-L. Sites of the negative charge responsible for β-arrestin recruitment are shown in blue and sites of phosphorylation of FFA4-S are shown in orange. Copied from Senatorov et al. 2020 [295].

High expression of GPR120 in the adipose tissue, pro-inflammatory macrophages, and eosinophils, as well as in brain regions implicated in depression and mood regulation may indicate that GPR120 plays important roles in these tissues and cell types. In addition, a group of synthetic agonists for GPR120 was determined for potential therapeutic applications in

metabolic and inflammation-related diseases, such as diabetes, inflammatory bowel diseases, cancer, and obesity. Together, these results may suggest a regulative potential of this receptor for the improvement of obesity, metabolic diseases, inflammation, and mood disorders [296].

### 5.3. GPR120 biological and physiological functions

There are accumulating data to support the various beneficial physiological and biological functions of GPR120 in the different cells types involved in the regulation of energy balance [297], the maturation of adipocytes and lipid accumulation, the regulation of gut hormone secretion [298], the regulation of insulin sensitivity and glucose metabolism [299], anti-inflammation functions, [260, 300], and the regulation of neuronal functions [301]. Consistent with this context, both loss-of-function GPR120 gene variants (R270H) in humans and GPR120 KO in mice result in development of obesity, increased inflammation, and insulin resistance [297, 299].

#### 5.3.1. Functions of GPR-120 in insulin sensitization

GPR120 was identified as a possible anti-diabetic and anti-obesity target. It could promotes glucagon-like peptide (GLP-1) secretion from the colon and also, increase circulating insulin levels in the mice model study [283]. Consistent with previous study, insulin resistance was higher in GPR120-KO mice compared with WT mice with a high-fat diet. It could be due to reduced insulin signaling as well as, increased pro-inflammatory gene expression in adipose tissue [281, 297].

Oh and their collaborators in an interesting experiment in 2010 reported that the GPR-120 specific agonist (CpdA) and DHA improved systemic insulin sensitivity by enhancing muscle and liver insulin action in HFD-fed mice (60% fat) [299]. Further, GPR120 agonist treatment improved insulin resistance and inflammation in high-fat-fed obese mice, and so for these

reasons the authors suggested GPR120 as a promising anti-diabetes and anti-obesity target [299].

In obesity, SFA and/or LPS activated inflammatory signaling via TLR4 or by pro-inflammatory cytokine receptors (IL-1βand TNF-α) can initiate upregulation of the NF-κB/IKKβ and MAPK/JNK pathway cascades that promote further inflammatory mediator release by macrophages and reduce insulin sensitivity in adipocytes [302, 303]. Insulin promotes the IRS-1/PI3K/AKT pathway, which is linked to insulin sensitivity and inflammatory signaling pathways (IKKβ and JNK), which could finally reduce the translocation of GLUT4 to the plasma membrane and lead to insulin resistance and reduced glucose uptake [304]. In parallel, this was confirmed in the brain where Glombik et al. showed that when depression (prenatal stress) and obesity (high-fat diet) coexist in rats models, the membrane form of GLUT4 is diminished in the frontal cortex which, could cause weaker insulin action in this region [305]. Peripherally, GPR120-Gq/11 binding activation by n-3 fatty acids (DHA, EPA) induced the phosphorylation of the phosphoinositide 3-kinase PI3K/AKT pathways, which leads to the translocation of GLUT4 to the plasma membrane, thereby enhancing glucose intake in primary adipose tissue and adipocytes, leading to the regulation of adipogenesis and adipogenic differentiation [263, 297]. Furthermore, n-3 FAs suppress inflammation by reducing NLRP3 inflammasome activation via a GPR120-dependent pathway in macrophages, which has been shown to have beneficial effects in T2D [306, 307]. All of these results illustrate that GPR-120 plays a role in insulin sensitivity.

### 5.3.2. Functions of GPR-120 on energy homeostasis, appetite control, and food intake

Some experiments have elucidated that GPR120 controls appetite and food intake by affecting the regulation of gut hormone secretions, including gut hormones GLP-1 [283], glucagon [308], and ghrelin [298]. Also, Ichimura et al. in 2012, showed that GPR120 plays a key role in sensing dietary fat and controlling energy balance in both humans and mice. Compared to WT mice, GPR120-KO mice exposed to a high fat diet have reduced basal metabolism and also gained more body weight. Moreover, GPR120 expression in the adipose tissue of obese humans was

higher than in lean individuals [297]. GLP-1 as an appetite suppressant is a gastrointestinal-derived neuropeptide that is made primarily in gut cells and the caudal brainstem [309], and also is secreted from microglia [310]. GLP-1 has a potent, critical role is in body

weight homeostasis maintenance by acting in the VTA/NAc to reduce high-fat diet intake [311, 312] as well as important function regulating emotions, including anxiety and depression [313, 314]. GLP-1 has been reported to improve glucose-dependent insulin action through its receptor, belonging to the G-protein-coupled receptor group [315]. In this context, rat models of depression and obesity had decreased GLP-1 receptor expression in the hippocampus [305]. These findings suggest a potential link between GPR-120's regulatory function on energy homeostasis and mood disorders through impacts on GLP1, however, more experiments are needed to evaluate this possibility. Schilperoort et al. have reported that the GPR-120 agonist (TUG-891) induced brown adipose tissue (BAT) activity and increased lipid oxidation and combustion, therefore reducing body weight and fat mass in mice [260]. For this reason, it could be a contributing mechanism for weight loss, and targeting GPR120 may be a promising strategy to reduce obesity.

### 5.3.3. Functions of GPR120 on inflammatory responses

GPR120 is highly expressed in innate immune cells, especially in peripheral macrophages. There are accumulating data to support the anti-inflammatory role of GPR120 in various cells. Oh et al. demonstrated anti-inflammatory functions of GPR120 in the presence of ligands (DHA and EPA) in RAW264.7 macrophage cells [279]. Additionally, GPR120 activation by DHA has been demonstrated to mediate anti-inflammatory actions in a hypothalamic neuronal line [316].GPR120 has been implicated in inducing an anti-inflammatory phenotype (a shift to M2) via increased expression of IL-10, and arginase (Arg-1) [263]. Also, three different parallel studies by Han et al., illustrated DHA could promote the proliferative activity of macrophages in mice and RAW264.7 cells. In addition, DHA and EPA treatment could activate macrophages by

stimulating GPR120/MAPKs to initiate NF-κB/p65 (in vivo and in vitro) and immunomodulatory activity in macrophages in a dose-dependent manner [317-319].

Indeed, our previous study in 2016 demonstrated that the direct activation of GPR120 via its agonist by ICV injections in the hypothalamus substantially reduced food intake 4-hours post injection, and central injections of a GPR-120 agonist (TUG-891) suppressed the short term rewarding effects of high-fat/-sugar food in chow-fed mice, as well as reduced SHFD-induced anxiety- and depressive-like behavior in mice [301]. However, our knowledge of the role of GPR120 in the central nervous system remains poorly understood, but collectively, these findings indicate the possible involvement of microglial GPR120 activation in the modulation of metabolic anti-inflammatory responses by their agonists, such as long chain unsaturated fatty acids (LC-UFA), which may control behavioral functions. Therefore in our current research, we directly examine the effect of activation GPR120 via specific agonist (cpdA) administration by ICV injections in the NAc on LPS-induced sickness behavior and neuro-inflammation.

#### 5.3.3.1 GPR120-Mediated Anti-Inflammatory Mechanisms

##### 5.3.3.1.1. Inhibition of TLR4/Nuclear Factor-κB (NFκB) pathway

Activation of toll-like receptor 4 (TLR4) or TNF-α receptor (TNFR) via binding with LPS or TNF-α, respectively, causes interactions between the transformation of growth factor-β-activated kinase 1 (TAK1) and the transformation of growth factor-β-activated kinase 1 binding protein 1 (TAB1). Activation of TAK1 induces the phosphorylation of mitogen-activated protein kinase (MAPK) and induces JNK phosphorylation, as well as IKK-β and NF-κB/p65, which promotes the expression of various chemokines and cytokines, such as TNF-α, IL-6, IL-1β, MCP-1 and iNOS [302, 303]. On the other hand, the activation of GPR120 by DHA and EPA can lead to β-arrestin-2 enhancement and recruitment of cytosolic β-arrestin 2 to the cell membrane, which detaches the TAK1-TAB1 protein complex formation and blocks interaction with TAK1 (Figure 7). This results in blockade of downstream activation of NF-κB and JNK inflammatory cascades, leading to anti-inflammatory effects in macrophages [281, 282, 299]. Also, Ren et al. demonstrated the

anti-inflammatory effects of DHA correlated with GPR120 interactions with β-arrestin2 in an in vitro model of oxygen-glucose deprivation (OGD)-induced inflammatory responses in primary microglia and murine microglial BV2 cells [320]. Of note, oxygen-glucose deprivation (OGD) is considered the most commonly used in vitro model for ischemia. Furthermore, n-3 FAs suppressed inflammation by reducing NLRP3 inflammasome activation by GPR120- β-arrestin-2 in macrophages [306, 307].

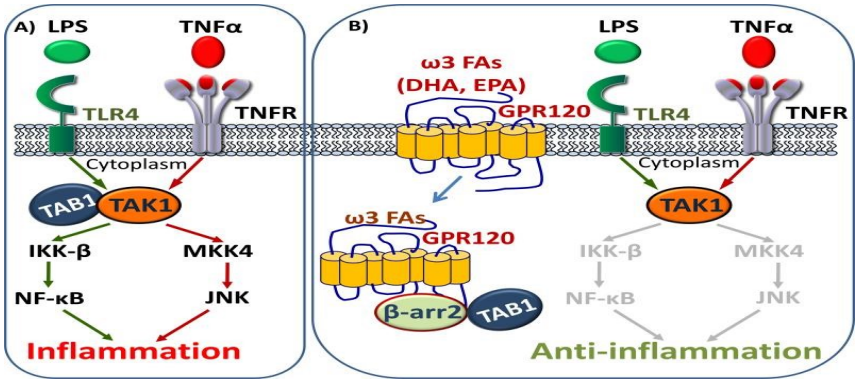



**Figure 7.** Mechanism of anti-inflammation upon GPR120 activation by n-3 fatty acids in macrophages, Copied from Talukdar et al,2011 [321]

#### 5.3.3.1.2. Inhibition of Nod-like receptor protein 3 (NLRP3) inflammasome

Recently, targeting the inflammasome has been taken into consideration as a novel therapeutic approach in the prevention of obesity-related inflammatory diseases, with the ability for microglial GPR120 to alter NLRP3 inflammasome activation [322]. DHA and EPA have been demonstrated to induce an inhibitory effect on the NLRP3 inflammasome activation in primary human and mouse macrophages and macrophage cell lines [306, 307, 323] particularly by reducing gene expression of NLRP3 inflammasome gene [306, 323, 324] and/or inhibiting NLPR3 inflammasome activation via GPR120 and GPR40 downstream scaffold protein β-arrestin-2 in macrophages [306, 307]. In addition, GPR120 activation via DHA was inhibited in NLRP3 inflammasome assembly/activity, NF-κB translocation, and IL-1β secretion in a manner dependent on both Gαq/11 and β-arrestin-2 signalling in macrophages [307, 324]. Lee and

colleagues in 2018, used human primary adipocytes and THP-1 macrophages treated with LC n-3 PUFAs to demonstrate a reduction of the NLRP3 inflammasome via EPA and DHA in human adipose tissue through downregulation of gene expression in adipocytes and monocytes/macrophages [323]. In the most recent experiment by Mo et al., (2020) GPR40 and GPR120 played essential roles in the inhibition of NLRP3 inflammasome activation induced by EPA in mice and BV2 microglia cells [322]. Based on these recent results, it seems GPR120 and GPR40 play crucial roles to regulate the NLRP3 inflammasome by n-3 fatty acid activation in macrophages and microglia [306, 307, 322, 323]. Furthermore, Lin Chao et al., in 2017 showed that traumatic brain injury (TBI) increases NLRP3 inflammasome activation and neuroinflammation levels in human brains [325]. In addition, they showed n-3 FAs markedly ameliorated neuronal death and behavioral deficits after traumatic brain injury in rats by inhibiting inflammation via the activation of β-arrestin-2 through direct binding of this downstream scaffold protein with NLRP3 in the n-3 treatment group [325]. Although we still know very little about the precise mechanisms in microglia, it seems that GPR120 via n-3 fatty acids may inhibit NLRP3 activation by directly binding to β-arrestin-2. However, more experiments are needed to better support this hypothesis.

# Chapter 2: BACKGROUND AND HYPOTHESIS

## 2.1. Background

This project is a part of an overarching effort aimed at proposing therapeutic alternatives based on nutritional (PUFAs) and pharmacological approaches targeting the GPR120 receptor to improve central neuroinflammatory outcomes and prevent the development of anxiodepressive behaviors associated with obesity. According to our previous study results (Appendix 3) [88], FO supplementation enriched in PUFAs improves energy homeostasis, glucose sensitivity, cerebral lipid composition, neuroinflammation and anxio-depressive behavior induced by DIO in mice. These findings support the beneficial effect of n-3 PUFA intake to alleviate metabolic dysregulation and mood disturbances associated with high fat intake and obesity.

In addition, long chain fatty acids such as n-3 are considered as potent activators of GPR120 receptors in different cells, and are involved in regulating different metabolic, pain, and inflammation responses. Previously, we reported that the activation of GPR120 in the brain substantially reduced SHFD-induced anxiety- and depressive-like behavior in mice [301]. Therefore, this receptor in brain cells, particularly in microglia as a critical cell type involved in neuroinflammation, can be activated by n-3 fatty acid in FO supplementation to reduce inflammatory tone in the brain, along with improved cognition and reduced sickness behavior related to induce neuroinflammation.

## 2.2. Hypothesis

### Global hypothesis

The negative impacts on metabolic and inflammatory parameters and anxio-depressive behaviors induced by neuroinflammation related to DIO or LPS administration are modulated by microglial GPR120 receptor activation.

**Specific hypotheses**

1- Activation of GPR120 by a synthetic agonist (cpdA) in primary microglial cells subjected to acute inflammation (LPS) in vitro will reduce the levels of pro-inflammatory cytokine in a manner similar to the effects of PUFA application.

2- Activation of GPR120 by a synthetic agonist (cpdA) in mice subjected to acute inflammation in vivo will reduce anxio-depressive behaviors and levels of pro-inflammatory markers in NAc.

**Objectives**

- To identify the effects of n-3 (DHA, EPA ,ALA) and n-9 (OA) fatty acids and GPR120 agonist application in primary microglial cells to alter neuroinflammatory processes elicited by acute LPS administration.

- To determine the impact of central GPR120 agonist treatment on the behavioral and neuroinflammatory effects caused by LPS administration in vivo.

**2.3. Contribution to the project**

As a part of this project, I completed several experiments that were conducted in vivo and in vitro. In the previous study (Appendix 3), we show that a prolonged saturated HFD elicits metabolic dysfunction and anxiodepressive behavior concurrent with excess pro-inflammatory brain lipids. Fish oil supplementation initiated following obesity development was found to improve certain negative metabolic and mood corollaries of a saturated HFD in conjunction with higher brain levels of anti-inflammatory fatty acids and reduced neuroglia activation. I contributed to mouse housekeeping and data collection of food intake and energy balance in DIO mice and associated statistical analyses.

In the microglial GPR120 study (inserted manuscript), we elucidated the role of microglial GPR120 stimulation in integrating anti-inflammatory signals in microglial cells and anxio-

depressive behavior. The experiments were conducted in collaboration with Arturo Israel Machuca parra, a postdoctoral fellow, and former MSc students Genevieve Demers and Diane Bairamian. In this study, I evaluated the protective role of the synthetic agonist of GPR120 on LPS induced inflammation in primary microglial cultures in vitro, as well as the effect of n-3 as a natural agonist for GPR120 in primary microglia cultures. The in vitro experiments involved the isolation of the microglial cells from pups, establishment of microglial cell cultures, treatments, and measurements of mRNA gene expression of inflammatory cytokines by qPCR, and measurements of cytokine secretion by ELISA, as well as completed the statistical analysis data (in vitro) which were performed completely by me. I also contributed to the writing of the presented manuscript as well as published manuscript. All in vivo experiments and behavioral tests were conducted by Genevieve Demers.

## Chapter 2: Unpublished manuscript

# GPR120 agonism protects against neuroinflammation-induced microglia activation and associated behavioral alterations

Geneviève Demers[1,2], Arturo Israel Machuca-Parra[1], Zahra Dashtehei Pour[1,2], Diane Bairamian[1,2], Khalil Bouyakdan[1], Demetra Rodaros[1], Cyril Laurent[1,4], Guillaume Ferreira[5], Nathalie Arbour[1,4], Thierry Alquier[1,3*], Stephanie Fulton[1,2*]

1- Centre de Recherche du Centre Hospitalier de l'Université de Montréal (CRCHUM), Departments of: 2-Nutrition, 3-Medicine, 4-Neuroscience, Université de Montréal, Montréal QC H3T1J4, Canada. 5- Nutrition and Integrative Neurobiology Unit, UMR 1286 INRA-Université de Bordeaux, Bordeaux, France.

* Equal contribution

**Corresponding author:**

Stephanie Fulton, PhD

Centre de Recherche du CHUM

900 rue Saint-Denis, R08-428

Montréal, QC H2X 0A9, Canada

Email: stephanie.fulton@umontreal.ca

**Acknowledgments**

This work was supported by research grants from Canadian Institutes of Health Research (SF and TA), the Société Francophone du Diabète (TA), Fonds de Recherche du Québec – Santé (FRQS), Cardiometabolic, Obesity and Diabetes research network (CMDO) and Merck Sharp Dohme Corp/Université de Montréal. GD was supported by a FRQS MSc scholarship and SF and TA hold FRQS salary awards.

**Conflict of interest**

The authors declare no conflict of interest.

## Abstract

GPR120 is a G protein-coupled receptor for long-chain fatty acids (LCFAs) including omega-3 polyunsaturated fatty acids (n-3 PUFA) that are known to have beneficial effects on inflammation, metabolism and mood. GPR120 partly mediates the anti-inflammatory and insulin-sensitizing effects of n-3 PUFA in peripheral tissues, findings that are generating considerable interest in GPR120 as a therapeutic target. Despite these results, the contribution of brain GPR120 to neuroimmune responses and associated behavioral changes is largely unknown. The aim of this study was to characterize the expression pattern of brain GPR120 and to investigate the impact of GPR120 agonism (Compound A, CpdA) on acute LPS-induced neuroinflammation in primary microglia and compare to the effects of n-3 PUFA and the monounsaturated fatty acid oleate. We also explored the influence of central *in vivo* GPR120 activation on anxiety and sickness-like behaviors elicited by LPS and on inflammation in the nucleus accumbens (NAc), a region involved in mood and motivation. We found that GPR120 is highly expressed in mouse primary microglia and microglia isolated by fluorescent-activated cell from CX3CR1$^{CreER\text{-}YFP}$ mice. In primary microglial cultures, GPR120 agonism attenuated morphological signs of microglia activation and substantially decreased mRNA expression of several LPS-induced pro-inflammatory mediators in a manner similar to or exceeding the effects of n-3 PUFA and Oleate. Intracerebroventricular administration of CpdA alleviated LPS-induced anxiety-like behavior and hypolocomotion and reduced cytokine mRNA expression in the NAc. Collectively, these findings suggest that GPR120 plays a key role in neuroimmune function by offsetting microglia-mediated inflammatory behavioral changes in response to systemic infection. GPR120 may well participate in the protective effects of n-3 PUFAs at the neural and behavioral levels and hold promise as a target for treating chronic neuroinflammatory conditions.

**Keywords**: Microglia, GPR120, free fatty acids, neuroinflammation, anxiety, sickness behavior

**Highlights**

- Brain GPR120 is predominantly expressed in mouse microglia
- Unsaturated fatty acids reduce LPS-induced pro-inflammatory responses in primary microglia
- GPR120 activation reduces LPS-induced pro-inflammatory responses in primary microglia
- Central GPR120 agonist treatment prevents LPS-induced anxiety and sickness-like behaviors

## Introduction

Neuroimmune responses ensuing from systemic inflammation are a fundamental mechanism underlying sickness-like behaviors, including psychomotor slowing and mood disturbances. As the intrinsic immune cells of the brain, microglia play a key role in the regulating neuroinflammation. Microglia activation can be triggered by a poor diet that includes excess consumption of saturated fat and sugars and ensuing metabolic inflammation associated with adipose expansion and/or gut-derived endotoxemia [1, 2]. Long-chain unsaturated fatty acids, particularly omega-3 polyunsaturated fatty acids (n-3 PUFA), are well described to have central actions that mitigate neuroinflammation, in part by suppressing microglial cytokines and chemokines[3]. Elevated n-3 PUFA consumption or central n-3 administration is well-documented to alleviate behavioral dysfunction associated with neuroinflammation, including anxiety-like behaviors [4]. N-3 PUFA dietary supplementation can protect against the pro-inflammatory effects of endotoxin-derived lipopolysaccharides (LPS), diet-induced obesity, neural injury and chronic stress both at the peripheral and central levels [5].

GPR120, also known as free fatty acid receptor 4 (FFAR4), is a G protein-coupled receptor preferentially activated by long-chain unsaturated fatty acids [6] such as the n-3 PUFA α-linolenic acid (ALA; 18:3n-3), eicosapentaenoic acid (EPA; 20:5n-3) and docosahexaenoic acid (DHA; 22:6n-3) [7-9]. GPR120 is expressed in the intestine and lung [10] among other peripheral tissues, and is particularly enriched in tissue-derived immune cells such as macrophages and dendritic cells [11]. GPR120 mRNA is also localized in several regions of the mouse brain, including in regions controlling motivation and mood such as the nucleus accumbens (NAc) [12]. GPR120 protein has been detected in neurons [13] and microglia [14] of hypothalamus, however the cellular expression profile of brain GPR120 remains uncertain due lack of antibody specificity. GPR120 activation has been shown to mediate the macrophage-mediated tissue inflammation and improve insulin resistance [15]. In addition, diet-induced obese mice receiving a GPR120 agonist exhibit reduced adipose tissue inflammation and improved glucose homeostasis [16], and thus GPR120 has garnered attention as a potential target for the treatment of metabolic disorders [17].

In view of implication of macrophage GPR120 in the resolution of systemic inflammation, we investigated the impact of GPR120 on acute LPS-induced neuroimmune activation in male mice. To this end, the present study set out to determine: (1) the cellular expression of GPR120 in mouse brain, (2) the effects of long-chain unsaturated FA, including n-3 PUFA and the monounsaturated FA oleate, and a GPR120 agonist to modulate the pro-inflammatory effects of LPS in primary microglial, and (3) the influence of central GPR120 agonism on LPS-induced anxiety- and sickness-like behaviors and neuroinflammation in the NAc.

## Methods

### *Animals*

All experiments were approved by the Institutional Animal Care Committee of the CRCHUM in accordance with the standards of the Canadian Council on Animal Care. Nine to ten-week-old C57Bl/6 male mice from Jackson Laboratories (Bar Harbor, Maine, USA) were used for gene expression and secretion measurements in culture and for *in vivo* experiments. Eight to ten-week-old CX3C chemokine receptor 1 (CX3CR1)-CRE-ER-YFP mice ($Cx3cr1^{CreER}$; stock 021160, Jackson Laboratories) were also used. $Cx3cr1^{CreER}$ mice express YFP in microglia and were used for fluorescence-activated cell sorting (FACS) of microglia (YFP+) and non-microglia (YFP-) neural cells. All animals were maintained in an environmentally controlled room (22–24ºC) with ad libitum access to standard chow and water. Mice were acclimatized to a reverse light/dark cycle for at least seven days prior to initiation of experiments.

### *Chemicals and reagents*

LPS from Escherichia coli (L-4516, serotype 0127:B8), ALA (L2376), EPA (E2011) DHA (D2534) and OA (O1008) were purchased from Sigma–Aldrich (St. Louis, MO, USA) and aliquoted for single freeze-thaw use. Compound A (CpdA) was purchased from Cayman Chemical (Ann Arbor, MI, USA). CpdA was selected for its high affinity and selectivity for GPR120 [18]. For cell culture,

LPS was dissolved in phosphate-buffered saline (PBS) and diluted to a final concentration of 100 ng/mL on testing days. DHA, EPA, ALA, OA and CpdA were dissolved in ethanol for stock solution at 100 mM and diluted in the culture media to a final concentration of 10 μM in 0.1% ethanol. For in vivo use, CpdA was dissolved in saline with 0.1% ethanol. Fatty acid (FA) doses were selected according to our previously studies [19, 20]. The CpdA dose selected for culture experiments was based on doses applied to cultured macrophages [16] whereas the dose applied *in vivo* was based on the report of Oh and colleagues and was adapted for ICV administration (peripheral vs ICV dose ratio: 10:1) [16, 21].

***Microglial Cell Culture and Treatment***

Primary cultures of microglia were derived from male and female wild type pups (P1-P2 postnatal day) (Supplemental Figure 1A). Briefly, after decapitation and meninges removal, whole brains were dissected and mechanically dissociated. Mixed glial cells were cultured in T75 flask and maintained in Dulbecco's modified Eagle's medium (DMEM) containing 25mM of glucose and supplemented with 10% heat-inactivated Fetal Bovine Serum (FBS) and 1% of antibiotics (Penicillin G (10,000U/ml)-Streptomycin Sulfate (10,000 μg/ml) at 37°C in 5% CO2. Media was replaced with fresh medium twice per week (every three days) to reach astrocyte confluence (10-14 days). Subsequently, microglia start growing on top of a single layer of astrocytes as small rounded cells. Primary microglia were finally harvested by transferring the supernatant (without shaking, centrifuging or enzymatic solution) and cultured in poly-$_L$-lysine coated 24-well plates for a maximum of five days prior to treatment. To evaluate the expression of GPR120 in different neural cell types in culture, primary astrocytes derived from the procedures above and an immortalized hypothalamic cell line (N46; Cellutions Biosystems, Toronto, ON, Canada) were used.

For FA and CpdA treatments, microglial cells were cultured in the DMEM without FBS and antibiotic for 24h as a starvation. Microglial cells were exposed to DHA, EPA, ALA, OA or CpdA (10μM) 1 h before adding LPS (100 ng/ml) for 6 hours. Between starvation, pre-treatment and treatment steps, cells were washed with 500 μl of DMEM without FBS and antibiotic (Appendix

2). As CpdA may rapidly react with albumin, serum-free medium was used in these steps. Supernatants were harvested for ELISA and cells were processed for RNA extraction.

***Microglial Fluorescence-Activated Cell Sorting***

As detailed by Legroux and colleagues [22], deeply anesthetized (Isoflurane, 5%) adult male Cx3cr1$^{CreER\text{-}YFP}$ mice were perfused with 50ml of saline 0.9% (w/v). Brains were collected in hibernate medium (Life technologies, Burlington, ON, Canada) and kept on ice. Organs from pooled mice (n=3) were finely minced with a scalpel followed by a 15 min digestion with a mixture of collagenase D (2mg/mL, Roche Diagnostic) and DNase I (14µg/mL, Roche Diagnostic) in a water-bath at 37°C. After homogenization and centrifugation by Percoll$^{TM}$ to remove myelin, microglial cells were isolated using a CD11b positive (remove no microglial brain cells) selection kit according to the instructions provided by the manufacturer (Stemcell Technologies, #18000). After, CD11b positive cells were incubated with blocking solution (0.02µg/mL of rat anti-mouse CD16/CD32, BD Bioscience) and FACS buffer was added (PBS+1%FBS+0.1% sodium azide) containing PECy7-conjugated antibody recognizing mouse CD45 prior to cell sorting (Microglial cells are CD45intCD11b+ and macrophages are CD45hiCD11b+). The gene encoding CreER for Cx3cr1$^{CreER}$ mice was followed by an IRES-YFP element [23]. This insertion, as already observed [24], revealed that YFP+ cells are microglia (Iba1+) (Supplementary Figure 1B). Data were acquired on an Arya III flow cytometer and data were analyzed using Diva software (BD Biosciences).

***Immunocytochemistry***

To evaluate microglial morphology changes induced by LPS treatment, we performed Iba-1 immunochemistry revealed by Vectastain Elite ABC HRP kit using DAB (3, 3'-diaminobenzidine) peroxidase (HRP) substrate (with nickel) purchased from Vector Laboratories (CA, USA). After treatment, microglia cell cultures were post-fixed in 4% paraformaldehyde. After blocking endogenous peroxidase (30 minutes in H2O2 0,3% + 0,3% normal goat serum (NGS)) and non-specific sites (30 minutes in 3% NGS + 0,1% Triton + 0,02% Azide in PBS 1X). Samples were

blocked and incubated with primary antibodies overnight (3% NGS + 0,1% Triton + 0,02% Azide in PBS 1X + Iba1:500 ; 1 ug/ml; Wako chemicals, Richmond, VA, USA) at 4°C followed by 2 h incubation at 22°C with a secondary biotinylated anti-rabbit IgG antibody (provided in the DAB kit: vector BA-1000 (rabbit). After the incubation with the peroxidase substrate solution and dehydration, coverslips were mounted and imaged with a Zeiss fluorescent microscope (Carl Zeiss AG, Jena, Germany) at a 20-fold magnification. Morphometric analysis of microglia images (Zeiss AxioImager.M2 ApoTome.2) were converted to 8-bit and measured using the analyze particles function of ImageJ. The total number of processes per cell and percentage of cells with processes was counted; in addition to the length of each process was measured (border of the soma to the end of the process).

**Quantitative PCR**

To examine inflammatory marker expression in the NAc, a brain region important for controlling locomotion and anxiodepressive behavior, microdissections from fresh brain sections derived from the mice used for behavioral testing were immediately frozen on dry ice before RNA extraction using TRIzol (Invitrogen). For cell culture, TRIzol was directly added into the wells (75-90% cell confluence). RNA concentration was quantified and 1000 ng of total RNA was reverse-transcribed by M-MuLV reverse transcriptase (Invitrogen) with random hexamers following the manufacturer's protocol. Quantitative gene expression was measured from 1:5 cDNA dilutions. RT-PCR were performed using the QuantiFast SYBR Green PCR kit (Qiagen, Valencia, CA, USA) according to the manufacturer's guidelines on a Corbett Rotor-Gene 6000. Quantitative real-time PCR for GPR120, glial fibrillary acid protein (GFAP, astrocyte marker), NeuN (neuronal marker), interleukin 1B (IL-1ß), interleukin 16 (IL6), tumor necrosis factor-alpha (TNF-α), monocyte chemoattractant protein-1 (MCP-1), ionized calcium binding adaptor molecule 1 (Iba-1, microglia marker), interleukin 10 (IL-10) and 18S (reference gene) was carried out using the primer sequences below. Each PCR reaction was performed in triplicate and relative gene expression was calculated using the ΔΔCT method using 18S as the housekeeping gene.

## Primer sequences

| Gene | Forward Primer (5'-3') | Reverse Primer (3'-5') |
|---|---|---|
| **18S** | TAG CCA GGT TCT GGC CAA CGG | AAG GCC CCA AAA GTG GCG CA |
| **GPR120** | TTT ACA GAT CAC GAA AGC ATC GC | GTG CGG AAG AGT CGG TAG TC |
| **Iba-1** | GGA TTT GCA GGG AGG AAA AG | TGG GAT CAT CGA GGA ATT G |
| **GFAP** | AAC GAC TAT CGC CAA CTG | CTC TTC CTG TTC CGC CAT TTG |
| **NeuN** | CCA GGC ACT GAG GCC AGC ACA CAG C | CTC CGT GGG GTC GGA AGG GTG G |
| **IL-1β** | GAC CCC AAA AGA TGA AGG GCT | ATG TGC TGC TGC GAG ATT TG |
| **IL6** | CAG AGT CCT TCA GAG AGA TAC | AGC TTA TCT GTT AGG AGA GC |
| **TNF-α** | CAC GCT CTT CTG TCT ACT G | AAG ATG ATC TGA GTC TGA GG |
| **MCP1** | ATT GGG ATC ATC TTG CTG GT | CCT GCT GTT CAC AGT TGC C |
| **IL-10** | CTG GCT CAG CAC TGC TAT GC | ACT GGG AAG TGG GTG CAG TT |

***Microglial culture ELISA***

After starvation and exposure to treatments, microglial cell culture media (75-90% confluency) was collected before the addition of TRIzol and immediately frozen. Murine TNF-α, IL-1β, IL6 and MCP-1 cytokines were measured using the antibodies and reference standards contained in R&D Systems (Minneapolis, MN, USA) enzyme-linked immunoabsorbent assay (ELISA) Duokits according to the manufacturer's protocol.

***Intracerebroventricular Cannula Implantation***

Animals were individually housed one week prior to ICV cannula implantation. Mice were anesthetized with Isoflurane (3% induction; 1–2% maintenance) and positioned in an Ultraprecise Mouse stereotaxic apparatus (Kopf Instruments). A single ICV cannula (guide cannula: C315GS-5-SP, 5 mm, 26 gauge, Plastics One) was implanted into the right cerebral ventricle using stereotaxic coordinates (+0.5 mm caudal and +1 mm lateral; -1.4 mm ventral from dura). The cannula was secured to the skull with cyanoacrylate glue and dental cement and closed with an adapted dust cap (Dummy cannula: C315DCS-5-SPC, 5 mm, Plastics One).

Correct positioning of the cannula was verified seven days after surgery by the drinking response elicited by injection of angiotensin II (20 ng/µL; Sigma). Behavioral testing and sacrifices were performed at the end of the light cycle and during the dark cycle, respectively. Mice were decapitated under Isoflurane anesthesia. Brains and blood samples were harvested and stored at -80$^{o}$C.

***Behavioral Tasks***

To assess the behavioral effects of central GPR120 agonism in the context of acute inflammation, mice were injected IP with LPS (0.83 mg/kg) after receiving daily intracerebroventricular (ICV) injections of CpdA (10 µg/day) or vehicle (DMSO) during three consecutive days. On day three, mice were euthanized 2h (gene expression studies) or 12h (behavior studies) after LPS injection. The LPS dose was chosen based on a report showing it is the minimal effective dose to induce anxiety and depressive-like behavior [25]. The CpdA dose was selected based on Oh et al. and was adapted for ICV administration (peripheral vs ICV dose ratio: 10:1) [16, 21]. Control mice received an IP injection of vehicle (endotoxin-free saline solution).

***Light/Dark Box***

The light/dark box (LDB) was used to assess anxiety-like behavior. The apparatus (Med Associates, Inc.) consisted of an illuminated compartment made of transparent plastic and a dark compartment made of black plastic, covered by a lid (both 13.7 cm X 13.7 cm X 20.3 cm). The two boxes were separated by a partition wall, with an opening at the bottom to allow the animal to pass freely between compartments. Number of entries and time spent in the lit compartment of the box were measured by an overhead video camera connected to a PC with Ethovision XT software (Med Associates, Inc.) for a period of five minutes.

### *Elevated-Plus Maze*

The elevated-plus maze (EPM) was used as a second test of anxiety-like behavior as previously reported [26]. In brief, each mouse was placed in the center of the maze facing an open arm opposing the experimenter. Distance travelled, velocity and percentage of time spent in open arms were measured by an overhead video camera connected to a PC with Ethovision XT software (Med Associates, Inc.) for a period of five minutes.

### *Statistical Analyses*

All data are expressed as mean ± SEM. Data were analyzed using GraphPad Prism 9 (San Diego, CA, USA). Between group comparisons were performed by ANOVA with Bonferroni or Sidak (Figure 4) posthoc tests. Criteria for statistical significance was set at $p \leq 0.05$.

## Results

### GPR120 is predominantly expressed in microglia

We first set out to determine the expression profile of GPR120 in different neural cell types using cultured cells. GPR120 mRNA was highly enriched in primary microglia derived from newborn mouse pups as compared to primary astrocytes and neurons (Figure 1A). We then investigated GPR120 cell type expression in adult mice using whole brain FACS-sorting of microglia and non-microglia cells isolated from Cx3cr1$^{CreER\text{-}YFP}$ mice. As shown is Figure 1B, we validated the cell sorting approach by verifying mRNA expression of a microglial marker (Iba1), but not astrocyte (GFAP) and neuronal (NeuN) markers, in the YFP+ cell fraction. In contrast, Iba-1 mRNA was almost absent in the YFP- fraction that expressed high levels of GFAP and NeuN. Corroborating findings in culture, we found that GPR120 expression is much higher in microglia (YFP+) as opposed to non-microglial cells isolated from adult Cx3cr1$^{CreER\text{-}YFP}$ mice (Figure 1B).

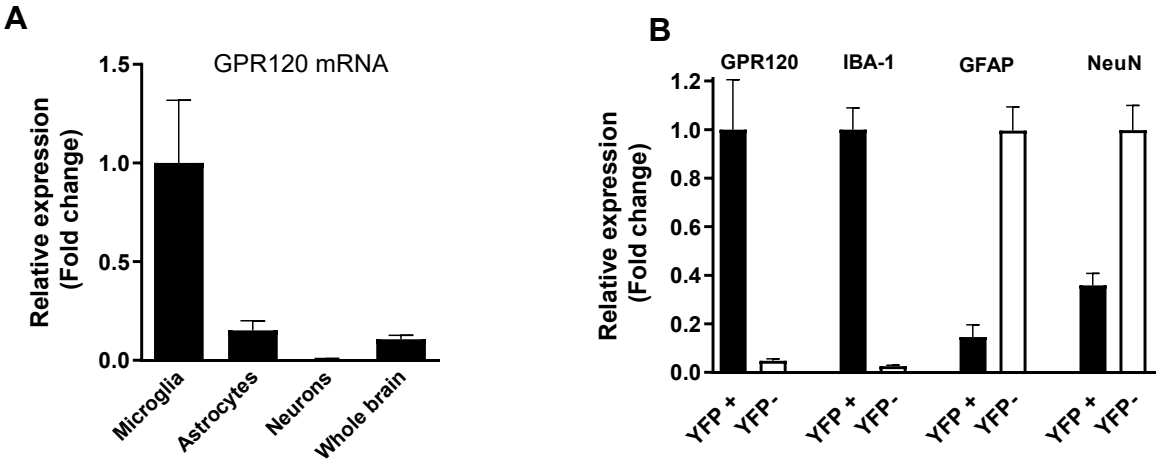



**Figure 1. GPR120 expression in neonatal and adult mouse brain.** (A) GPR120 RNA expression in primary microglia, primary astrocytes, immortalized hypothalamic neurons (N46) and whole brain by real time PCR. Fold change vs microglia (n=5-6/group). (B) Expression of GPR120 and cell-type markers for microglia (Iba1), astrocytes (GFAP) and neurons (NeuN) in FACS-sorted cells derived from adult Cx3cr1$^{CreER\text{-}YFP}$ mice. n=4-6/group. Mean ± SEM.

**GPR120 activation attenuates morphological signs of microglial activation**

We next set out to study the effects of GPR120 activation on morphological parameters of LPS-treated primary microglia. Using chromogen-based immunocytochemistry to stain microglial cells, we found that LPS decreased microglial process protrusions, a feature characteristic of active amoeboid microglia (Figure 2A). Accordingly, LPS-treated cultures had a lower percentage of cells with processes (Figure 2B) and a higher number of processes per cell (Figure 2C) indicating microglia reactivity. Pro-inflammatory morphological changes were attenuated by acute GPR120 activation (Figure 2A-C).

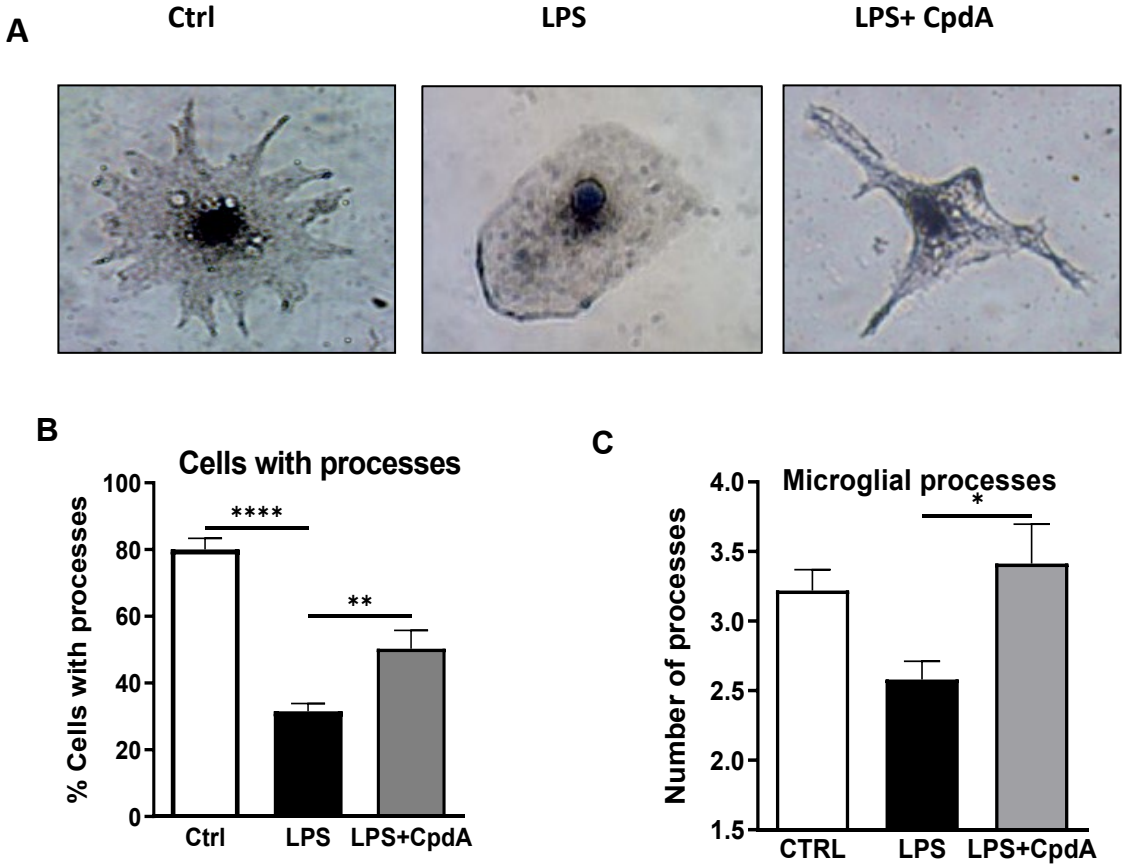



**Figure 2. Effects of acute GPR120 activation on LPS-induced changes in microglial morphology** (A) Image shows microglia in resting state (Ctrl), activated by acute LPS incubation and pre-treated with CpdA. (B) Percentage of cells with processes, (C) Number of processes per cell. Mean ± SEM; One-way ANOVA, Bonferroni post hoc; * p≤ 0.05, ** p≤ 0.01, *** p ≤ 0.001, **** p ≤ 0.0001vs Ctrl/ctrl vs Ctrl/LPS. N=7-9 per condition.

**GPR120 agonism by CpdA decreases LPS-induced microglial inflammatory markers**

To study the effect of GPR120 agonism on cytokine gene expression and protein release we treated primary cultures as above and collected cells and culture medium for analysis. Pre-treatment with the CpdA reduced LPS-induced mRNA expression and protein secretion of IL-1β, TNF-α and MCP-1 (Figures 3A-F). IL1B mRNA expression was also inhibited (Figure 3G) but not IL1B protein secretion (Figure 3H).

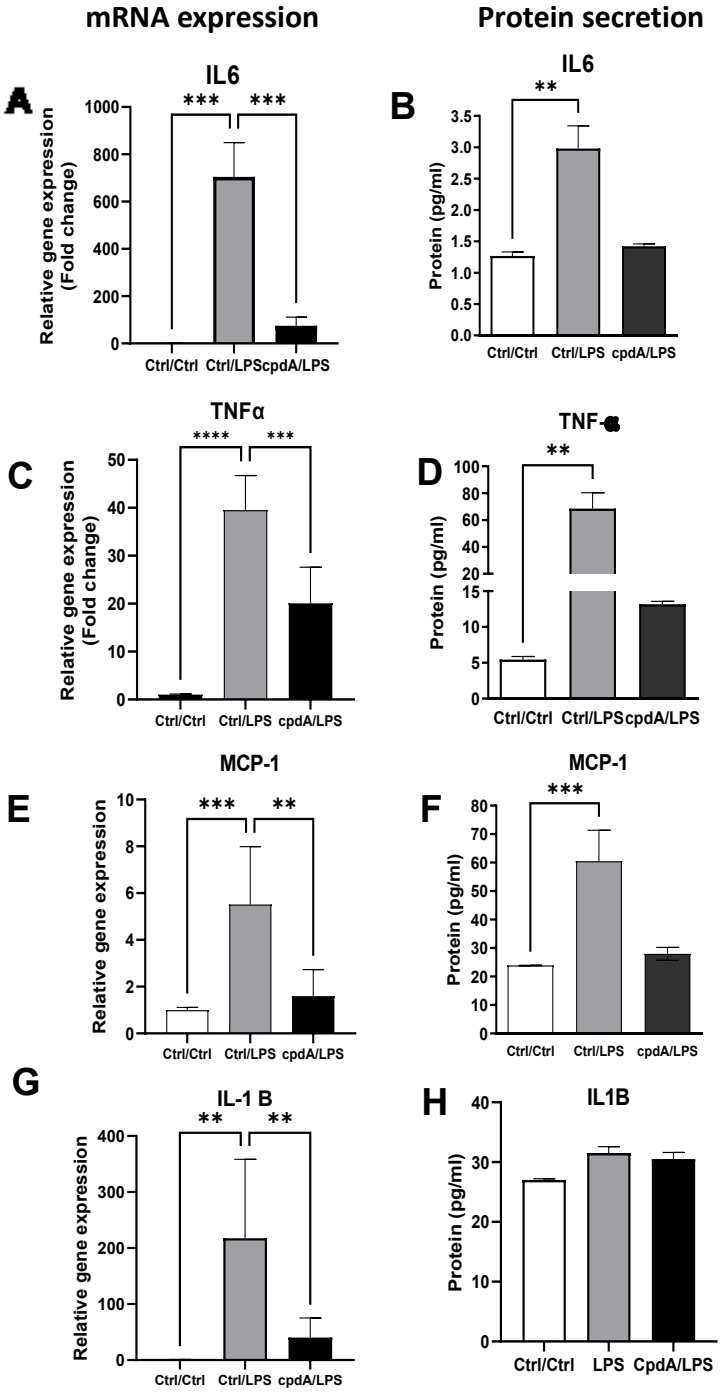



**Figure 3. GPR120 activation downregulates LPS-induced pro-inflammatory gene expression and cytokines secretion in cultured microglia.** Effect of GPR120 activation on LPS-induced gene expression (left) and protein secretion (right) for (A-B) IL6; (C-D) TNF-α; (E-F) MCP-1; (G-H) IL1B. Mean ± SEM; One-way ANOVA, Bonferroni post hoc; * p≤ 0.05, ** p≤ 0.01, *** p ≤ 0.001, **** p ≤ 0.0001. n=4-8 / condition

**GPR120 agonism by CpdA has effects that exceed or are similar to the effects of LCUFA to suppress LPS-induced microglial inflammatory markers**

We next studied the impact of GPR120 agonism and compared to the effects of long-chain unsaturated fatty acids (LCUFA). As above, cell cultures were pretreated with CpdA, DHA, EPA, ALA or OA (n-9) prior to LPS application. There was a significant reduction in LPS-induced gene expression for IL6, IL1B, MCP-1 and NFkB by LCUFA, and particularly n-3 PUFA and CpdA (Figure 4A, C, D, F). Notably, CpdA had greater effects on inflammatory marker expression than most LCUFA on these inflammatory markers, but never exceeded the attenuating effect of EPA. CpdA treatment produced a greater suppression of: IL-6 expression (Fig. 4A) relative to OA ($p<.0001$) and ALA ($p<.001$), TNFα expression (Fig. 4B) relative to OA ($p<.0001$), ALA ($p<.01$) and DHA ($p<.05$), MCP1 expression (Fig. 4C) relative to OA ($p<.0001$), ALA ($p<.01$), and DHA ($p<.0001$), IL1B expression (Fig. 4D) relative to OA ($p<.0001$) and ALA ($p<.001$), and NFkB expression (Fig. 4F) relative to OA ($p<.0001$) and ALA ($p<.001$). CpdA had effects similar to LCUFA to reduce Iba1 expression (Fig. 4F). Only DHA had substantially increased IL10 (Fig. 4E) and GPR120 (Fig. 4H) expression.

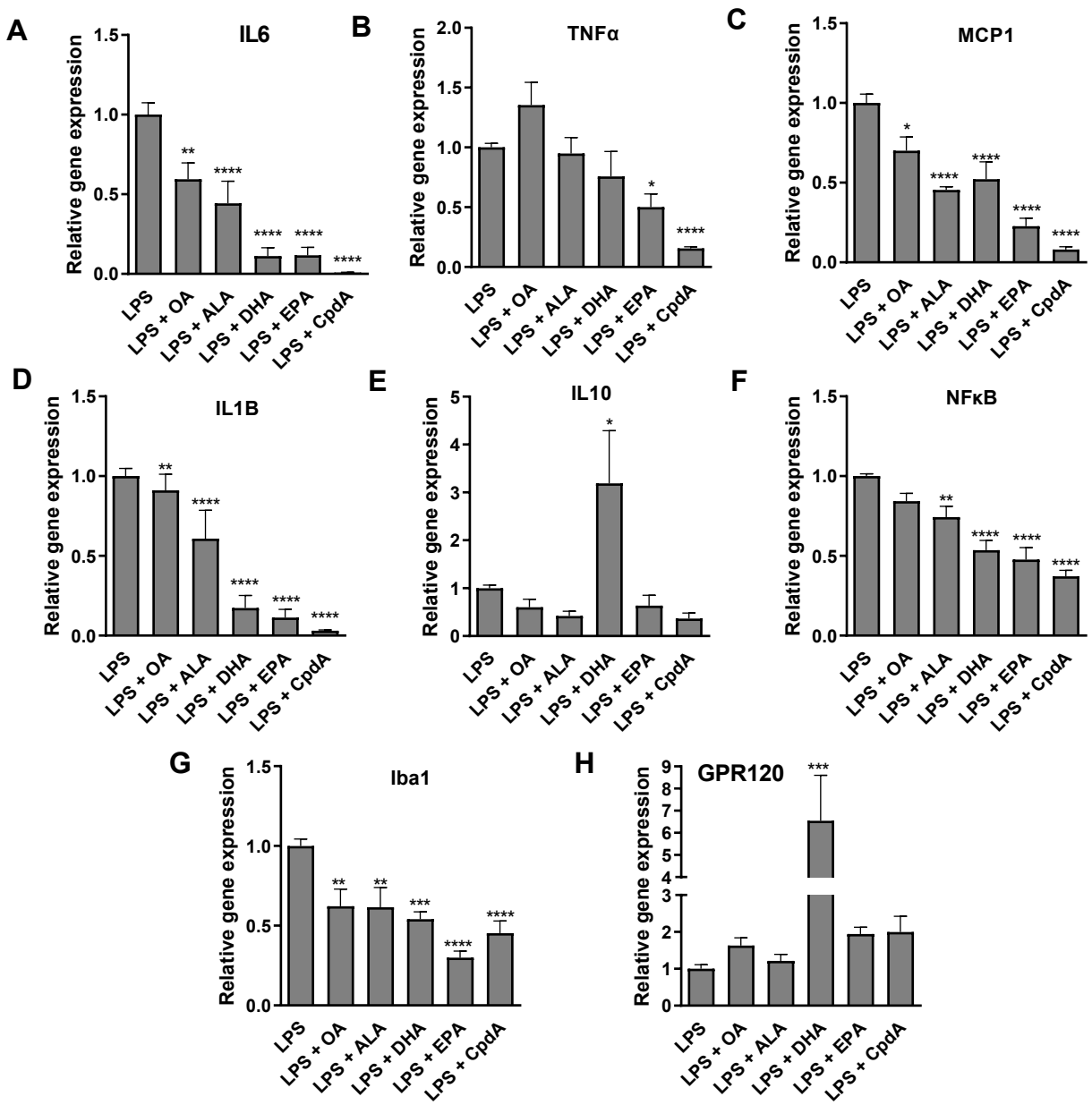



**Figure 4. GPR120 activation suppresses LPS-induced pro-inflammatory gene expression and cytokines secretion in cultured microglia.** (A) Effect of GPR-120 agonists CpdA, DHA, EPA, ALA and OA on LPS-induced gene expression of IL6 (A), TNFα (B), MCP-1 (C), IL-1β (D), IL-10 (E), Nf-kb (F), Iba-1 (G) and GPR-120 (H) in primary microglial cells derived from WT pups. Data are expressed as the mean ± SEM. *, P<0.05 versus LPS treatment in WT mice to OA+LPS or DHA+LPS or EPA+LPS or ALA+LPS or CpdA + LPS. Data is a representative image from four independent experiments. One way ANOVA with Bonferroni post hoc test**,** * p≤ 0.05, ** p≤ 0.01, *** p ≤ 0.001, **** p ≤ 0.0001, vs LPS. n= 6-8 / condition

**Central GPR120 activation inhibits NAc neuroinflammation and LPS-induced sickness behaviors**

We next evaluated if central administration of CpdA could blunt LPS-induced decreases in locomotion and anxiety-like behavior using two well-established tests. Three days of ICV CpdA pretreatment completely prevented systemic LPS-induced hypolocomotion (distance) (Figure 5A) and reduced locomotor velocity (Figure 5b); however, LPS and CpdA did not significantly alter the proportion of time spent in the open arms (Figure 5C). Mice demonstrated anxiety-like behavior in the light-dark box test following LPS injection as shown by reduced time spent (Figure 5D) and entries made (Figure 5E) in the lit compartment. Increased anxiety-like responses were abrogated by CpdA administration in the light-dark box suggesting that CpdA has anxiolytic actions (Figure 5D,E).

As a final step we measured two inflammatory markers in the NAc, a region in which we previously demonstrated neuroimmune activation elicited by diet-induce obesity and it role in anxiety-like behavior [27]. Systemic LPS administration significantly increased NAc mRNA expression of IL6 and IL1B 2-hour post-injection whereas three day CpdA pretreatment blocked this elevation: mice receiving CpdA resembled controls (Figure 5F, G).

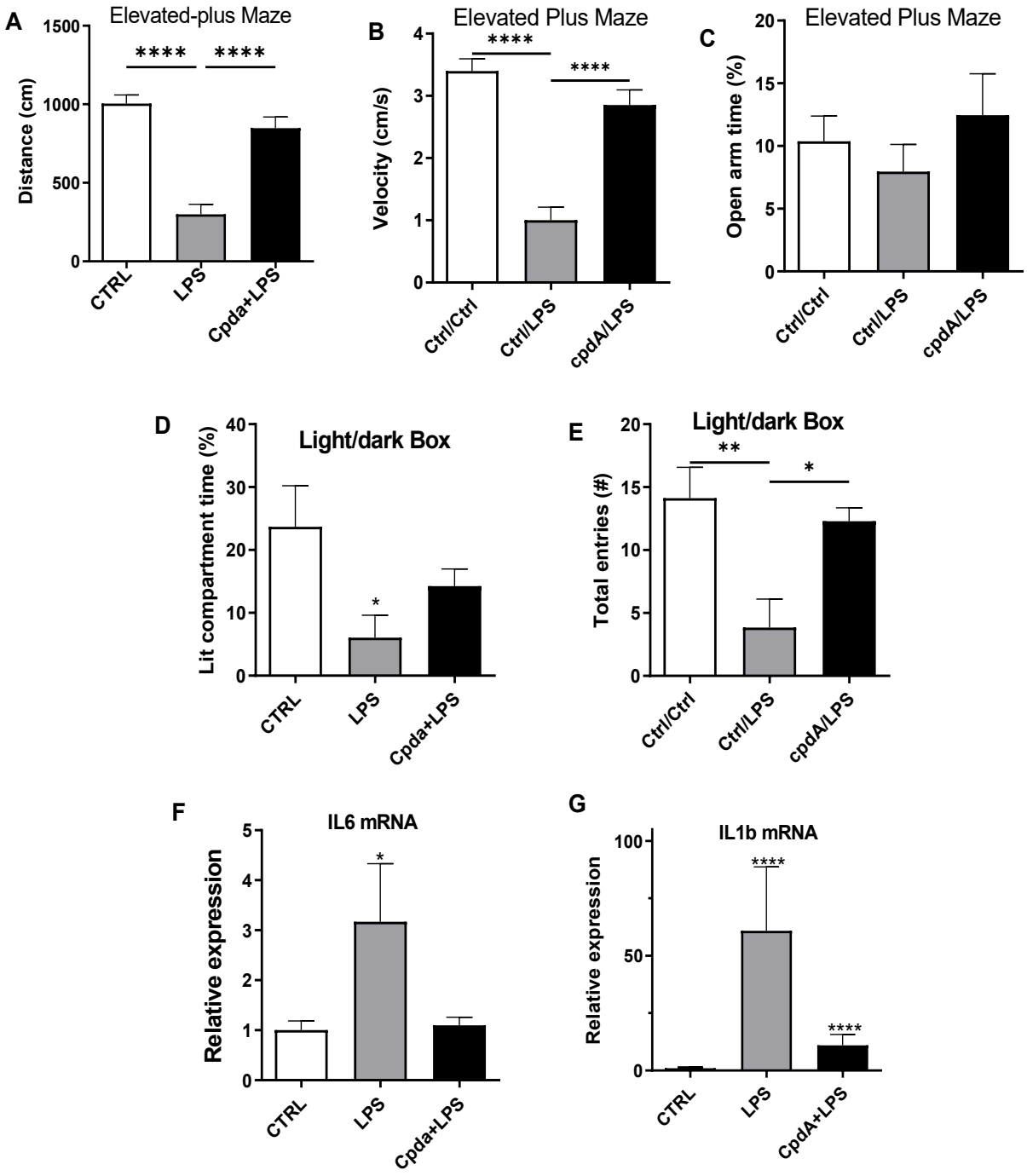



**Figure 5. Central GPR120 activation diminishes LPS-induced hypolocomotion, anxiety-like responses and NAc inflammation.** (A)Total distanced travelled, (B) locomotor velocity, and (C) open arm time in the elevated plus maze. (D) Light compartment time and (E) light compartment entries in the light-dark box (n=7-9/group). CpdA prevented LPS-induced increases in NAc mRNA expression of (F) IL6 and (G) IL1B (n=7-8/group). Mean ± SEM; One-way ANOVA, Tukey post hoc; * p≤ 0.05, ** p≤ 0.01, **** p ≤ 0.0001.

**Discussion**

The present study provides the first evidence that brain GPR120 is enriched in microglial cells and has a considerable impact on acute microglia-mediated inflammation. These results are consistent with Dragano et al. showing that GPR120 protein expression in hypothalamic microglia [28]. Further, we showed that activation of GPR120 by the specific agonist CpdA was effective in abrogating LPS-induced inflammation of primary microglia whereas central GPR120 activation suppressed anxiety- and sickness-like behaviors elicited by LPS. Together, these results suggest brain GPR120 could be a valuable target for the improvement of neuroinflammation associated with systemic infections and show promise for its use in the treatment of neuroinflammatory disorders.

Microglia morphology is highly associated with its function [29-31] and neuronal activity in the microenvironment [32, 33]. Here we established an association between GPR120 activation and a state of microglia surveillance. While LPS reduced the number of cell processes, leading to amoeboid shaped microglia, CpdA visibly blunted these characteristic microglia morphological changes. Our results are in agreement with previous findings demonstrating the activation of expression and release of pro-inflammatory cytokines following an LPS administration [34]. LPS-induced microglial IL-1β, IL-6, MCP-1 and TNF-α expression was reduced by GPR120 selective agonist treatment. We report here an absence of correlation between IL-1β expression and secretion levels. IL-1β does not follow the conventional ER-Golgi secretion mechanism, as opposed to IL-6 and TNF-α [35, 36]. We can hypothesize that our results are consistent with other teams who have observed an excursion of IL-1β secretion, and this, minutely 2 hours after administration of LPS in primary microglia [37].

DHA and EPA have been shown to inhibit inflammatory responses in macrophages [16]. In the present study we also, demonstrated that LCUFAs reduced LPS-induced inflammation in primary microglial cultures. EPA showed more potent to inhibit microglial activation and inflammation in compare with the other fatty acids ligands. In the current experience, OA decreases IL-1β, IL-6, and MCP-1 pro-inflammatory cytokine expression but it has shown much less anti-inflammatory potent than n-3. Both two presented in vitro experiments in primary

microglia culture suggest that the activation Gpr-120 may be a potent regulator for neuroinflammation.

Our study has shown that GPR120 activation by LCUFAs and CpdA is strongly decreased Iba-1 gene expression in microglial cultures. The anti-inflammatory effects of GPR-120 agonist may be accompanied by microglia deactivation [37]. EPA has been shown better regulatory effect than DHA on microglial activation caused by chronic unpredictable mild stress and neuroinflammation by down-regulating NF-KB expression in rats, which is consistent with our primary culture study [38]. The gene expression of GPR-120 and anti-inflammatory cytokine interleukin (IL-10) was strongly induced by DHA in cultured microglia. However the exact mechanism of activation in microglia is not clear but IL-10 was able to disrupt the expression of TNF-α, IL-1β and IL-6 in microglia by inducing anti-inflammatory elements, like Bcl3 (anti-apoptotic factor) and Socs3 to subsequently inhibit NF-κB-dependent signals [39].

These results support the idea that n-3 fatty acids may have a role to the reduction of neuroinflammation in the context of acute inflammation by the activation of microglial GPR120. This results not only in reducing the expression of inflammatory cytokines, but also in change of polarization of cells of microglia. Microglial morphological alteration and activation may have influence on brain function [40].

At the brain levels, neuroinflammation is associated with a myriad of complications including sickness and depressive-like behaviors [41, 42]. In animal studies, an activation of immune system through the administration of LPS as shown increased pro-inflammatory cytokines levels [43], These cytokines mediate a pattern of responses termed sickness behaviors that include weakness, hypolocomotion and increased anxiety and depressive-like behaviors [42, 44-48]. In agreement, we found that peripheral LPS administration promoted anxiety-like behaviors and induced the expression of IL6 and IL-1ß gene expression in the NAc [43]. The pro-inflammatory response to LPS in the NAc is similar to the effect of LPS reported in the prefrontal cortex and hippocampus [43, 49, 50]. Our data show that LPS significantly evoked characteristics of sickness behaviors as evidenced by a decrease in velocity and distance travelled during the EPM test as well as fewer entries and percentage of time spent in lit compartment of the LDB test.

Remarkably, ICV pre-treatment with CpdA significantly blunted LPS-induced anxiety and IL-1β expression in the NAc. There was a non-significant trend towards reduced IL-6 expression. These results suggest that GPR120 agonism restricted to the brain has beneficial effects on sickness behavior that may be dependent on its central anti-inflammatory effect. Therefore, these findings suggest that central GPR120 activation is sufficient to abrogate sickness behavior in the context of an acute whole body inflammation. Also, peripheral cytokines promoted by LPS injection can access the central nervous system and increase the production of local inflammatory mediators including IL-1β and TNF-α by endothelial cells, perivascular macrophages, microglia and astrocytes [51]. Moreover, increased IL-1β and TNF-α expression in the brain is one of the hallmarks of rodent depressive-like behavior enhanced by LPS [52-55]. Consistently, several investigations highlight the major implication of the NAc in the response to stress [56] and the regulation of anxiety and depressive-like behavior [57, 58] that are highly associated to neuroplasticity. We previously found that prolonged chronic inflammation by high-fat feeding resulting in diet-induced obesity elicits anxiodepressive behavior and NAc molecular adaptations that correlate with the extent of behavioral despair [59].

Immune-to-brain communication also occurs in humans by generating mood changes that are associated with modified activity in particular brain regions, following systemic inflammation or infection [60-62]. While these modifications are part of and homeostatic process, it is increasingly evident that inflammation has a detrimental effect that can promote numerous psychiatric and neurological conditions in human [63, 64]. Several studies provide evidence that communication from the site of inflammation to the brain is systemically generated via a response in the perivascular macrophages and microglia [46, 65, 66]. In rodents, depressive-like behaviors, secondary to an elevation of pro-inflammatory cytokines, develop over a background of sickness behavior, similar to those observed in depressed patients [42, 45, 47]. Importantly, our findings suggest that CpdA treatment can abolish LPS-induced locomotor impairments and anxiety-like traits. We argue that these protective effects of CpdA are due to its capacity to subdue neural inflammation via GPR120 activation in microglia, but cannot rule of that other cell populations expressing GPR120, such as astrocytes and neurons, could be involved given evidence of some expression in these cell types.

**Conclusion**

Taken together, this work demonstrates the enrichment of GPR120 in microglial cells and its capacity to moderate inflammatory responses. These findings identify GPR120 as a promising target for the prevention of microglia-mediated neuroinflammation. In a consistent manner, these results also suggest a role of central GPR120 agonism in the prevention of inflammation-related sickness behaviors. Collectively, this work implicates GPR120 targeting in moderating immune response following systemic inflammation and potentially offers an innovative therapeutic approach for neuroinflammation-associated diseases such as anxiety and depression. Further studies will be required to elucidate the role of GPR120 activation in the resolution of obesity and associated metabolic inflammation.

**References**


1. Kim, J.S., B.J.P. Claire, and behavior, *Diet, gut microbiota composition and feeding behavior.* 2018. 192: p. 177-181.
2. Erny, D., et al., *Host microbiota constantly control maturation and function of microglia in the CNS.* Nature Neuroscience, 2015. 18(7): p. 965-977.
3. Leyrolle, Q., S. Layé, and A. Nadjar, *Direct and indirect effects of lipids on microglia function.* Neurosci Lett, 2019. 708: p. 134348.
4. Larrieu, T. and S. Layé, *Food for Mood: Relevance of Nutritional Omega-3 Fatty Acids for Depression and Anxiety.* Front Physiol, 2018. 9: p. 1047.
5. Layé, S., et al., *Anti-Inflammatory Effects of Omega-3 Fatty Acids in the Brain: Physiological Mechanisms and Relevance to Pharmacology.* Pharmacol Rev, 2018. 70(1): p. 12-38.
6. Sassa, T. and A. Kihara, *Metabolism of very long-chain Fatty acids: genes and pathophysiology.* Biomolecules & therapeutics, 2014. 22(2): p. 83-92.
7. Davenport, A.P., et al., *International Union of Basic and Clinical Pharmacology. LXXXVIII. G protein-coupled receptor list: recommendations for new pairings with cognate ligands.* Pharmacol Rev, 2013. 65(3): p. 967-86.
8. Burns, R. and N. Moniri, *Agonism with the omega-3 fatty acids alpha-linolenic acid and docosahexaenoic acid mediates phosphorylation of both the short and long isoforms of the human GPR120 receptor.* Biochemical and biophysical research communications, 2010. 396: p. 1030-5.
9. Bjursell, M., et al., *The beneficial effects of n-3 polyunsaturated fatty acids on diet induced obesity and impaired glucose control do not require Gpr120.* PLoS One, 2014. 9(12): p. e114942.
10. Hirasawa, A., et al., *Free fatty acids regulate gut incretin glucagon-like peptide-1 secretion through GPR120.* Nat Med, 2005. 11(1): p. 90-4.
11. Oh, D.Y. and J.M. Olefsky, *Omega 3 fatty acids and GPR120.* Cell Metab, 2012. 15(5): p. 564-5.

12. Auguste, S., et al., *Central Agonism of GPR120 Acutely Inhibits Food Intake and Food Reward and Chronically Suppresses Anxiety-Like Behavior in Mice.* Int J Neuropsychopharmacol, 2016. 19(7).
13. Cintra, D.E., et al., *Unsaturated fatty acids revert diet-induced hypothalamic inflammation in obesity.* PLoS One, 2012. 7(1): p. e30571.
14. Dragano, N.R.V., et al., *Polyunsaturated fatty acid receptors, GPR40 and GPR120, are expressed in the hypothalamus and control energy homeostasis and inflammation.* J Neuroinflammation, 2017. 14(1): p. 91.
15. Walenta, E., et al., *A Gpr120-selective agonist improves insulin resistance and chronic inflammation in obese mice.* Nature medicine, 2014. 20(8): p. 942-947.
16. Oh, D.Y., et al., *GPR120 is an omega-3 fatty acid receptor mediating potent anti-inflammatory and insulin-sensitizing effects.* Cell, 2010. 142(5): p. 687-698.
17. Oh, D.Y., et al., *A Gpr120-selective agonist improves insulin resistance and chronic inflammation in obese mice.* Nat Med, 2014. 20(8): p. 942-7.
18. Oh, D.Y., et al., *GPR120 is an omega-3 fatty acid receptor mediating potent anti-inflammatory and insulin-sensitizing effects.* Cell, 2010. 142(5): p. 687-98.
19. Taïb, B., et al., *Glucose regulates hypothalamic long-chain fatty acid metabolism via AMP-activated kinase (AMPK) in neurons and astrocytes.* J Biol Chem, 2013. 288(52): p. 37216-29.
20. Bouyakdan, K., et al., *A novel role for central ACBP/DBI as a regulator of long-chain fatty acid metabolism in astrocytes.* J Neurochem, 2015. 133(2): p. 253-65.
21. Oh, D.Y., et al., *A Gpr120-selective agonist improves insulin resistance and chronic inflammation in obese mice.* Nature medicine, 2014. 20(8): p. 942-947.
22. Legroux, L., et al., *An optimized method to process mouse CNS to simultaneously analyze neural cells and leukocytes by flow cytometry.* J Neurosci Methods, 2015. 247: p. 23-31.
23. Jung, S., et al., *Analysis of fractalkine receptor CX(3)CR1 function by targeted deletion and green fluorescent protein reporter gene insertion.* Mol Cell Biol, 2000. 20(11): p. 4106-14.

24. Parkhurst, C.N., et al., *Microglia promote learning-dependent synapse formation through brain-derived neurotrophic factor.* Cell, 2013. 155(7): p. 1596-609.
25. Martin, S.A., et al., *Voluntary wheel running does not affect lipopolysaccharide-induced depressive-like behavior in young adult and aged mice.* Neuroimmunomodulation, 2014. 21(1): p. 52-63.
26. Sharma, S. and S. Fulton, *Diet-induced obesity promotes depressive-like behaviour that is associated with neural adaptations in brain reward circuitry.* Int J Obes (Lond), 2013. 37(3): p. 382-9.
27. Décarie-Spain, L., et al., *Nucleus accumbens inflammation mediates anxiodepressive behavior and compulsive sucrose seeking elicited by saturated dietary fat.* Mol Metab, 2018. 10: p. 1-13.
28. Dragano, N.R.V., et al., *Polyunsaturated fatty acid receptors, GPR40 and GPR120, are expressed in the hypothalamus and control energy homeostasis and inflammation.* Journal of Neuroinflammation, 2017. 14(1): p. 91.
29. Suzumura, A., T. Marunouchi, and H. Yamamoto, *Morphological transformation of microglia in vitro.* Brain Research, 1991. 545(1): p. 301-306.
30. Stence, N., M.L. Waite, and M.E. Dailey, *Dynamics of microglial activation: a confocal time-lapse analysis in hippocampal slices.* Glia, 2001. 33 3: p. 256-66.
31. Szabo, M. and K. Gulya, *Development of the microglial phenotype in culture.* Neuroscience, 2013. 241: p. 280-95.
32. Chamak, B. and M. Mallat, *Fibronectin and laminin regulate thein vitro differentiation of microglial cells.* Neuroscience, 1991. 45(3): p. 513-527.
33. Tam, W.Y. and C.H.E. Ma, *Bipolar/rod-shaped microglia are proliferating microglia with distinct M1/M2 phenotypes.* Scientific reports, 2014. 4: p. 7279-7279.
34. Dantzer, R., et al., *From inflammation to sickness and depression: when the immune system subjugates the brain.* Nat Rev Neurosci, 2008. 9(1): p. 46-56.
35. Lopez-Castejon, G. and D. Brough, *Understanding the mechanism of IL-1β secretion.* Cytokine Growth Factor Rev, 2011. 22(4): p. 189-95.

36. O'Callaghan, P., et al., *Microglial Heparan Sulfate Proteoglycans Facilitate the Cluster-of-Differentiation 14 (CD14)/Toll-like Receptor 4 (TLR4)-Dependent Inflammatory Response.* The Journal of biological chemistry, 2015. 290(24): p. 14904-14914.

37. Biesmans, S., et al., *Systemic immune activation leads to neuroinflammation and sickness behavior in mice.* Mediators Inflamm, 2013. 2013: p. 271359.

38. Peng, Z., et al., *EPA is More Effective than DHA to Improve Depression-Like Behavior, Glia Cell Dysfunction and Hippcampal Apoptosis Signaling in a Chronic Stress-Induced Rat Model of Depression.* Int J Mol Sci, 2020. 21(5).

39. Wu, H.Y., et al., *Spinal interleukin-10 produces antinociception in neuropathy through microglial β-endorphin expression, separated from antineuroinflammation.* Brain Behav Immun, 2018. 73: p. 504-519.

40. Chunchai, T., N. Chattipakorn, and S.C. Chattipakorn, *The possible factors affecting microglial activation in cases of obesity with cognitive dysfunction.* Metabolic Brain Disease, 2018. 33(3): p. 615-635.

41. Huang, Y., et al., *Exaggerated sickness behavior and brain proinflammatory cytokine expression in aged mice in response to intracerebroventricular lipopolysaccharide.* Neurobiology of Aging, 2008. 29(11): p. 1744-1753.

42. Dantzer, R., *Cytokine-Induced Sickness Behavior: Where Do We Stand?* Brain, Behavior, and Immunity, 2001. 15(1): p. 7-24.

43. O'Connor, J.C., et al., *Lipopolysaccharide-induced depressive-like behavior is mediated by indoleamine 2,3-dioxygenase activation in mice.* Molecular psychiatry, 2009. 14(5): p. 511-522.

44. Kelley, K.W., et al., *Cytokine-induced sickness behavior.* Brain Behav Immun, 2003. 17 Suppl 1: p. S112-8.

45. Konsman, J.P., P. Parnet, and R. Dantzer, *Cytokine-induced sickness behaviour: mechanisms and implications.* Trends Neurosci, 2002. 25(3): p. 154-9.

46. Yuan, T.F., et al., *Neural Mechanisms of Exercise: Anti-Depression, Neurogenesis, and Serotonin Signaling.* CNS Neurol Disord Drug Targets, 2015. 14(10): p. 1307-11.

47. Yeh, C.-H., et al., *Dexmedetomidine reduces lipopolysaccharide induced neuroinflammation, sickness behavior, and anhedonia.* PloS one, 2018. 13(1): p. e0191070-e0191070.

48. Andre, C., et al., *Diet-induced obesity progressively alters cognition, anxiety-like behavior and lipopolysaccharide-induced depressive-like behavior: focus on brain indoleamine 2,3-dioxygenase activation.* Brain Behav Immun, 2014. 41: p. 10-21.

49. Norden, D.M., et al., *Sequential activation of microglia and astrocyte cytokine expression precedes increased Iba-1 or GFAP immunoreactivity following systemic immune challenge.* Glia, 2016. 64(2): p. 300-16.

50. Ek, M., et al., *Inflammatory response: pathway across the blood-brain barrier.* Nature, 2001. 410(6827): p. 430-1.

51. O'Connor, J.C., et al., *Induction of IDO by bacille Calmette-Guérin is responsible for development of murine depressive-like behavior.* Journal of immunology (Baltimore, Md. : 1950), 2009. 182(5): p. 3202-3212.

52. Zhang, Y., et al., *Involvement of inflammasome activation in lipopolysaccharide-induced mice depressive-like behaviors.* CNS neuroscience & therapeutics, 2014. 20(2): p. 119-124.

53. Aguilar-Valles, A., et al., *Role of brain transmigrating neutrophils in depression-like behavior during systemic infection.* Mol Psychiatry, 2014. 19(5): p. 599-606.

54. Cazareth, J., et al., *Molecular and cellular neuroinflammatory status of mouse brain after systemic lipopolysaccharide challenge: importance of CCR2/CCL2 signaling.* J Neuroinflammation, 2014. 11: p. 132.

55. Gold, P.W., *The organization of the stress system and its dysregulation in depressive illness.* Mol Psychiatry, 2015. 20(1): p. 32-47.

56. Williams, Z.M., *Good vibrations with deep brain stimulation.* Nat Neurosci, 2015. 18(5): p. 618-9.

57. Francis, T.C. and M.K. Lobo, *Emerging Role for Nucleus Accumbens Medium Spiny Neuron Subtypes in Depression.* Biol Psychiatry, 2017. 81(8): p. 645-653.

58. Russo, S.J. and E.J. Nestler, *The brain reward circuitry in mood disorders.* Nat Rev Neurosci, 2013. 14(9): p. 609-25.

59. Hryhorczuk, C., et al., *Dampened Mesolimbic Dopamine Function and Signaling by Saturated but not Monounsaturated Dietary Lipids.* Neuropsychopharmacology, 2016. 41(3): p. 811-821.

60. Krabbe, K.S., et al., *Low-dose endotoxemia and human neuropsychological functions.* Brain, behavior, and immunity, 2005. 19(5): p. 453-460.

61. Bucks, R.S., et al., *Selective effects of upper respiratory tract infection on cognition, mood and emotion processing: a prospective study.* Brain Behav Immun, 2008. 22(3): p. 399-407.

62. Cunningham, C., et al., *Systemic inflammation induces acute behavioral and cognitive changes and accelerates neurodegenerative disease.* Biological psychiatry, 2009. 65(4): p. 304-312.

63. Holmes, C., et al., *Systemic inflammation and disease progression in Alzheimer disease.* Neurology, 2009. 73(10): p. 768-74.

64. Cunningham, C., et al., *Central and Systemic Endotoxin Challenges Exacerbate the Local Inflammatory Response and Increase Neuronal Death during Chronic Neurodegeneration.* The Journal of Neuroscience, 2005. 25(40): p. 9275-9284.

65. Yamagata, K., et al., *Coexpression of microsomal-type prostaglandin E synthase with cyclooxygenase-2 in brain endothelial cells of rats during endotoxin-induced fever.* J Neurosci, 2001. 21(8): p. 2669-77.

66. Reichenberg, A., et al., *Cytokine-associated emotional and cognitive disturbances in humans.* Arch Gen Psychiatry, 2001. 58(5): p. 445-52.

## Supplementary Figure 1

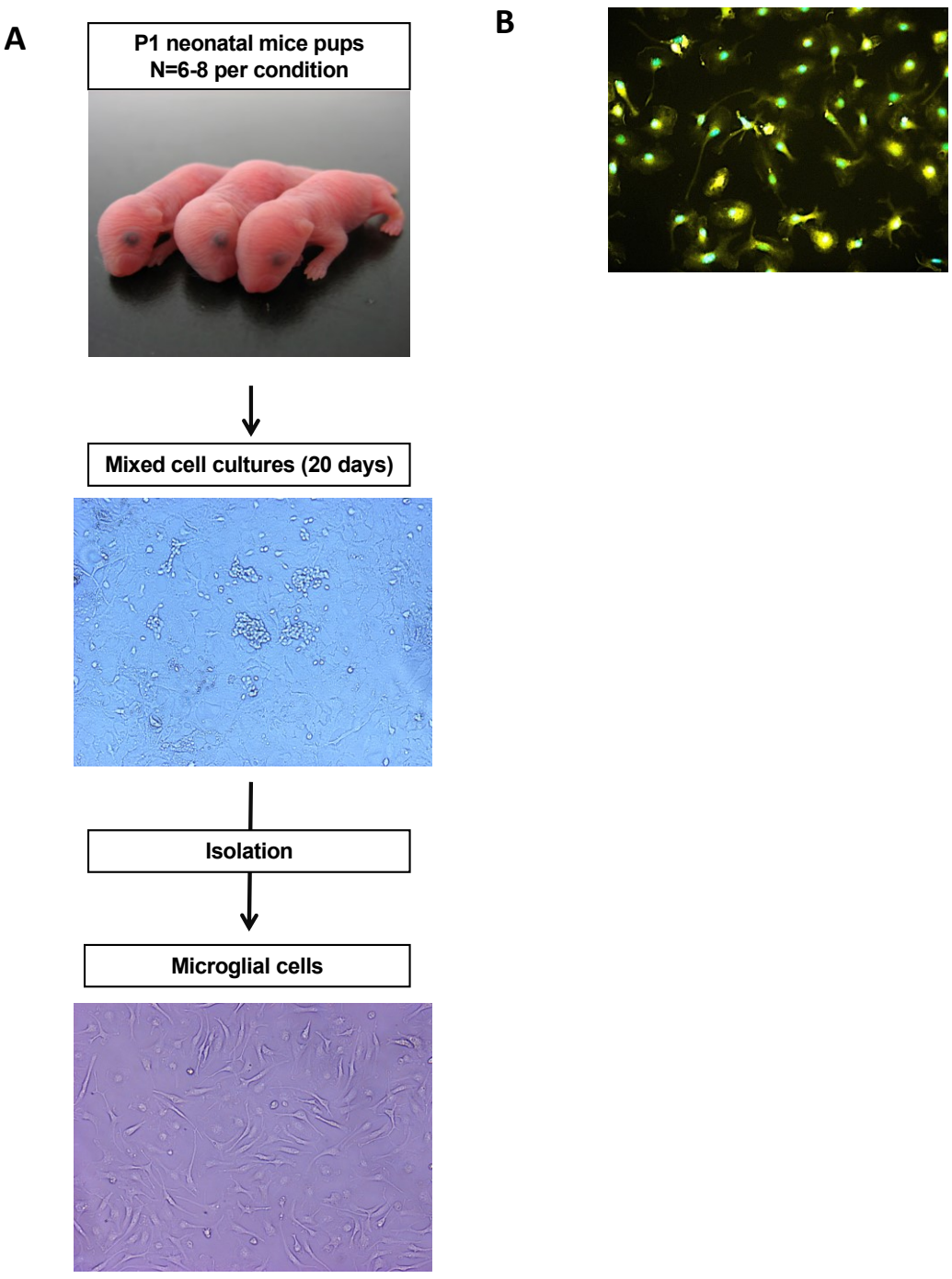
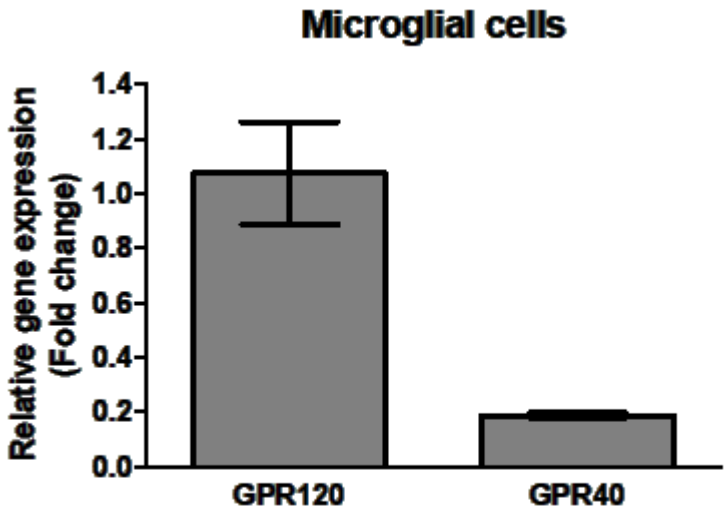



**Figure S1.** A. Protocol for primary microglia cell culture. B. Validation of microglia cultures derived from Cx3cr1$^{CreER-YFP}$ mice that express a YFP fusion protein (yellow) in microglia. Microglia were labeled with an Iba-1 antibody coupled to a secondary fluorescent antibody (green) to validate the purity of cultures and specificity of the YFP reporter.

## Appendix 1.

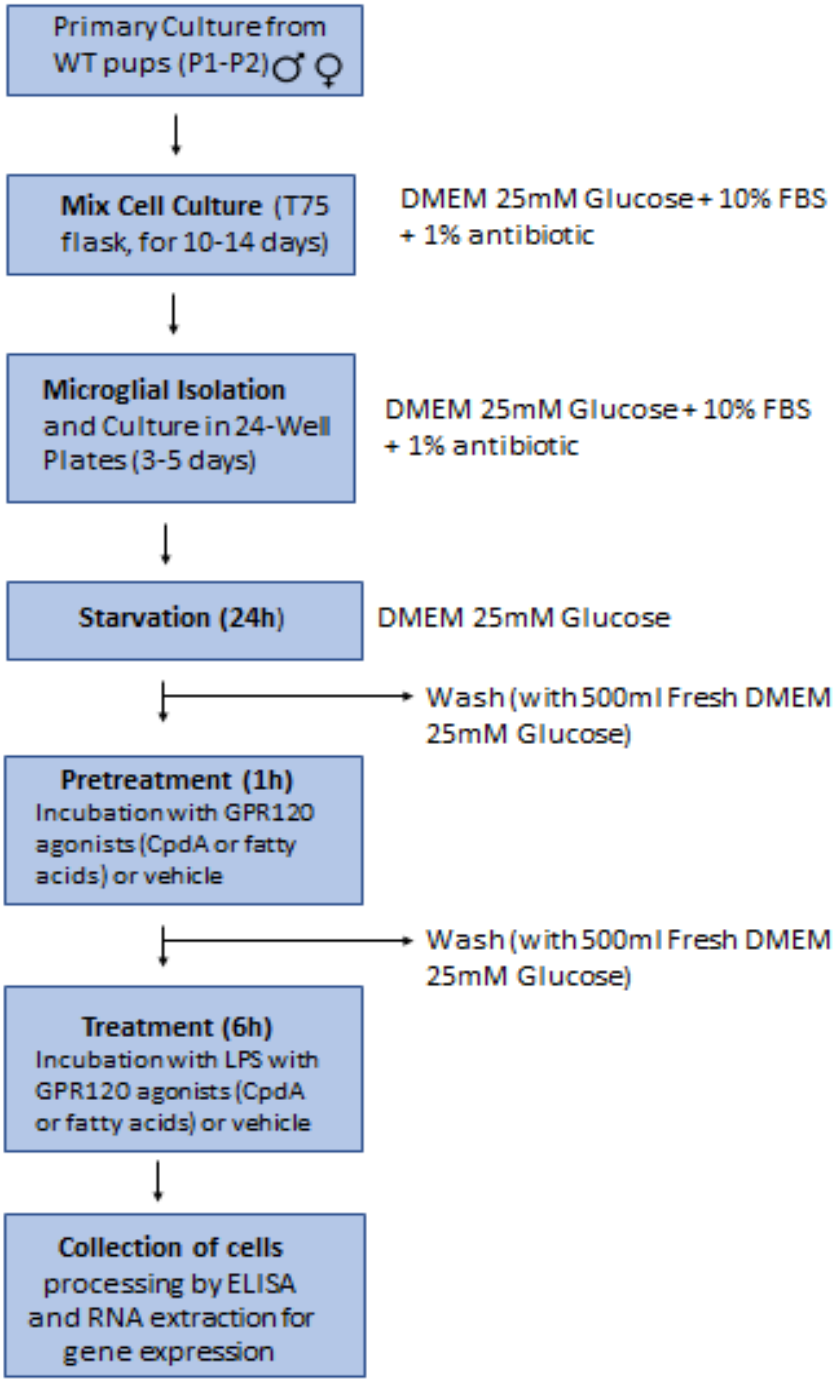
**Microglia GPR120 and GPR-40 mRNA expression**




**Appendix 1.** GPR120 and GPR-40 mRNA expression in primary microglia cell culture from neonatal mouse brain by RT-PCR. Results are expressed as mean ± SEM, fold change vs GPR120 N=4-6.

## Appendix 2.

**Microglial cell culture isolation and treatment flow chart (in vitro)**



## Appendix 3.

Published Article in International Journal of Obesity (2020) 44:1936–1945

**Fish oil supplementation alleviates metabolic and anxiodepressive effects of diet-induced obesity and associated changes in brain lipid composition in mice**

Geneviève Demers1,2, Jerome Roy1, Arturo Israel Machuca-Parra1,3, Zahra Dashtehei pour1,2, Diane Bairamian 1,2 , Caroline Daneault4, Christine Des Rosiers2,4 , Guillaume Ferreira 5,6 , Thierry Alquier 1,3,6 ,Stephanie Fulton1,2,6 ,

International Journal of Obesity (2020) 44:1936–1945
https://doi.org/10.1038/s41366-020-0623-6

ARTICLE

Animal Models

# Fish oil supplementation alleviates metabolic and anxiodepressive effects of diet-induced obesity and associated changes in brain lipid composition in mice

Geneviève Demers[1,2] · Jerome Roy[1] · Arturo Israel Machuca-Parra[1,3] · Zahra Dashtehei pour[1,2] · Diane Bairamian[1,2] · Caroline Daneault[4] · Christine Des Rosiers[2,4] · Guillaume Ferreira[5,6] · Thierry Alquier[1,3,6] · Stephanie Fulton[1,2,6] · Representative of consortium

Received: 21 October 2019 / Revised: 1 May 2020 / Accepted: 22 May 2020 / Published online: 16 June 2020


## Abstract

**Objective** Obesity significantly elevates the odds of developing mood disorders. Chronic consumption of a saturated high-fat diet (HFD) elicits anxiodepressive behavior in a manner linked to metabolic dysfunction and neuroinflammation in mice. Dietary omega-3 polyunsaturated fatty acids (n-3 PUFA) can improve both metabolic and mood impairments by relieving inflammation. Despite these findings, the effects of n-3 PUFA supplementation on energy homeostasis, anxiodepressive behavior, brain lipid composition, and gliosis in the diet-induced obese state are unclear.

**Methods** Male C57Bl/6J mice were fed a saturated high-fat diet (HFD) or chow for 20 weeks. During the last 5 weeks mice received daily gavage ("supplementation") of fish oil (FO) enriched with equal amounts of docosahexaenoic (DHA) and eicosapentaenoic acid (EPA) or control corn oil. Food intake and body weight were measured throughout while additional metabolic parameters and anxiety- and despair-like behavior (elevated-plus maze, light–dark box, and forced swim tasks) were evaluated during the final week of supplementation. Forebrain lipid composition and markers of microglia activation and astrogliosis were assessed by gas chromatography–mass spectrometry and real-time PCR, respectively.

**Results** Five weeks of FO supplementation corrected glucose intolerance and attenuated hyperphagia in HFD-induced obese mice without affecting adipose mass. FO supplementation also defended against the anxiogenic and depressive-like effects of HFD. Brain lipids, particularly anti-inflammatory PUFA, were diminished by HFD, whereas FO restored levels beyond control values. Gene expression markers of brain reactive gliosis were supressed by FO.

**Conclusions** Supplementing a saturated HFD with FO rich in EPA and DHA corrects glucose intolerance, inhibits food intake, suppresses anxiodepressive behaviors, enhances anti-inflammatory brain lipids, and dampens indices of brain gliosis in obese mice. Together, these findings support increasing dietary n-3 PUFA for the treatment of metabolic and mood disturbances associated with excess fat intake and obesity.

## Introduction

While often associated with heightened risk for cardiovascular and metabolic diseases, obesity also significantly increases the incidence of mood disorders [1–3]. Anxiety and depressive

**Supplementary information** The online version of this article (https://doi.org/10.1038/s41366-020-0623-6) contains supplementary material, which is available to authorized users.

✉ Stephanie Fulton
stephanie.fulton@umontreal.ca

[1] Centre de Recherche du CHUM and Montreal Diabetes Research Center, Montreal, QC H2X 0A9, Canada

[2] Departments of Nutrition, Université de Montréal, MontrealQC, QC H2X 0A9, Canada

[3] Departments of Medicine, Université de Montréal, Montréal, QC, Canada

[4] Montreal Heart Institute, Montréal, QC, Canada

[5] Nutrition and Integrative Neurobiology Unit, UMR1296 INRA - Université de Bordeaux, Bordeaux, France

[6] Food4BrainHealth France-Canada International Research Network, Bordeaux, France

disorders impair quality of life, motivation and occupational functioning, and these consequences diminish treatment adherence to enhance metabolic dysfunction and associated complications [4]. As metabolic and mood deficits exacerbate the threat of obesity there is an imminent need to identify suitable therapeutic interventions.

Several lines of evidence suggest that enhanced immune activation concurrent with abdominal obesity contributes to metabolic impairments and mood deficits such as depression [5–8]. Similarly, rodent models of diet-induced obesity (DIO) exhibit anxiety- and depressive-like behaviors alongside metabolic dysfunction [9, 10]. Excess consumption of saturated fat is known to aggravate metabolic dysfunction in obesity by generating physiological responses that favor deposition of pro-inflammatory abdominal adipose tissue [11, 12]. We recently reported that a saturated (but not monounsaturated) high-fat diet (HFD), which promotes visceral obesity, peripheral inflammation, and glucose intolerance, triggers anxiodepressive behavior via NFκB-mediated neuroinflammatory processes in mice [13]. These findings along with clinical and epidemiological data linking poor diet quality, metabolic inflammation, and depressive symptomology [6, 14, 15] as well as the protective effects of a Mediterranean diet [16] suggest that the type and amount of dietary lipids contribute to mood disorders development.

Omega-3 polyunsaturated fatty acids (n-3 PUFA), primarily eicosapentaenoic acid (EPA, 20:5), and docosahexaenoic acid (DHA, 22:6), are essential fatty acids that play an important role in inflammation. Increased dietary n-3 PUFA can improve insulin sensitivity and significantly diminish peripheral inflammation as evidenced by reductions in plasma C-reactive protein (CRP), IL-6, and TNF-α levels [17, 18]. Moreover elevated intake of n-3 PUFA via fish oil (FO) supplementation can diminish cerebral inflammation and attenuate behavioral indices of mood deficits in rodents [19–21]. Consistently, epidemiological studies show that high n-3 PUFA consumption is associated with lower prevalence and severity of mood disturbances [22], whereas clinical studies show improvements in major depressive disorder with n-3 PUFA (primarily EPA) supplementation [23, 24]. In turn, numerous reports demonstrate that dietary n-3 PUFA deficiency and associated decreases in brain n-3 PUFA levels stimulate neuroinflammation and anxiety- and depressive-like behaviors in rodents [20, 25, 26].

Despite the connection between diet, obesity, and mood impairments, the impact of n-3 PUFA supplementation during DIO on brain lipid composition and metabolic and mood endpoints has not been fully explored. In the present study, we show that a prolonged saturated HFD elicits metabolic dysfunction and anxiodepressive behavior concurrent with excess pro-inflammatory brain lipids. FO supplementation initiated following obesity development was found to improve certain negative metabolic and mood corollaries of a saturated HFD in conjunction with higher brain levels of anti-inflammatory fatty acid and reduced neuroglia activation.

## Methods

### Animals

All experimental procedures were approved by the Institutional Animal Care Committee of the CRCHUM in accordance with the standards of the Canadian Council on Animal Care. Seven to eight-week-old male C57BL/6J mice were obtained from Jackson Laboratories (Bar Harbor, Maine, USA). Upon arrival, mice were individually housed and maintained in an environmentally controlled room (22–24 °C) with ad libitum access to standard chow and water. Mice were acclimatized to a reverse light/dark cycle for at least 7 days prior to initiation of experiments. Euthanasia was carried out under isoflurane anesthesia. Brains and blood samples were harvested and stored at −80 °C.

### Diets and supplements

Mice received ad libitum access to either a saturated HFD containing 50% kcal palm oil or an ingredient-matched, control diet ("chow") containing 16.8% kcal soybean oil for 20 weeks (Supplemental Fig. 1A). During the last 5 weeks (16th–20th week) of the diet protocol, mice from the HFD and chow groups received daily gavage of FO (Omega Protein; Houston, TX, USA) or control corn oil ("Ctrl"; Sigma–Aldrich; St. Louis, MO, USA) described in Table 1. This defined four experimental groups ($n = 12$/group): HFD + control ($HFD^{Ctrl}$); HFD + FO ($HFD^{FO}$); chow + control ($chow^{Ctrl}$); chow + FO ($chow^{FO}$). The amount of FO administered (0.7 mg/kg) was based on *Dietitians of Canada* recommendations for humans of 500 mg of combined DHA and EPA per day [27]. Assuming an average human body weight of 60 kg, this recommended human dose of 8.33 mg/kg was converted for mice by dividing by 12.3 (according to published standards [28]) to provide 0.7 mg/kg of combined DHA and EPA. At an average body weight of 30 g, each mouse received a daily gavage of 80 µl of the FO (Table 1). Diets and supplementation continued throughout testing and until sacrifice.

### Metabolic profiling

Body weight and food intake were measured weekly, whereas additional metabolic measurements were performed the last week of the diet protocol. For glucose tolerance tests, mice were fasted 4 h prior to intraperitoneal (IP) injection of glucose (2 g/kg of body weight). Blood glucose was measured at 0, 15, 30, 60, and 120-min post

**Table 1** Composition of supplements.

| Fatty acid | C:D | Corn oil (%) | Fish oil (%) |
|---|---|---|---|
| Myristic | C14:0 | <0.1 | 8.20 |
| Palmitic | C16:0 | 8.6–16.5 | 18.43 |
| Stearic | C18:0 | 1.0–3.3 | 3.36 |
| Arachidonic | C20:0 | <0.8 | 0.20 |
| Palmitoleic | C16:1 | <0.5 | 11.51 |
| Oleic | C18:1 | 20.0–42.2 | 9.16 |
| Linolenic | C18:2 | 39.4–62.0 | 1.67 |
| γ-linolenic | C18:3 | 0.5–1.5 | 0.28 |
| Gondoic | C20:1 | <0.5 | 0.19 |
| Eicosapentaenoic (EPA) | C20:5 | | 13.93 |
| Heneicosapentaenoic (HPA) | C21:5 | | 0.52 |
| Docosapentaenoic (n-6 DPA) | C22:5 | | 0.23 |
| Docosapentaenoic (n-3 DPA) | C22:5 | | 2.30 |
| Docosahexaenoic | C22:6 | | 13.35 |
| Other | | <0.9 | 16.67 |
| Total | | 100.0 | 100.0 |

injection. Lean and fat mass were measured using Echo MRI (Echo Medical Systems; Houston, Texas). Ambulatory activity and energy expenditure (normalized to lean mass) were assessed for 24 h in the Comprehensive Lab Animal Monitoring System (CLAMS; Columbus Instruments) set at 22 °C after a 24-h habituation period to cages.

## Enzyme-linked immunosorbent assay (ELISA)

For plasma protein measurements, blood was collected after decapitation, kept on ice and then centrifuged at 8000 rpm for 10 min at 4 °C. Plasma was stored at −80 °C until further use. Insulin was measured using the R&D Systems Duokits ELISA kits (Minneapolis, MN, USA) according to the manufacturer's protocol. CRP was measured using a mouse ELISA kit (Abcam, USA).

## Anxiodepressive behavior

All behavioral tests were video recorded and analyzed using Ethovision XT software system (Noldus). Behavioral testing was carried out during the last week of the protocol. All testing was performed in the light just before dark cycle onset to control for any differences in basal locomotion.

## Elevated-plus maze

The elevated-plus maze (EPM) was used to assess anxiety-like behavior as previously reported [9]. In brief, each mouse was placed in the center of the maze facing an open arm opposing the experimenter. Number of open arm entries, percentage of open arm time, and distance traveled were measured over a period of 5 min.

## Light/Dark box

The light/dark box (LDB) was used as an additional measure of anxiety-like behavior. The mouse LDB apparatus (Med Associates, Inc.) consists of an illuminated compartment adjoined to a dark compartment covered by a lid (both 13.7 × 13.7 × 20.3 cm). The two sides were separated by a partition with an opening at the bottom to allow mice to pass freely between compartments. The number of entries and time spent in the lit compartment were measured for 5 min.

## Forced swim test

The forced swim test (FST) was used as a measure of behavioral despair as described in detail previously [9]. Mice are placed in a beaker of water (24 °C) for 6 min. Velocity and locomotor capacity were evaluated during the first 2 min while despair-related mobility was measured during the last 4 min.

## Brain lipid quantification

Forebrains were used for quantitative profiling of fatty acids, both free and bound to triglycerides and phospholipids, by gas chromatography–mass spectrometry using previously described methods [29–31]. In brief, samples containing 25 mg of pulverized of tissue were incubated overnight at 4 °C in a solution of chloroform/methanol (2:1) containing 0.004% butylated hydroxytoluene, filtered through gauze and dried under nitrogen gas. Fatty acids were analyzed as their methyl esters after a direct transesterification with acetyl chloride/methanol on a 7890B gas chromatograph coupled to a 5977A Mass Selective Detector (Agilent Technologies, Santa Clara, USA) equipped with a capillary column (J&W Select FAME CP7420; 100 m × 250 µm inner diameter; Agilent Technologies Inc.) and operated in the PCI mode using ammonia as the reagent gas. Samples (0.4 µL) were analyzed under the following conditions: injection at 270 °C in a split mode (split ratio: 50:1) using high-purity helium as the carrier gas (constant flow rate: 0.44 mL/min) and the following temperature gradient: 190 °C for 25 min, increased by 1.5 °C/min until 236 °C. Fatty acids were analyzed as their $[M + NH_4]^+$ ion by selective ion monitoring and concentrations were calculated using standard curves and isotope-labeled internal standards.

## Quantitative PCR

As markers of neuroglia responses, we measured glial fibrillary acidic protein (GFAP) and ionized calcium-binding adapter molecule 1 (IBA1) mRNA levels in

forebrain tissue. IBA1 is expressed microglia, the innate immune cells of the brain, and upregulated during the activation of these cells. GFAP upregulation is tied with astrogliosis, the reactivity of astrocytes that occurs in response to neural injury and/or inflammation. Forebrain samples were processed for mRNA extraction with TRIzol. RT-PCR were performed using the Rotor-Gene SYBR Green PCR Kit (Qiagen, Valencia, CA, USA) using a Corbett Rotor-Gene 6000. IBA1 forward: GGATTTGC AGGGAGGAAAAG, reverse: TGGGATCATCGAGGAA TTG; GFAP forward: AACGACTATCGCCGCCAACTG, reverse: CTCTTCCTGTTCGCGCATTTG; Cyclophilin forward: GCTTTTCGCCGCTTGCTGCA, reverse: TGCA AACAGCTCGAAGGAGACGC. Each PCR reaction was performed in triplicate. Expression was calculated using the ΔΔCT method using cyclophilin as the housekeeping gene.

### Statistical analyses

Data were analyzed using GraphPad Prism 6 software (San Diego, CA, USA). Outliers were removed using Grubbs' test set at $p \leq 0.05$. A two-way ANOVA with Sidak post-tests was used. Main effects (diet and FO) and interaction effects are described in the text whereas pairwise (simple) effects are noted by symbols in graphs and tables. All data are presented as mean ± SEM. $p < 0.05$ was set as criterion for statistical significance.

## Results

### Fish oil supplementation attenuates metabolic disturbances

We first sought to determine the impact of FO supplementation on energy metabolism in mice fed a saturated HFD or control diet. As shown in Fig. 1a, body weights of HFD mice significantly increased relative to control mice (diet effect at 5 weeks; $F_{(1,38)} = 94.09$, $p < 0.0001$); however, FO supplementation did not affect body weights in either diet group. While there was a trend for reduced body weight in $HFD^{FO}$ compared with $HFD^{Ctrl}$ mice by the 5th week of supplementation (Fig. 1a), fat and lean mass were unchanged (Fig. 1b). Caloric intake was elevated in $HFD^{Ctrl}$ mice (diet effect; $F_{(3,38)} = 78.44$; $p < 0.0001$) and FO resulted in a small but significant decrease in food intake in HFD mice by the 5$^{th}$ week (FO effect; $F_{(1,38)} = 4.87$, $p = 0.03$; interaction, $p = 0.23$; Fig. 1c). FO increased locomotor activity in $HFD^{FO}$ relative to $HFD^{Ctrl}$ mice only at the peak of dark cycle activity (Fig. 1d), yet total dark and light cycle activity (area under curve) were not affected by supplementation or diet. As expected, HFD increased energy expenditure; however, FO did not influence this parameter (Supplemental Fig. 1). Glucose intolerance caused by HFD was corrected by FO supplementation: glucose excursion curves for $HFD^{FO}$ mice were similar to those of $Chow^{Ctrl}$ mice (FO effect on AUC; $F_{(3,36)} = 9.60$, $p = 0.001$; interaction AUC, $p = 0.08$; Fig. 1e). Mice consuming the HFD were hyperinsulinemic (HFD effect: $F_{(1,32)} = 15.14$, $p = 0.0005$; Fig. 1f) and had higher plasma CRP levels ($F_{(1,33)} = 7.79$, $p = 0.008$; Fig. 1g), but FO did not decrease fed-state plasma insulin or CRP levels.

### Obesity-induced anxiety and despair is attenuated by fish oil supplementation

We next determined the influence of DIO with or without FO supplementation on anxiety and depressive-like behavior using three behavioral tasks. In the EPM, chronic high-fat feeding increased signs of anxiety as reflected by reduced open arm entries (diet effect; $F_{(1,35)} = 7.946$; $p = 0.008$; Fig. 2a) and proportion of time spent in the open arms for $HFD^{Ctrl}$ mice as compared with $Chow^{Ctrl}$ mice (diet effect; $F_{(1,35)} = 7.31$, $p = 0.01$; Fig. 2a). FO attenuated this difference between groups: open arm entries (FO effect; $F_{(1,35)} = 2.21$, $p = 0.15$; interaction, $p = 0.09$; Fig. 2a) and proportion of time spent (FO effect; $F_{(1,35)} = 0.49$, $p = 0.49$; interaction, $p = 0.05$; Fig. 2a). HFD also decreased distance traveled in the EPM (diet effect; $F_{(1,34)} = 5.20$, $p = 0.028$), an effect reversed by FO (FO effect; $F_{(1,34)} = 3.68$, $p = 0.06$, interaction, $p = 0.04$).

In a second test of anxiety, the LDB, high-fat feeding again triggered anxiety-like behavior as demonstrated by a lower number of entries (diet effect; $F_{(1,35)} = 2.001$, $p = 0.1660$; Fig. 2b) and proportion of time spent in the lit compartment in $HFD^{Ctrl}$ mice relative to $Chow^{Ctrl}$ mice (diet effect; $F_{(1,36)} = 10.98$, $p = 0.002$; Fig. 2b). In contrast, the $HFD^{FO}$ group had similar entries (FO effect; $F_{(1,35)} = 4.26$, $p = 0.046$; interaction, $p = 0.003$; Fig. 2b) and lit compartment time as compared with the $Chow^{Ctrl}$ group (FO effect; $F_{(1,36)} = 0.05$, $p = 0.81$; interaction, $p = 0.12$; Fig. 2b).

In the FST, HFD enhanced behavioral despair: immobility time was increased in $HFD^{Ctrl}$ mice as compared with $Chow^{Ctrl}$ mice (diet effect; $F_{(1,36)} = 9.987$, $p = 0.003$; Fig. 2c). On the other hand, while there was no main effect of FO (FO effect; $F_{(1,36)} = 0.76$, $p = 0.387$) FO reduced immobility time in $HFD^{FO}$ versus $HFD^{Ctrl}$ mice (interaction, $p = 0.03$; Fig. 2c). As an index of locomotor capacity, swim velocity was comparable across the four groups.

### Brain fatty acid content is modulated by high-fat feeding and fish oil supplementation

To evaluate the impact of HFD and FO supplementation on brain lipid composition, we performed quantitative profiling

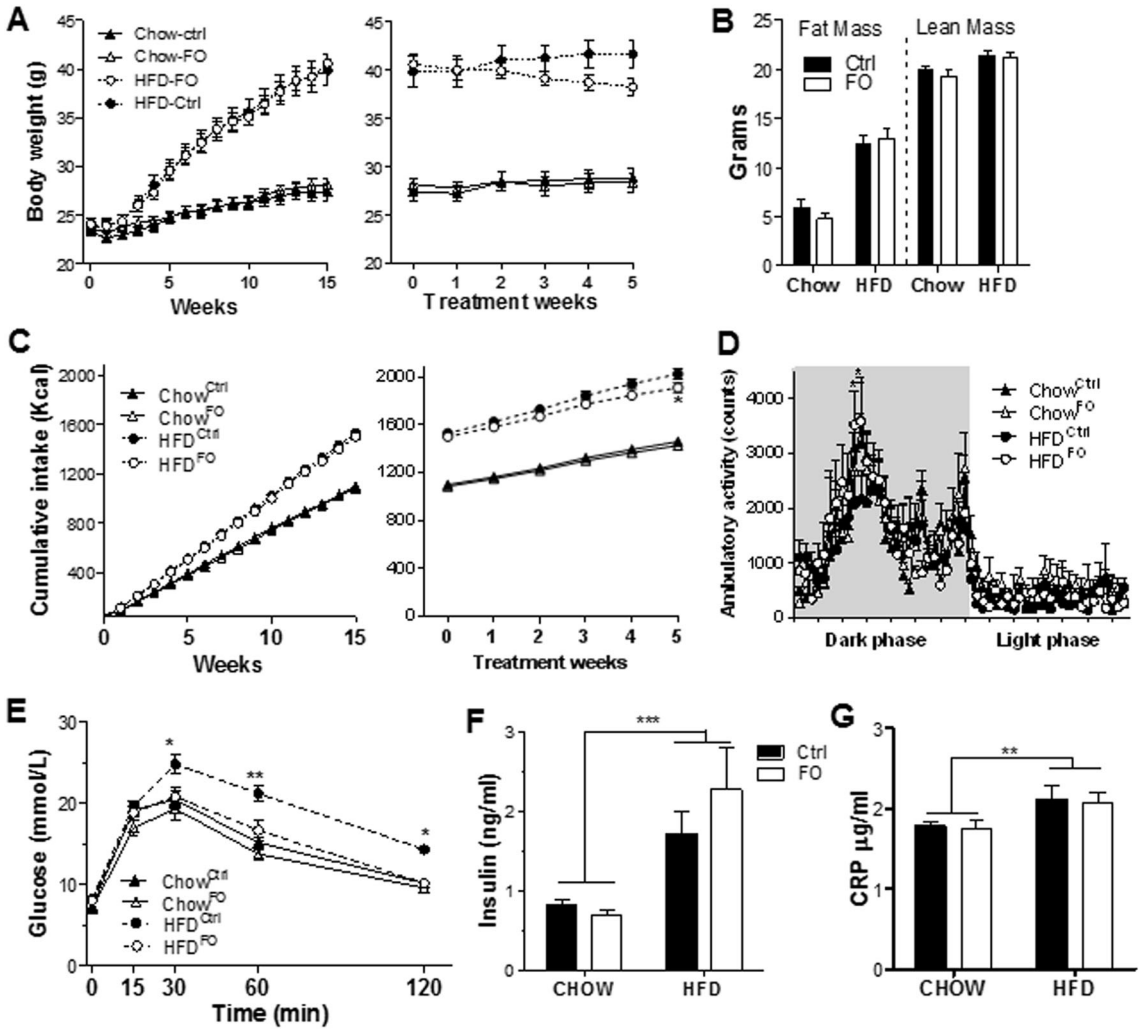



**Fig. 1 Fish oil supplementation protects against diet-induced glucose intolerance and attenuates hyperphagia without affecting body composition.** **a** Body weight during 15 weeks of chow or saturated HFD (left panel) and during 5 following weeks of daily gavage with corn oil (Ctrl) or fish oil (FO) (right panel). **b** Fat and lean mass following supplementation. **c** Cumulative caloric intake before (left panel) and during supplementation (right panel). **d**, **e** Ambulatory activity, **f**, **g** 24 h energy expenditure corrected by metabolic mass (**h**) glucose excursion curves following IP-GTT (**i**) plasma insulin, and (**j**) plasma c-reactive protein. Values are expressed as mean ± SEM; $*p \le 0.05$, $**p \le 0.01$, $***p \le 0.001$, $****p \le 0.0001$. $N = 9$–12/condition.

of forebrain fatty acids. As shown in Fig. 3a, total fatty acids were not modified by HFD; however, FO elevated total fatty acid content (FO effect: $F_{(1,36} = 4.15$; $p = 0.04$). On the other hand, HFD increased the proportion of saturated fatty acids (SFA) (Fig. 3b; diet effect: $F_{(1,35)} = 5.57$, $p = 0.023$) while decreasing polyunsaturated fatty acids (PUFA) (Fig. 3c; diet effect; $F_{(1,34)} = 16.63$; $p = 0.0003$). FO supplementation did not alter the level of either of these fatty acid families. However, as illustrated in Fig. 3d, individual PUFA were significantly modified by both HFD and FO. HFD decreased DHA (C22:6n-3) (diet effect: $F_{(1,36)} = 7.72$, $p = 0.008$), DGLA (C20:3n-6) (diet effect: $F_{(1,37)} = 49.74$, $p < 0.0001$) and LA (C18:2n-6) (diet effect; $F_{(1,27)} = 15.07$, $p = 0.0006$) concentrations. As shown in Fig. 3d, EPA (C20:5n-3) and docosapentaenoic acid (DPA, C22:5n-3) substantially increased with FO supplementation in the HFD group (EPA FO effect: $F_{(1,27)} = 7.71$, $p = 0.009$, interaction, $p = 0.001$; DPA FO effect: $F_{(1,29)} = 50.51$, $p <$ 0.0001, interaction, $p = 0.013$; Fig. 3d). Similarly, two n-6 fatty acids, LA (C18:2n-6) (FO effect: $F_{(1,28)} = 5.81$, $p =$ 0.02) and DGLA (C20:3n-6) (FO effect: $F_{(1,37)} = 12.3$, $p =$ 0.001) were elevated by FO in both diet groups. In contrast, arachidonic acid (AA; C20:4n-6) was decreased by FO in both chow and HFD mice (FO effect: $F_{(1,36)} = 15{,}14$, $p =$ 0.0004).

Additional fatty acid species are presented in Table 2. Of note, palmitate levels were unchanged by HFD, instead the SFA stearate significantly increased with HFD (diet effect; $F_{(1,37)} = 7.87$; $p = 0.008$). FO supplementation increased the levels of the SFA acid myristate (FO effect: $F_{(1,34)} = 9.88$, $p =$

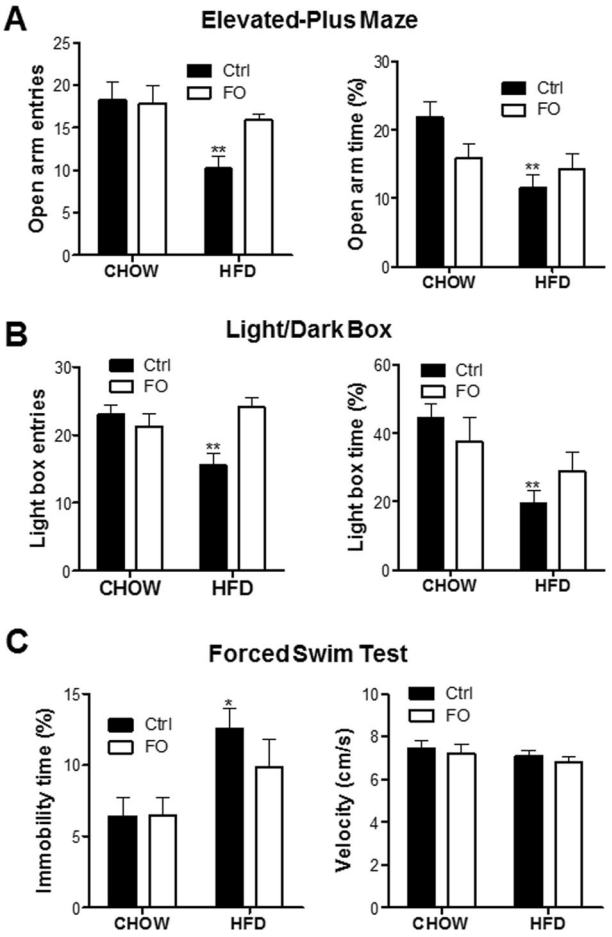



**Fig. 2 Fish oil supplementation alleviates anxiety- and despair-like behavior triggered by a saturated high-fat diet and obesity. a** Number of entries in the open arms and percent of open-arms time. **b** Total entries and percentage of time spent in the lit compartment of the light/dark box. **c** Immobility time and swim velocity in the forced swim test. Values are expressed as mean ± SEM; $*p \le 0.05$, $**p \le 0.01$. $N = 9$–12/group.

0.003; interaction, $p = 0.06$) and lead to a trend for increased stearate levels ($F_{(1,37)} = 3.48$, $p = 0.07$; interaction, $p = 0.12$). While the n-3:n-6 ratio was unaffected, HFD and FO influenced the EPA:AA ratio (marker of chronic inflammation): HFD diminished (diet effect: $F_{(1,28)} = 13.41$, $p = 0.001$), whereas FO caused a substantial increase in the EPA:AA ratio in HFD mice (FO effects: $F_{(1,28)} = 8.38$, $p = 0.007$; interaction, $p = 0.0001$).

### Fish oil supplementation dampens reactive gliosis

As illustrated in Fig. 4, while HFD did not alter GFAP and Iba-1 mRNA expression in the forebrain, FO supplementation reduced GFAP expression across diets (FO effect; $F_{(1,37)} = 10.44$; $p = 0.003$; interaction, $p = 0.32$) and decreased Iba-1 levels in $HFD^{FO}$ relative to $HFD^{Ctrl}$ mice (FO effect: $F_{(1,38)} = 0.06$, $p = 0.8$, interaction, $p = 0.005$).

## Discussion

The last several decades have witnessed an increase in the consumption of SFA to the detriment of n-3 PUFA, a change in dietary pattern that is posited to contribute to increased immune activity and greater obesity-associated risks. Among the findings associating n-3 PUFA intake to the regulation of energy balance and mood, both body mass index [32] and depression rates [33] were shown to be inversely related to circulating n-3 PUFA levels. As metabolic impairments arising from poor dietary lifestyle and obesity development increase the risk of anxiety and depression, the present work determined the impact of FO supplementation in a DIO setting on metabolic, mood and brain fatty acid measures. Consistent with earlier work [9, 10, 13], our results demonstrate increased anxiety and indices of despair by saturated HFD. In addition to restoring glycemic control and blunting hyperphagia, FO supplementation was effective at alleviating anxiety- and despair-like behavioral responses to the saturated HFD while increasing individual anti-inflammatory PUFA and attenuating signs of reactive gliosis.

Although several reports assessed the protective effects of n-3 PUFA in the development of obesity using different diets [34–36], the effects of FO supplementation initiated following obesity development and during a saturated HFD on metabolic and emotional measures has not been investigated. Moreover, to our knowledge, no study has employed an administration protocol that is comparable with taking a FO supplement (gavage) with n-3 PUFA doses selected to biologically resemble those recommended for humans. FO was used as it is more commonly consumed, both in diet and supplementation form, and due to its content in DHA, EPA and DPA, n-3 PUFA that are more potent than n-3 alpha-linolenic acid which has a limited ability to convert to long-chain n-3 PUFA.

In accordance with previous reports [37, 38], the present study demonstrates protective effects of n-3 PUFA on HFD-induced glucose intolerance in mice. Prior findings were obtained in conditions where body weight and fat mass deviated with n-3 PUFA dietary intervention. Here, we demonstrate that daily FO supplementation defends against the deleterious effects of DIO on glucose tolerance and anxiodepressive behavior in conditions where body composition and energy expenditure are unchanged. Thus, the benefits of FO for glucose homeostasis are unlikely to be largely secondary to catabolic actions of n-3 PUFA, a result that is in agreement with recent studies [39, 40]. Improved glucose tolerance by FO did not coincide with changes in

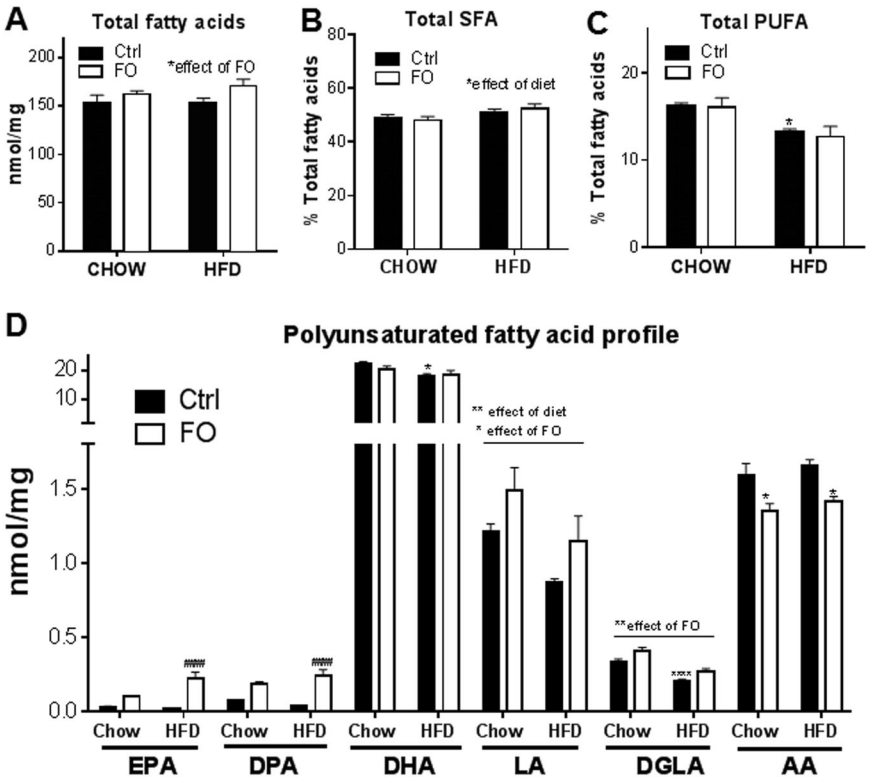



**Fig. 3 Forebrain lipid composition changes associated with saturated high-fat feeding and fish oil supplementation. a** Total fatty acid levels. **b** Proportion of saturated fatty acids (SFA). **c** Proportion of polyunsaturated fatty acids (PUFA). **d** Concentration of individual PUFA. EPA eicosapentaenoic acid, DPA docosapentaenoic acid, DGLA dihomo-γ-linolenic acid, LA linoleic acid, AA arachidonic acid, DHA docosahexaenoic acid. Values are expressed as the mean ± SEM (nmol/mg or %). $Chow^{Ctrl}$ vs. $HFD^{Ctrl}$: $^{****}p \le 0.0001$, $HFD^{Ctrl}$ vs. $HFD^{FO}$: $^{\#\#\#}p \le 0.001$. $N = 4–12$/group. Main effects: $^{*}p \le 0.05$, $^{**}p \le 0.01$.

insulin levels, suggesting that FO supplementation may improve glucose tolerance independent of modulating insulin sensitivity. These findings contrast those showing that FO supplementation in HFD-fed obese mice improves insulin sensitivity [39]. This discrepancy may be related to differences in n-3 type, dose and animal model tested. Nonetheless, the absence of changes in body weight and insulin levels are in agreement with the influence of FO supplementation observed in randomized clinical trials of obese adolescents [41] and overweight adults [42].

In agreement with prior observations [43], we found that total brain SFA content to be elevated by HFD. While the palm oil HFD we used is palmitate-enriched (~44% of total fatty acids) and substantially increases plasma palmitate levels [44], there was no difference in brain palmitate content between HFD- and chow-fed mice. This contrasts with results of Rodrigues-Navas et al. of increased brain palmitate by milk-fat HFD that contains less palmitate [43]. The discrepancy may be due to the higher amount of sucrose in the milk-fat HFD and the longer duration of diet regimen implemented by these authors (16 vs. 12 weeks). On the other hand, we observed that the most abundant SFA in the brain, stearate, was increased by the palm oil HFD. Thus, despite the positive association between plasma palmitate levels and depression severity in humans [45], it is not clear higher circulating palmitate gives rise to increased brain palmitate. Moreover, central SFA content was not affected by FO supplementation despite the favorable effects of FO on glucose homeostasis and anxiodepressive behavior. Rather, FO augmented total fatty acids and the long-chain SFA myristate and produced a trend for higher stearate levels. These results suggest that higher brain composition of SFAs alone does not underlie neuroimmune responses and associated emotional and metabolic impairments produced by saturated high-fat feeding and DIO.

Our observations suggest that a saturated HFD and obesity diminish brain PUFA levels. Although DHA is plentiful in the brain, central DHA levels can remain relatively stable in response to short-term dietary interventions [46] as opposed to longer n-3 PUFA nutritional interventions [47]. These findings are consistent with our lipidomic results showing no change in DHA with 5 weeks of FO supplementation, but reduced DHA in HFD mice. In contrast, EPA is much less abundant in the brain and fluctuates more rapidly and according to ongoing nutritional status and cellular metabolism [48]. Indeed, despite stability in total PUFA levels, select PUFA species such as EPA and DPA were markedly increased by FO. It is important to note in this regards that EPA rather than DHA supplementation ameliorates major depressive disorder symptomology in randomized controlled trials [23, 49] and blunts rodent depressive behavior [50] and the deleterious behavioral and neuroinflammatory effects of central interleukin-1β injection [51].

Several mechanisms have been proposed to explain the increased risk of anxiety and depression in individuals with

**Table 2** Forebrain fatty acid profile.

| Fatty acid | | $Chow^{Ctrl}$ | $Chow^{FO}$ | $HFD^{Ctrl}$ | $HFD^{FO}$ |
|---|---|---|---|---|---|
| Myristic | C14:0 | 0.25 ± 0.02 | 0.27 ± 0.01 | 0.24 ± 0.02 | $0.34 \pm 0.13^{\#\#}$ |
| Palmitic | C16:0 | 31.97 ± 4.78 | 32.36 ± 1.57 | 31.96 ± 1.91 | 34.38 ± 1.76 |
| Palmitoleic | C16:1 n-7 | 0.72 ± 0.11 | 0.75 ± 0.1 | 0.65 ± 0.11 | 0.61 ± 0.13 |
| Stearic | C18:0 | 42.12 ± 11.65 | 43.1 ± 10.95 | 46.37 ± 9.96 | 58.39 ± 11.65 |
| Oleic | C18:1 n-9 | 49.55 ± 7.33 | 51.97 ± 6.63 | 49.79 ± 5.3 | 57.24 ± 4.16 |
| Vaccenic | C18:1 n-7 | 4.63 ± 0.44 | 4.29 ± 0.57 | 4.27 ± 0.61 | 3.66 ± 0.56 |
| Mead | C20:3 n-9 | 0.22 ± 0.04 | 0.22 ± 0.03 | 0.35 ± 0.05*** | 0.33 ± 0.05 |
| Ratio n-3/n-6 | | 7.33 ± 0,56 | 6.97 ± 2,5 | 7.31 ± 1,34 | 10.46 ± 5,58 |
| Ratio EPA/AA | | 0.02 ± 0.001 | 0.07 ± 0.006 | 0.01 ± 0.001*** | $0.16 \pm 0.036^{\#\#\#}$ |
| Total % SFA | | 48.87 ± 4.48 | 43.74 ± 18.77 | 50.9 ± 4.2 | 47.65 ± 20.89 |
| Total % MUFA | | 35.96 ± 2.48 | 34.67 ± 14.48 | 35.6 ± 3.1 | 32.25 ± 13.23 |
| Total % PUFA | | 16.27 ± 1.08 | 15.15 ± 6.07 | 13.3 ± 0.7* | 12.0 ± 5.25 |

Values are expressed as the mean ± SEM (nmol/mg or %); $Chow^{Ctrl}$ vs. $HFD^{Ctrl}$ $*p \le 0.05$; $**p \le 0.01$, $***p \le 0.0001$, $HFD^{Ctrl}$ vs. $HFD^{FO}$: $^{\#\#}p \le 0.01$, $^{\#\#\#}p \le 0.001$.

*AA* arachidonic acid, *EPA* eicosapentaenoic acid, *MUFA* monounsaturated fatty acids, *PUFA* polyunsaturated fatty acids, *SFA* saturated fatty acids.

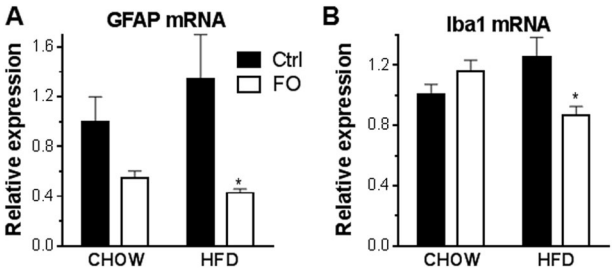



**Fig. 4 Fish oil supplementation suppresses forebrain markers of gliosis. a** mRNA expression of glial fibrillary acidic protein (GFAP) and **b** ionized calcium-binding adapter molecule 1 (Iba-1). Forebrain samples measured by RT-PCR normalized to beta-actin. Values are expressed as the mean ± SEM. Fold change vs. $Chow^{Ctrl}$; $*p \le 0.05$, $n = 9$–12/group.

obesity, including immune activation, impaired hypothalamic–pituitary–adrenal axis activity and neuroendocrine dysfunction [5, 6]. Altered membrane fatty acid composition in individuals with mood disorders has been repeatedly reported and given rise to the phospholipid PUFA hypothesis of depression [52]. In line with our previous observations of striatal inflammation by saturated HFD [13], we hypothesize that elevated brain SFA generates pro-inflammatory responses that can be mitigated by increasing central n-3 PUFA concentrations. One way this can happen is via the effects of phospholipid-derived PUFAs to modulate inflammatory derivatives that contribute to neuroimmune and neural functions. N-3 PUFA and n-6 fatty acids can compete for the same enzymes to significantly regulate these inflammatory products.

Although found in only trace amounts in the brain, DGLA yields anti-inflammatory eicosanoids and competes with AA for cyclooxygenase (COX) and lipoxygenase (LO), inhibiting the production of AA-derived pro-inflammatory eicosanoids. In the present study, DGLA was reduced by HFD and potentiated by FO supplementation, whereas AA was decreased by FO. EPA also competes with AA for COX and LO enzymes to generate less inflammatory products. As a result, the EPA:AA ratio has been used as a marker of chronic inflammation, with a lower ratio corresponding to higher inflammation. Prolonged high-fat feeding reduced the EPA:AA ratio by half, whereas FO produced a near 15-fold increase in this ratio in the HFD condition. Along these lines, new findings demonstrates that over most of the range of plasma levels, AA positively correlates with depression severity and reduced serotonin transport binding in the brain [53]. Thus, our results suggest that increased brain EPA, DPA and DGLA and reduced AA by FO supplementation participate toward dampened neuroimmune activity and alleviation of diet-induced anxiety and despair. This possibility is supported by the observed effects of FO to decrease expression markers of microglia reactivity and astrogliosis, neuroimmune responses to invasion or injury known as reactive gliosis, and are consistent with the neuroprotective effects of increased n-3 PUFA in pro-inflammatory conditions [20, 54]. While DIO has been shown to stimulate a pro-inflammatory state coincident with reactive gliosis [13, 55, 56], Iba-1and GFAP mRNA levels were not significantly elevated by HFD in the present study. We suspect that this is due to assessment of forebrain samples rather than specific brain regions (e.g., hypothalamus or striatum) that are more sensitive to obesity-induced neuroinflammation [57].

In addition to stimulating cellular production of eicosanoid lipid mediators with well-known anti-inflammatory actions [20], EPA can activate GPR120, a G protein-coupled receptor

implicated in the anti-inflammatory and insulin-sensitizing effect of n-3 PUFA in the periphery [39]. Such a possibility is consistent with our previous observations of the anxiolytic actions of central pharmacological GPR120 activation [58]. In parallel or alternatively, n-3 PUFA are known to be agonists and activators of nuclear peroxisome proliferator activated receptor gamma, a receptor that when activated in adipocytes promotes the expression of genes improving glucose metabolism. Nevertheless, in view of the capacity of FO to reduce brain gliosis and enhance select brain n-3 PUFA with established antidepressant actions, the anti-inflammatory impact of n-3 PUFA is likely involved. Future research will be required to determine which of these mechanisms could be involved in the protective effects of FO supplementation on metabolism and mood. Collectively, these data add to a growing literature on the beneficial effects of n-3 PUFA and encourage FO intake to mitigate mood and glycemic dysregulation in obesity.

**Acknowledgements** This work was supported by grants from the Natural Sciences and Engineering Research Council of Canada to SF, Merck Sharp Dohme Corp to SF, TA, and CDR and Réseau cardiométabolique, diabète & obésité from Fonds de Recherche Québec-Santé (CMDO-FRQS) to TA and CDR. GD was supported by a FRQS graduate fellowship, JR by a FRQS postdoctoral fellowship, GF by an INRA fund for mobility/sabbatical stay, and SF and TA are supported by FRQS senior salary awards.

## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

**Publisher's note** Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

## References

1. Gariepy G, Nitka D, Schmitz N. The association between obesity and anxiety disorders in the population: a systematic review and meta-analysis. Int J Obes. 2010;34:407–19.
2. Faith MS, Butryn M, Wadden TA, Fabricatore A, Nguyen AM, Heymsfield SB. Evidence for prospective associations among depression and obesity in population-based studies. Obesity Rev. 2011;12:e438–53.
3. Mannan M, Mamun A, Doi S, Clavarino A. Prospective associations between depression and obesity for adolescent males and females—a systematic review and meta-analysis of longitudinal studies. PLoS ONE. 2016;11:e0157240.
4. Luppino FS, de Wit LM, Bouvy PF, Stijnen T, Cuijpers P, Penninx BW, Zitman FG. Overweight, Obesity, and Depression: A Systematic Reviewand Meta-Analysis of Longitudinal Studies. Arch Gen Psychiatry. 2010;67:220–9.
5. Capuron L, Lasselin J, Castanon N. Role of adiposity-driven inflammation in depressive morbidity. Neuropsychopharmacology. 2017;42:115–28.
6. Hryhorczuk C, Sharma S, Fulton SE. Metabolic disturbances connecting obesity and depression. Front Neurosci. 2013;7:177.
7. Lee ES, Kim YH, Beck SH, Lee S, Oh SW. Depressive mood and abdominal fat distribution in overweight premenopausal women. Obes Res. 2005;13:320–5.
8. Milaneschi Y, Simonsick EM, Vogelzangs N, Strotmeyer ES, Yaffe K, Harris TB, et al. Leptin, abdominal obesity, and onset of depression in older men and women. J Clin Psychiatry. 2012;73:1205–11.
9. Sharma S, Fulton S. Diet-induced obesity promotes depressive-like behaviour that is associated with neural adaptations in brain reward circuitry. Int J Obes. 2013;37:382–9.
10. Yamada N, Katsuura G, Ochi Y, Ebihara K, Kusakabe T, Hosoda K, et al. Impaired CNS leptin action is implicated in depression associated with obesity. Endocrinology. 2011;152:2634–43.
11. Tchernof A, Despres JP. Pathophysiology of human visceral obesity: an update. Physiol Rev. 2013;93:359–404.
12. Santos S, Oliveira A, Lopes C. Systematic review of saturated fatty acids on inflammation and circulating levels of adipokines. Nutr Res. 2013;33:687–95.
13. Decarie-Spain L, Sharma S, Hryhorczuk C, Issa-Garcia V, Barker PA, Arbour N, et al. Nucleus accumbens inflammation mediates anxiodepressive behavior and compulsive sucrose seeking elicited by saturated dietary fat. Mol Metab. 2018;10:1–13.
14. Lai JS, Oldmeadow C, Hure AJ, McEvoy M, Hiles SA, Boyle M, et al. Inflammation mediates the association between fatty acid intake and depression in older men and women. Nutr Res. 2016;36:234–45.
15. Chan KL, Cathomas F, Russo SJ. Central and peripheral inflammation link metabolic syndrome and major depressive disorder. Physiology. 2019;34:123–33.
16. Lassale C, Batty GD, Baghdadli A, Jacka F, Sanchez-Villegas A, Kivimaki M. et al. Healthy dietary indices and risk of depressive outcomes: a systematic review and meta-analysis of observational studies. Mol Psychiatry. 2019;24:965–86 .
17. Li K, Huang T, Zheng J, Wu K, Li D. Effect of marine-derived n-3 polyunsaturated fatty acids on C-reactive protein, interleukin 6 and tumor necrosis factor alpha: a meta-analysis. PLoS One. 2014;9:e88103.
18. Gao H, Geng T, Huang T, Zhao Q. Fish oil supplementation and insulin sensitivity: a systematic review and meta-analysis. Lipids Health Dis. 2017;16:131.
19. Zemdegs J, Rainer Q, Grossmann CP, Rousseau-Ralliard D, Grynberg A, Ribeiro E, et al. Anxiolytic- and antidepressant-like effects of fish oil-enriched diet in brain-derived neurotrophic factor deficient mice. Front Neurosci. 2018;12:974.
20. Laye S, Nadjar A, Joffre C, Bazinet RP. Anti-inflammatory effects of omega-3 fatty acids in the brain: physiological mechanisms and relevance to pharmacology. Pharmacol Rev. 2018;70:12–38.
21. Dang R, Zhou X, Tang M, Xu P, Gong X, Liu Y, et al. Fish oil supplementation attenuates neuroinflammation and alleviates depressive-like behavior in rats submitted to repeated lipopolysaccharide. Eur J Nutr. 2018;57:893–906.
22. Grosso G, Micek A, Marventano S, Castellano S, Mistretta A, Pajak A, et al. Dietary n-3 PUFA, fish consumption and depression: a systematic review and meta-analysis of observational studies. J Affect Disord. 2016;205:269–81.
23. Grosso G, Pajak A, Marventano S, Castellano S, Galvano F, Bucolo C, et al. Role of omega-3 fatty acids in the treatment of depressive disorders: a comprehensive meta-analysis of randomized clinical trials. PLoS ONE. 2014;9:e96905.
24. Deacon G, Kettle C, Hayes D, Dennis C, Tucci J. Omega 3 polyunsaturated fatty acids and the treatment of depression. Crit Rev Food Sci Nutr. 2017;57:212–23.
25. Auguste S, Sharma S, Fisette A, Fernandes MF, Daneault C, Des Rosiers C, et al. Perinatal deficiency in dietary omega-3 fatty acids potentiates sucrose reward and diet-induced obesity in mice. Int Soc Dev Neurosci. 2018;64:8–13.
26. Mathieu G, Denis S, Lavialle M, Vancassel S. Synergistic effects of stress and omega-3 fatty acid deprivation on emotional response and brain lipid composition in adult rats. Prostaglandins Leukot Essent Fatty Acids. 2008;78:391–401.

27. Innis PMK-EaS. Position of the american dietetic association and dietitians of canada: dietary fatty acids. J Am Dietetic Association. 2007;107:1599.e1–15.
28. Nair AB, Jacob S. A simple practice guide for dose conversion between animals and human. J Basic Clin Pharm. 2016;7:27–31.
29. Gelinas R, Thompson-Legault J, Bouchard B, Daneault C, Mansour A, Gillis MA, et al. Prolonged QT interval and lipid alterations beyond beta-oxidation in very long-chain acyl-CoA dehydrogenase null mouse hearts. Am J Physiol Heart Circ Physiol. 2011;301:H813–23.
30. Turcot V, Brunet J, Daneault C, Tardif JC, Des Rosiers C, Lettre G. Validation of fatty acid intakes estimated by a food frequency questionnaire using erythrocyte fatty acid profiling in the Montreal Heart Institute Biobank. J Hum Nutr Dietetics. 2015;28:646–58.
31. Thompson JA, Larion S, Mintz JD, Belin de Chantemele EJ, Fulton DJ, Stepp DW. Genetic deletion of NADPH oxidase 1 rescues microvascular function in mice with metabolic disease. Circ Res. 2017;121:502–11.
32. Howe P, Buckley J. Metabolic health benefits of long-chain omega-3 polyunsaturated fatty acids. Military Med. 2014;179:138–43.
33. Thesing CS, Bot M, Milaneschi Y, Giltay EJ, Penninx B. Omega-3 and omega-6 fatty acid levels in depressive and anxiety disorders. Psychoneuroendocrinology. 2018;87:53–62.
34. Belzung F, Raclot T, Groscolas R. Fish oil n-3 fatty acids selectively limit the hypertrophy of abdominal fat depots in growing rats fed high-fat diets. Am J Physiol. 1993;264:R1111–8.
35. Huang XF, Xin X, McLennan P, Storlien L. Role of fat amount and type in ameliorating diet-induced obesity: insights at the level of hypothalamic arcuate nucleus leptin receptor, neuropeptide Y and pro-opiomelanocortin mRNA expression. Diabetes Obes Metab. 2004;6:35–44.
36. Burdge GC, Calder PC. Dietary alpha-linolenic acid and health-related outcomes: a metabolic perspective. Nutr Res Rev. 2006;19:26–52.
37. Oliveira V, Marinho R, Vitorino D, Santos GA, Moraes JC, Dragano N, et al. Diets Containing alpha-linolenic (omega3) or Oleic (omega9) fatty acids rescues obese mice from insulin resistance. Endocrinology. 2015;156:4033–46.
38. Kasbi Chadli F, Andre A, Prieur X, Loirand G, Meynier A, Krempf M, et al. n-3 PUFA prevent metabolic disturbances associated with obesity and improve endothelial function in golden Syrian hamsters fed with a high-fat diet. Br J Nutr. 2012;107:1305–15.
39. Oh DY, Talukdar S, Bae EJ, Imamura T, Morinaga H, Fan W, et al. GPR120 is an omega-3 fatty acid receptor mediating potent anti-inflammatory and insulin-sensitizing effects. Cell. 2010;142:687–98.
40. Datilo MN, Sant'Ana MR, Formigari GP, Rodrigues PB, de Moura LP, da Silva ASR, et al. Omega-3 from Flaxseed Oil Protects Obese Mice Against Diabetic Retinopathy Through GPR120 Receptor. Sci Rep. 2018;8:14318.
41. Lopez-Alarcon M, Inda-Icaza P, Marquez-Maldonado MC, Armenta-Alvarez A, Barbosa-Cortes L, Maldonado-Hernandez J. A randomized control trial of the impact of LCPUFA-omega3 supplementation on body weight and insulin resistance in pubertal children with obesity. Pediatr Obes. 2019;14:e12499.
42. Helland A, Bratlie M, Hagen IV, Mjos SA, Sornes S, Ingvar Halstensen A, et al. High intake of fatty fish, but not of lean fish, improved postprandial glucose regulation and increased the n-3 PUFA content in the leucocyte membrane in healthy overweight adults: a randomised trial. Br J Nutr. 2017;117:1368–78.
43. Rodriguez-Navas C, Morselli E, Clegg DJ. Sexually dimorphic brain fatty acid composition in low and high fat diet-fed mice. Mol Metab. 2016;5:680–9.
44. Hryhorczuk C, Florea M, Rodaros D, Poirier I, Daneault C, Des Rosiers C, et al. Dampened mesolimbic dopamine function and signaling by saturated but not monounsaturated dietary lipids. Neuropsychopharmacology. 2016;41:811–21.
45. Tsuboi H, Watanabe M, Kobayashi F, Kimura K, Kinae N. Associations of depressive symptoms with serum proportions of palmitic and arachidonic acids, and alpha-tocopherol effects among male population–a preliminary study. Clin Nutr. 2013;32:289–93.
46. Holman HMaRT. Alteration of the fatty acid composition of brain lipids by varying levels of dietary essential fatty acids. J Neurochem. 1963;10:523–30.
47. Naliwaiko K, Araujo RL, da Fonseca RV, Castilho JC, Andreatini R, Bellissimo MI, et al. Effects of fish oil on the central nervous system: a new potential antidepressant? Nutr Neurosci. 2004;7:91–9.
48. Chen CT, Bazinet RP. beta-oxidation and rapid metabolism, but not uptake regulate brain eicosapentaenoic acid levels. Prostaglandins Leukot Essent Fatty Acids. 2015;92:33–40.
49. Su KP, Lai HC, Yang HT, Su WP, Peng CY, Chang JP, et al. Omega-3 fatty acids in the prevention of interferon-alpha-induced depression: results from a randomized, controlled trial. Biol Psychiatry. 2014;76:559–66.
50. Dantzer R, O'Connor JC, Freund GG, Johnson RW, Kelley KW. From inflammation to sickness and depression: when the immune system subjugates the brain. Nat Rev Neurosci. 2008;9:46–56.
51. Song C, Manku MS, Horrobin DF. Long-chain polyunsaturated fatty acids modulate interleukin-1beta-induced changes in behavior, monoaminergic neurotransmitters, and brain inflammation in rats. J Nutr. 2008;138:954–63.
52. Lin PY, Huang SY, Su KP. A meta-analytic review of polyunsaturated fatty acid compositions in patients with depression. Biol Psychiatry. 2010;68:140–7.
53. Gopaldas M, Zanderigo F, Zhan S, Ogden RT, Miller JM, Rubin-Falcone H, et al. Brain serotonin transporter binding, plasma arachidonic acid and depression severity: a positron emission tomography study of major depression. J Affect Disord. 2019;257:495–503.
54. Delpech JC, Madore C, Joffre C, Aubert A, Kang JX, Nadjar A, et al. Transgenic increase in n-3/n-6 fatty acid ratio protects against cognitive deficits induced by an immune challenge through decrease of neuroinflammation. Neuropsychopharmacology. 2015;40:525–36.
55. Thaler JP, Yi CX, Schur EA, Guyenet SJ, Hwang BH, Dietrich MO, et al. Obesity is associated with hypothalamic injury in rodents and humans. J Clin Investig. 2012;122:153–62.
56. Morselli E, Fuente-Martin E, Finan B, Kim M, Frank A, Garcia-Caceres C, et al. Hypothalamic PGC-1alpha protects against high-fat diet exposure by regulating ERalpha. Cell Rep. 2014;9:633–45.
57. de Mello AH, Schraiber RB, Goldim MPS, Garcez ML, Gomes ML, de Bem Silveira G, et al. Omega-3 fatty acids attenuate brain alterations in high-fat diet-induced obesity model. Mol Neurobiol. 2019;56:513–24.
58. Auguste S, Fisette A, Fernandes MF, Hryhorczuk C, Poitout V, Alquier T. et al. Central agonism of GPR120 acutely inhibits food intake and food reward and chronically suppresses anxiety-like behavior in mice. Int J Neuropsychopharmacol. 2016;19:1–10.

## Chapter 4: DISCUSSION and CONCLUSION

### 4.1. Discussion

Several lines of evidence demonstrate the protective effects of n-3 supplementation on metabolism and mood. We previously showed that FO supplementation in male mice protects against some of the metabolic and behavioral consequences of diet-induced obesity [88]. GPR120 partly mediates the anti-inflammatory effects of n-3 in peripheral tissues, which has generated considerable interest in GPR120 as a therapeutic target. As a LCFA receptor, GPR120 can be activated by natural and synthetic agonists. We previously reported that chronic pharmacological stimulation of GPR120 in the brain suppresses anxiety-like behavior in high-fat fed mice [301]. It remained unclear how GPR120 regulates SHFD-induced changes in mood-related behaviors. Fish oil supplementation provides n-3, which can act as a natural agonist for GPR120 that is hypothesized to reduce inflammatory tone in the brain, particularly in microglia as a central player in neuroinflammation.

Furthermore, we showed that FO supplementation in DIO improves mood and reduced sickness-like behaviors that are known to be related to neuroinflammation. In the current study, we aim to characterize the expression pattern of brain GPR120 and to investigate its potential role in neuroinflammatory responses in microglia in vitro. To further explore these actions, we also sought to elucidate the role of central GPR120 to attenuate neuroinflammation and regulate LPS-induced sickness behaviors.

**GPR120 is predominantly expressed in microglia**

GPR120 is expressed in different regions of brain with varying levels of mRNA expression [127, 149]. Our current study provides evidence of enriched expression of GPR120 in primary microglia isolated from pups' brain, compared to other glial cells, including primary astrocytes, neuronal hypothalamic cell line (N46) and whole brain in mice. Moreover, we demonstrated that GPR120 expression is much higher in microglia (YFP+) as opposed to non-microglial cells (astrocyte and neuron) isolated from adult Cx3cr1CreER-YFP mice.

On the other hand, we showed, both GPR120 and GPR40, as the two main LCFA receptors, are expressed in primary microglia. The mRNA expression level of GPR-40 is much lower (approximately four times less) than GPR120 (Appendix 1). Consistent with our results, Dragano et al. study showed that both receptors are expressed in the hypothalamus, but the hypothalamic expression of GPR120 corresponds to microglia, and GPR40 corresponds to neurons, especially POMC and NPY neurons in obese mice [280]. Indeed, our previous studies demonstrated relatively high expression of GPR120 mRNA in the ARC and NAc, a brain region well implicated in the control of motivation and mood, with lesser and minimal expression in the hippocampus and VTA. Additionally, GPR120 was found to be co-expressed with neuropeptide-Y in the ARC of the hypothalamus. Furthermore, direct activation of GPR120 via an agonist (GPR120 Agonist III) by ICV injections in the hypothalamus showed substantially reduced anxiety-like behaviors in SHFD mice [301]. From these lines of evidence, we could hypothesize that GPR120 is more related to anti-inflammatory responses and the modulation of depression in comparison to GPR40, and for this reason greater expression of GPR120 in microglia may correspond to functional roles in inflammation and regulation of anxiety-like behaviors.

**GPR120 activation alters LPS-induced changes in microglial morphology**

The activation of microglia has been noted in many studies to induce inflammation and brain function [326]. In addition, studies demonstrated microglia morphology is highly associated with its function [361-363]. However, the possibility exists that microglia morphological changes may impact neural functions independently of observed reactivity characteristics [326]. In the current study, we also report the morphological differences in cultured primary microglia following treatment with LPS and LPS + cpdA. According to the previous results, we chose low concentration of LPS (<100 ng/mL) for its reliability to alter microglial morphology and functions [327, 328]. Microglia assume an amoeboid form with fewer branches when exposed to LPS, alone. LPS-mediates alterations in microglia morphometric features at the single-cell level [329]. Pre-treatment with CpdA changes microglia morphology and

downregulated rounded/amoeboid-like shape to favor the transition of microglia. Also, we examined the percentage of cells with processes as a feature of active amoeboid cells, as well as the number of the microglial cell processes that were significantly increased by cpdA treatment in comparison to LPS treatment alone. These findings demonstrate that GPR120 stimulation may regulate neuroinflammation and neural function by microglial reactivation and pro-inflammatory morphological changes. Of note, these morphological analysis results based on visual characterization of microglia found that while LPS reduced the number of cells with processes, leading to amoeboid-like shaped microglia, CpdA visibly blunted these characteristic microglial morphological changes.

**GPR120 agonism by CpdA decreases LPS-induced microglial inflammatory markers**

The anti-inflammatory function of microglial GPR120 was tested in vitro by stimulating the receptor on WT cells. In our in vitro experiments, primary microglial cells were initially exposed to CpdA (10 μM) [330, 331] for 1h, followed by LPS (100 ng/ml) for 6h [331] as a positive control to induce acute inflammation. Consistently, LPS stimulation increased the levels of TNF-α, IL-6, IL-1β, and MCP-1 expression. Furthermore, our results agree with prior findings demonstrating the activation of expression and release of pro-inflammatory cytokines following an LPS administration [262, 332]. Stimulation of GPR120 by CpdA resulted in a significant reduction in the gene expression IL-1β, IL-6, MCP-1, and TNF-α, and the protein expression of IL-6, MCP -1 and TNF-α. But, we didn't see significant changes in IL-1β secretion. Thus, by these results, microglial GPR120 has anti-inflammatory properties and can reduce neuroinflammation by its agonist. Our first hypothesis to explain the lack of protein secretion of IL-1β may relate to the specifics of the IL-1β secretion mechanism. IL-1β is secreted through an unconventional secretion pathway or non-classical protein export. This means that it does not have a leader sequence required for secretion through the ER/Golgi pathway [333]. In addition, IL-1β secretion is related to the NLRP3 activation mechanism by two signals, including priming and activation signals [334]. Our result demonstrated the Nf-kb gene expression decreases by CpdA in LPS –induced inflammation microglia (figure 4F). CpdA may have the regulatory effect to

decrease pro-IL-1β mRNA gene expression by inhibit Nf-kb pathway in microglia. However, for the secretion of mature IL-1β, the NLRP3 inflammasome complex activation is needed, and the regulatory effect of GPR120 agonism is not clear in NLRP3 inflammasome complex activity. Thus, more experiments are needed to explore the related mechanisms.

**Long-chain unsaturated fatty acids suppress LPS-induced microglial inflammatory markers**

In the in vitro experiment, primary microglial cells were exposed to the same amount of synthetic (cpdA) and natural agonist (EPA, DHA, ALA, OA) of GPR120 (10 μM) [330, 331] for 1h, followed by LPS (100 ng/ml) for 6h [331] to induce acute inflammation. Consistently, LPS stimulation increased the levels of TNF-α, IL-6, IL-1β, NFkB and MCP-1 whereas cpdA treatments reduced cytokines and chemokine expression in primary microglial cells. There was a significant reduction in LPS-induced gene expression for IL6, IL-1β, MCP-1, and NFkB by LCUFA. Our results agree with previous findings demonstrating that DHA and EPA inhibit inflammatory responses in macrophages and microglia [330, 335]. Overall, the anti-inflammatory effect between treatments on LPS-induced pro-inflammatory cytokine expression in microglia suggests that cpdA is more effective than EPA, DHA, ALA, and OA. It was predictable by the previous experiments results in macrophages which shown, CpdA agonist was more potential than DHA to stimulate GPR120 [330, 336, 337].

**DHA demonstrated anti-inflammatory effect on microglia**

In the primary cultured microglia, DHA reduces LPS-induced pro-inflammatory cytokines mRNA expression. In this regard, some evidence demonstrated that DHA decreased inflammation levels by different pathways in microglia, including inhibition of pro-inflammatory microglial activation [338], promotion of anti-inflammatory microglial responses [339], and increased microglial phagocytosis [340]. However, to explain the anti-inflammatory activity of n-3 PUFAs, the different mechanisms have been suggested which are at least partly connected and act in parallel. The line of evidences from studies, have shown the protective effect of DHA by transformed into resolvins and protectins, which are considered to mostly regulate the extent

and magnitude of inflammatory processes. DHA derived SPMs which demonstrated some anti-inflammatory effect on microglia. For example, RvD1 [339] and RvD2 [264] decreased LPS-induced pro-inflammatory cytokine (TNF-α, IL-6 and IL-1β) gene expression in microglial BV2 cells via the modulation of miRNAs, and also, in primary microglia via the TLR4/ NFκB pathway, respectively, with RvD1 enhancing anti-inflammatory cytokine (IL-4, Arg1, and Ym1) expression [339]. By the evidence from the other experiment result in cultured microglial cells, DHA reduced LPS-induced pro-inflammatory cytokines TNF-α, IL-1β, IL-6 and MCP-1 production through inhibition of p38/MAPK activation [338]. In addition, EPA and DHA have been found to directly interact with their receptors such as GPR120 and PPAR-γ [341] which are critical regulators of inflammation. In this context, Ren et al. demonstrated the anti-inflammatory effects of DHA correlated with GPR120 interactions with β-arrestin2 in an in vitro model of oxygen-glucose deprivation (OGD)-induced inflammatory responses in primary microglia and murine microglial BV2 cells [320]. With these result, we could hypothesize GPR120 activation by DHA may be related to the observed anti-inflammatory effect. However, for mechanistic understanding of DHA anti-inflammatory effects on inflammation induced on microglial cells more experiments are needed. Experiments with GPR120 knockout in microglia could be used to more precisely evaluate the role of GPR120 with cellular and molecular specificity.

**EPA has shown anti-inflammatory effect on microglia**

EPA was another n-3 fatty acid that we used in vitro treatments. EPA reduced all cytokines mRNA expression, which we tested. Among the FAs treatments, only EPA significantly decreased TNF-α gene expression. It may be related to the more anti-inflammatory potential of this FA in microglia regulation. In consist with our result, the treatments with EPA (200 μM) alone and EPA plus DHA (100 μM each) significantly suppressed the LPS-induced TNF-α pro-inflammatory cytokine release in the BV-2 microglial cell line [342]. Microglia are one of the main sources of TNF-α in response to an inflammatory stimulus in the CNS [343]. TNF-α is an early cytokine expressed by cells in response to LPS stimulation, and TNF upregulates the NF-κB signaling pathway and the expression of inflammatory cytokine production, including IL-6 and

IL-1β [344, 345]. The microglia-derived TNF-α autocrine activation occurs via TNFR1 signaling pathway that amplifying its production and release [343].

On the other hand, LPS induced pro-inflammatory cytokines impaired autophagy processes in microglial cells through the activation of the PI3KI/AKT/mTOR pathway [346]. Microglial activity downregulates autophagy [180], which facilitates the shift of microglia from an M1-to-M2 phenotype and suppresses inflammation in microglia [347]. Also, there are negative interactions between autophagy and NALP3 inflammasome activation in the activation of microglia and the subsequent release of active IL-1β [348]. Further, EPA supplementation deactivated microglia activation in aged rats and mice [349, 350] and their derived compounds, such as RvE1, that demonstrated strong anti-inflammatory effects by inhibiting microglial activation via the NFκB signaling pathway and reduction of LPS-induced pro-inflammatory cytokine (TNF-α, IL-6 and IL-1β) gene expression in murine BV-2 microglial cells [339] as well as the regulation of the M2 phenotype in microglial [339, 351]. Also, RvE1 alleviates depression-like behavior through the activation of mammalian target of rapamycin complex 1(mTORC1) as a regulator of mitochondrial functions in models of depression.

Furthermore, EPA induced autophagy through GPR120-mediated AMPK/mTOR signaling in murine bone marrow-derived mesenchymal stem cells [352]. In the most recent experiment by Mo et al. (2020), GPR40 and GPR120 activation induced by EPA played an essential role in the inhibition of the NLRP3 inflammasome, in mice and BV2 microglia cells [322]. It seems EPA and activation of GPR40 and GPR120 receptors in macrophages and microglia attenuates microglial activity and upregulates autophagy (AMPK/mTOR pathway) [180], which is involved in facilitating the shift of microglia from an M1-to-M2 phenotype and suppression of inflammation (inhibition of TLR4 and NLRP3 inflammasome pathway) in microglia to alleviate depression-like behavior. These evidences suggest that EPA suppression of the inflammatory signaling pathway, deactivation of microglial activation and regulation of the autophagy are involved, to regulate microglial function**.** In the interesting research report by Rapaport et al., it was demonstrated

that subjects with major depression and a high number of inflammatory biomarkers had a better response to EPA than the placebo and a lower response to DHA than the placebo [258].

Also, in the other recent study by Peng et al., for the first time, a comparison of EPA and DHA anti-depressant effects was reported on a chronic stress-induced rat model of depression [353]. Their findings indicated both EPA and DHA supplementation significantly attenuated microglia marker CD11b and NF-κB expression in the hippocampus and modulated microglial activity, but EPA was better than DHA to down-regulate NF-KB expression in depression rat models. Base on their experiment results, EPA supplementation, reduced IL-1β, IL-6, and TNF-α pro-inflammatory cytokine levels, but DHA only decreased IL-1β. On the other hand, the EPA was shown more efficacious than DHA to regulate apoptosis signaling in the hippocampus and depression-like behaviors in a rat model of depression [353].

EPA is far less abundant than DHA in the brain and many other tissues. However, the exact mechanism that is responsible remains unclear, but it may support the more sensitive and anti-inflammatory effect of EPA that other n-3 fatty acids exert on microglia in the control of neuroinflammation and depression. Given all of these results together, we could hypothesize that a greater potential effect of EPA in comparison to other n-3 fatty acids in the control of depression may depend on the more potent anti-inflammatory effect of this fatty acid on the microglia as a main player of neuroinflammation and depression symptoms.

**ALA Impacts on Inflammation in microglial cells**

Another n-3 fatty acid treatment for in vitro study was ALA, which showed a significant reduction in LPS-induced gene expression of pro-inflammatory cytokines IL-1β, IL-6, MCP-1, and Nf-kB. Our results did not show a substantial decrease in TNF-α mRNA expression by ALA. In contrast, in the recent study by Carey et al., in 2020 [354], walnut extract (containing approximately 2.01 μM of ALA and 12.48 μM of LA attenuated LPS-induced increases in TNF-α when used both before and after LPS exposure in BV-2 murine microglial cells. However, as they used walnut extract and not pure ALA, it is possible this result could be due to the synergic effect of other compounds and not only ALA. Our results highlighted the anti-inflammatory effect of ALA in microglia but in lower levels in comparison to EPA and DHA in response to LPS.

**Oleic Acid Impacts on Inflammation in microglial cells**

In the current experience, OA (n-9), the main fatty acid of olive oil sources decreases IL-1β, IL-6, and MCP-1 pro-inflammatory cytokine expression in microglial cells but with much less anti-inflammatory potential than n-3. Consistent with our results, an in vitro study using OA (100 μM) shows its anti-inflammatory effect by inhibiting NF-κB signaling pathways and pro-inflammatory mediators induced by LPS in BV2 microglia [355]. A study by Khatchadourian et al. demonstrated that OA strongly induced lipid droplet formation and enhancement of lipid droplet size distribution in microglia [356], which led to protection against AA release [356] as well as prevented lipid peroxidation at the membrane level and reduced oxidative stress-mediated inflammatory signaling [357]. Another study by Rohwedder and colleges showed that lipid droplet formation in response to OA is mediated by GPR120 in human hepatoma cell lines (Huh-7 cells) [358]. By all this evidence, we can hypothesize that OA may generate a protective phenotype in microglia by inducing enrichment in PUFA within lipid droplets in microglia via GPR120 activation and oxidative balance [359].

**IL-10 anti-inflammatory cytokine expression highly induced by DHA**

In our experiment, the IL-10 mRNA expression level was highly expressed only by DHA treatment in vitro. It may suggest DHA is more potent in increasing IL-10 expression in comparison to other n-3. IL-10 is considered one of the most critical anti-inflammatory cytokines, mainly produced by astrocytes and microglia to balance pro and anti-inflammatory cytokine levels in the CNS [360, 361]. IL-10 is considered a potent negative autocrine regulator of microglia [362, 363]. Also, IL-10 is critical for maintaining regular neuro-immune communication and behavioral effects such as sickness behavior during infection [364]. LPS upregulated the IL-10 signaling molecules in primary microglia of mice [365]. In addition, IL-10 acts through the activation of its receptor (IL-10R). It can disrupt the LPS-mediated increases in the expression of IL-1β, and IL-6 in microglia [360, 366], by inducing anti-inflammatory elements, such as Bcl3 and Socs3 to inhibit NF-κB-dependent signals [367-369] subsequently

and also promote cell survival by expressing anti-apoptotic factors (e.g., Bcl-2 and Bcl-xl) [367-369]. Overall, IL-10 KO microglia showed a significantly higher number of TNF-α- and IL-6 after LPS stimulation than WT microglia [370]. Furthermore, IL-10 altered microglial morphology and restored their migration in LPS-treated microglial cells [370, 371]. DHA pre-treatment decreased pro-inflammatory cytokines (TNF-α and IL-6) and increased IL-10 expression in human monocyte cell line THP-1-derived macrophages [372]. On the other hand, hypothalamic GPR120 activation by intracerebroventricular (ICV) injection of a GPR120 agonist (TUG1197) promoted IL-10 anti-inflammatory gene expression and decreased the production of pro-inflammatory cytokines TNFα and IL-1β [280]. Therefore, based on these lines of evidence, we can hypothesize that the microglial GPR120 activation may correlate our results about DHA-induced changes in IL-10 expression and anti-inflammation responses. However, more research is needed to find a suitable mechanism because DHA has different anti-inflammatory pathways to explain this observation.

**Iba-1 gene expression significantly decreased in all GPR120 agonists**

Ionized calcium binding adaptor molecule 1 (Iba1) is considered a microglia activation marker, with expression strongly upregulated in activated microglia [373]. Iba1 participates in membrane ruffling and phagocytosis in activated microglia. Microglia membrane ruffling is essential for the morphological changes in microglia from quiescent ramified to amoeboid [374]. Our study has shown that GPR120 activation by LCUFAs and CpdA strongly decreased Iba-1 gene expression in microglial cultures. This reduction is consistent with our morphological differences seen in cultured primary microglia following treatment with LPS+CpdA. We could suggest that these anti-inflammatory effects may be accompanied by microglia deactivation. [375].

**Central GPR120 activation inhibits brain inflammation and LPS-induced sickness behaviors**

To determine the precise role of the GPR120 receptor, we used a specific synthetic ligand for this receptor to assess the anti-inflammatory and behavioral changes in an acute model of inflammation in male mice. Numerous studies have demonstrated that inflammation is involved in the pathogenesis of depression. In the animal study, we used LPS (0.83 mg/kg at 2h post-

systemic injection) to induce the peripheral and acute inflammation. This dose was selected as a minimal effective dose to induce anxiety and depressive-like behavior in mice [376]. Zhao et al. reported that IP injections of LPS at 0.5 mg/kg and 0.75 mg/kg doses (6 h before each daily test) was sufficient to reveal the occurrence of inflammation TNF-α and IL-1β gene expression in the serum and brain homogenates [377]. These effects were accompanied by microglial activation along with associated sickness behavior and cognitive impairments in C57BL/6J mice [378]. In another study, IP injections of 1.0 mg/kg LPS (24 h after the first injection) significantly increased pro-inflammatory cytokines expression (TNF-α and IL-1β) in the hypothalamus, microglial activation in the NAc and induced mild sickness behavior in C57BL/6J mice [378]. Our finding demonstrated IP injections of LPS significantly induced IL-1β and IL-6 gene expression level in comparison to control gene expression in the NAc [379]. The present study result demonstrated LPS injection significantly decreases the velocity and distance travelled in elevated plus-maze behavior tests and showed the fewer entire and time spent percentage in the lit compartment in light/dark box (LDB) test, as well. Therefore, based on these results, we hypothesize that IP injection of LPS may induce microglial activation and neuroinflammation in the NAc with associated behavior alterations in mice. Moreover, pre-treatment with cpdA (ICV injection) modulated cytokines expression in LPS induced anxiety-like behavior and hypolocomotion in the mice NAc. These results suggest that cpdA may alleviate sickness behavior through the anti-inflammatory effect.

### 4.2. Limitation and future perspectives

LC-UFAs are agonists for multiple receptors and are not exclusive agonists to GPR120; thus, the observed reduction in inflammation could represent a synergistic effect between them. On the other hand, the protective and anti-inflammatory effect of n-3 fatty acids passes through various pathways, including those of the PPAR family [283]. Therefore, it is difficult to conclude microglial GPR120 as entirely responsible for the anti-inflammatory effect of n-3 FAs. Thus, to validate the involvement of GPR120 in the in vivo part, we can repeat the same diet experiment on transgenic CX3CR1CreER / +; GPR120 fl / fl mice whose GPR120 gene is invalidated. To

validate the involvement of microglial GPR120 in the in vitro part, we can use KO microglial cells come from transgenic CX3CR1CreER / +; GPR120fl / fl mice in which the invalidation of GPR120 is specific to CX3CR1 + cells, or microglia cells at the central level. By comparing the effect of FAs in these conditional KO mice compared to WT mice, will reflect the impact of the selective absence of the receptor on metabolism and behavioral changes. Also, the in vitro experiments on the microglial knock-out GPR120 gene made it possible to invalidate only the microglial GPR120 in comparison to the results obtained on the WT cells and its possible role in inflammation regulation.

In addition, for in vitro experiments, the preventive effect of GPR120 on inflammation was studied by exposing microglia first to agonists (CpdA and UFAs) in the same amount to compare inflammation regulation than to LPS. It would be interesting to repeat these experiments by LPS followed by CpdA and see if the results obtained are comparable. It would allow studying the effect of stimulating GPR120 in a medium with significant inflammation and seeing if it can reverse inflammation.

Indeed, in our study, we use IP injection of LPS, but we couldn't determine with certainty whether the microglia present in the CNS is exposed to LPS injected into the periphery or by pro-inflammatory cytokines and if activation of their receptors can diffuse through the BBB. However, LPS consider as a more commonly used pro-inflammatory stimulus for microglia (both in vitro and in vivo) and induces neuroinflammation, but it is not representative for physiological for DIO situation. Because the plasma level of cytokines is induced in parallel with LPS, which can affect neuroinflammation. It would be interesting to repeat a similar methodology by applying a mixture of cytokines to the brain through ICV infusion/injection (in vivo) [380] to check whether the observed phenotype is comparable. LPS is the most common stimulus to pro-inflammatory responses in microglia, both in vitro and in vivo; but, SFAs such as palmitate, in high-fat DIO can increase other pro-inflammatory stimuli such as cytokines (e.g., IFNγ, TNFα) that may be more relevant to the stimulation of microglia [371]. The recent study by Zhao et al, induced the pro-inflammatory reaction in mice serum and brain by LPS, ICV (12 µg), or Ip (500 mg/kg and 750g/kg) injection. LPS group in compared to the saline group in per

group of injection (ICV and IP) were shown significant neuronal cell loss and microglial activation (Iba-1) in the hippocampus [377]. We can use the ICV infusion/injection of a cytokine or mixture of some stimulus cytokines that bind to receptors on microglia, including IFNγ and TNFα, to induce neuroinflammation [371]. We can also use the ICV inject of GPR120 agonists (synthetic and natural) to determine and compare the anti-inflammatory function and neuroinflammation regulation. On the other hand, we can use the mixture of cytokines as a stimulus for inflammation in primary microglia culture to assess the GPR120 agonist anti-inflammatory function.

On the other hand, there is a multi-complex cross-talk between glial cells and neurons to regulate metabolism in the brain. Microglial function results cannot indicate whole-brain function and action in the same situation; thus, it is better than in the subsequent research; we use a co-culture of microglial cells with astrocytes and neurons.

Moreover, the mixture of EPA + DHA shows a more effective inhibitory effect than either DHA or EPA alone on the expression of inflammatory genes in LPS-stimulated human THP-1 macrophages. DHA shows more potential than EPA [381]. Also, EPA and DHA show synergic effects in depression modulation. Therefore, it will be interesting to study the impact of the combination of UFAs in comparison to each FA used alone on the microglial activity, inflammation control and mood regulation. Although n-3 FAs are agonists of GPR120, it is difficult at this time to confirm the involvement of this receptor in the in vivo changes induced by these fatty acids. Subsequent studies should focus more on how GPR120 is involved in the effects of n-3 and obesity/mood in vivo and the mechanisms in place.

### 4.3. Conclusion

While the role of dietary n-3 FAs in regulating both mood and metabolic function through impacts on neuroinflammation is increasingly appreciated, the precise role of GPR120 function within the brain in these processes remains incompletely understood. In this study the role of central GPR120 to attenuate neuroinflammation and regulate mood-related neuro-behavioral processes was investigated using a combination of dietary, pharmacological, biochemical, and behavioral approaches. In the present study, we find that the central GPR120 activation attenuated neuroinflammation in the NAc. We also showed that GPR120 expression is enriched within microglial cells and that GPR120 moderates morphological alteration and inflammatory responses in microglial cells. Consistent with this, we observed that central GPR120 prevents inflammation-related sickness behavior. These findings advance our knowledge on the role of GPR120 within the brain, present new avenues for research into the interconnections between neuroinflammation, diet, metabolism, and mood, and suggest GPR120 may potentially serve as a promising novel target relevant for the treatment. Further studies will be required to elucidate the role of GPR120 activation in the resolution of obesity and associated metabolic inflammation.

**Chapter 5: REFERENCES**


1. Bluher, M., *Metabolically Healthy Obesity.* Endocr Rev, 2020. 41(3): p. 405-420.
2. Chooi, Y.C., C. Ding, and F. Magkos, *The epidemiology of obesity.* Metabolism, 2019. 92: p. 6-10.
3. García-Jiménez, C., et al., *From obesity to diabetes and cancer: epidemiological links and role of therapies.* Br J Cancer, 2016. 114(7): p. 716-22.
4. Kivimäki, M., et al., *Body mass index and risk of dementia: Analysis of individual-level data from 1.3 million individuals.* Alzheimers Dement, 2018. 14(5): p. 601-609.
5. Emery, C.F., et al., *Evidence of Bidirectional Associations Between Depressive Symptoms and Body Mass Among Older Adults.* The Journals of Gerontology: Series B, 2019.
6. Fourrier, C., et al., *Brain tumor necrosis factor-α mediates anxiety-like behavior in a mouse model of severe obesity.* Brain Behav Immun, 2019. 77: p. 25-36.
7. Bray, G.A., et al., *Obesity: a chronic relapsing progressive disease process. A position statement of the World Obesity Federation.* Obes Rev, 2017. 18(7): p. 715-723.
8. Vittengl, J.R., *Which body shape dimensions and sizes predict depression?* Journal of Affective Disorders, 2019. 250: p. 193-198.
9. Pereira-Miranda, E., et al., *Overweight and Obesity Associated with Higher Depression Prevalence in Adults: A Systematic Review and Meta-Analysis.* Journal of the American College of Nutrition, 2017. 36(3): p. 223-233.
10. Jorm, A.F., et al., *Association of obesity with anxiety, depression and emotional well-being: a community survey.* Aust N Z J Public Health, 2003. 27(4): p. 434-40.
11. Lim, S.Y., L.M. da Conceição, and S.G. Camorlinga, *A Study on the Modeling of Obesity*, in *Embracing Complexity in Health*. 2019, Springer. p. 307-320.
12. Kelly, T., et al., *Global burden of obesity in 2005 and projections to 2030.* International Journal of Obesity, 2008. 32(9): p. 1431-1437.
13. Bancej, C., et al., *Evidence Brief--Trends and projections of obesity among Canadians.* Health Promot Chronic Dis Prev Can, 2015. 35(7): p. 109-12.
14. Finkelstein, E.A., et al., *Obesity and severe obesity forecasts through 2030.* American journal of preventive medicine, 2012. 42(6): p. 563-570.
15. Collaboration, N.C.D.R.F., *Worldwide trends in body-mass index, underweight, overweight, and obesity from 1975 to 2016: a pooled analysis of 2416 population-based measurement studies in 128.9 million children, adolescents, and adults.* Lancet, 2017. 390(10113): p. 2627-2642.
16. Gurunathan, U. and P.S. Myles, *Limitations of body mass index as an obesity measure of perioperative risk.* Br J Anaesth, 2016. 116(3): p. 319-21.
17. Kadouh, H.C. and A. Acosta, *Current paradigms in the etiology of obesity.* Techniques in Gastrointestinal Endoscopy, 2017. 19(1): p. 2-11.

18. Lee, E.Y. and K.-H.J.F.o.m. Yoon, *Epidemic obesity in children and adolescents: risk factors and prevention.* 2018. 12(6): p. 658-666.
19. Yatham, S., et al., *Depression, anxiety, and post-traumatic stress disorder among youth in low and middle income countries: A review of prevalence and treatment interventions.* Asian Journal of Psychiatry, 2018. 38: p. 78-91.
20. James, S.L., et al., *Global, regional, and national incidence, prevalence, and years lived with disability for 354 diseases and injuries for 195 countries and territories, 1990–2017: a systematic analysis for the Global Burden of Disease Study 2017.* The Lancet, 2018. 392(10159): p. 1789-1858.
21. Ljungberg, T., E. Bondza, and C. Lethin, *Evidence of the Importance of Dietary Habits Regarding Depressive Symptoms and Depression.* Int J Environ Res Public Health, 2020. 17(5).
22. Mnookin, S., *Out of the shadows: Making mental health a global development priority*. 2016, The World Bank.
23. Disease, G.B.D., I. Injury, and C. Prevalence, *Global, regional, and national incidence, prevalence, and years lived with disability for 310 diseases and injuries, 1990-2015: a systematic analysis for the Global Burden of Disease Study 2015.* Lancet, 2016. 388(10053): p. 1545-1602.
24. Salk, R.H., J.S. Hyde, and L.Y. Abramson, *Gender differences in depression in representative national samples: Meta-analyses of diagnoses and symptoms.* Psychological Bulletin, 2017. 143(8): p. 783-822.
25. Disease, G.B.D., I. Injury, and C. Prevalence, *Global, regional, and national incidence, prevalence, and years lived with disability for 354 diseases and injuries for 195 countries and territories, 1990-2017: a systematic analysis for the Global Burden of Disease Study 2017.* Lancet, 2018. 392(10159): p. 1789-1858.
26. Knoll, A.D. and R.N. MacLennan, *Prevalence and correlates of depression in Canada: Findings from the Canadian Community Health Survey*. 2017, Educational Publishing Foundation: US. p. 116-123.
27. Fried, E.I., *The 52 symptoms of major depression: Lack of content overlap among seven common depression scales.* Journal of Affective Disorders, 2017. 208: p. 191-197.
28. Shin, L.M. and I. Liberzon, *The Neurocircuitry of Fear, Stress, and Anxiety Disorders.* Neuropsychopharmacology, 2010. 35(1): p. 169-191.
29. Bandelow, B., S. Michaelis, and D. Wedekind, *Treatment of anxiety disorders.* Dialogues Clin Neurosci, 2017. 19(2): p. 93-107.
30. Baxter, A.J., et al., *The global burden of anxiety disorders in 2010.* Psychol Med, 2014. 44(11): p. 2363-74.
31. Hendriks, S.M., et al., *Long-term disability in anxiety disorders.* BMC psychiatry, 2016. 16: p. 248-248.

32. Bandelow, B. and S. Michaelis, *Epidemiology of anxiety disorders in the 21st century.* Dialogues Clin Neurosci, 2015. 17(3): p. 327-35.
33. Prina, A., et al., *Co-occurrence of anxiety and depression amongst older adults in low- and middle-income countries: findings from the 10/66 study.* Psychological Medicine, 2011. 41(10): p. 2047.
34. Gariepy, G., D. Nitka, and N. Schmitz, *The association between obesity and anxiety disorders in the population: a systematic review and meta-analysis.* Int J Obes (Lond), 2010. 34(3): p. 407-19.
35. Pan, A., et al., *Bidirectional association between depression and obesity in middle-aged and older women.* International journal of obesity (2005), 2012. 36(4): p. 595-602.
36. Brumpton, B., et al., *The associations of anxiety and depression symptoms with weight change and incident obesity: The HUNT Study.* Int J Obes (Lond), 2013. 37(9): p. 1268-74.
37. Brunault, P., et al., *High preoperative depression, phobic anxiety, and binge eating scores and low medium-term weight loss in sleeve gastrectomy obese patients: a preliminary cohort study.* Psychosomatics, 2012. 53(4): p. 363-70.
38. Gaysina, D., et al., *Symptoms of depression and anxiety, and change in body mass index from adolescence to adulthood: results from a British birth cohort.* Psychological medicine, 2011. 41(1): p. 175.
39. Dawes, A.J., et al., *Mental health conditions among patients seeking and undergoing bariatric surgery: a meta-analysis.* Jama, 2016. 315(2): p. 150-163.
40. Luppino, F.S., et al., *Overweight, obesity, and depression: a systematic review and meta-analysis of longitudinal studies.* Archives of general psychiatry, 2010. 67(3): p. 220-229.
41. Marmorstein, N.R., W.G. Iacono, and L. Legrand, *Obesity and depression in adolescence and beyond: reciprocal risks.* International journal of obesity, 2014. 38(7): p. 906-911.
42. Faith, M.S., et al., *Evidence for prospective associations among depression and obesity in population-based studies.* Obes Rev, 2011. 12(5): p. e438-53.
43. Moazzami, K., et al., *Independent and joint association of obesity and metabolic syndrome with depression and inflammation.* Health Psychology, 2019. 38(7): p. 586.
44. Atlas, D., *International diabetes federation.* IDF Diabetes Atlas, 7th edn. Brussels, Belgium: International Diabetes Federation, 2015.
45. Bastien, M., et al., *Overview of epidemiology and contribution of obesity to cardiovascular disease.* Prog Cardiovasc Dis, 2014. 56(4): p. 369-81.
46. Ostchega, Y., et al., *Abdominal obesity, body mass index, and hypertension in US adults: NHANES 2007–2010.* 2012. 25(12): p. 1271-1278.
47. Lukács, A., et al., *Abdominal obesity increases metabolic risk factors in non-obese adults: a Hungarian cross-sectional study.* BMC Public Health, 2019. 19(1): p. 1533.
48. Skilton, M.R., et al., *Associations between anxiety, depression, and the metabolic syndrome.* Biological psychiatry, 2007. 62(11): p. 1251-1257.

49. Gibson-Smith, D., et al., *The relation between obesity and depressed mood in a multi-ethnic population. The HELIUS study.* Social psychiatry and psychiatric epidemiology, 2018. 53(6): p. 629-638.
50. Hidese, S., et al., *Association of depression with body mass index classification, metabolic disease, and lifestyle: a web-based survey involving 11,876 Japanese people.* Journal of psychiatric research, 2018. 102: p. 23-28.
51. Vancampfort, D., et al., *Metabolic syndrome and metabolic abnormalities in patients with major depressive disorder: a meta-analysis of prevalences and moderating variables.* Psychological medicine, 2014. 44(10): p. 2017-2028.
52. Brody, D.J., L.A. Pratt, and J.P. Hughes, *Prevalence of depression among adults aged 20 and over: United States, 2013-2016*. 2018: US Department of Health and Human Services, Centers for Disease Control and ….
53. Milano, W., et al., *Depression and Obesity: Analysis of Common Biomarkers.* Diseases, 2020. 8(2): p. 23.
54. Robinson, M.R., et al., *Genotype–covariate interaction effects and the heritability of adult body mass index.* Nature genetics, 2017. 49(8): p. 1174.
55. Milaneschi, Y., et al., *Genetic association of major depression with atypical features and obesity-related immunometabolic dysregulations.* Jama Psychiatry, 2017. 74(12): p. 1214-1225.
56. Locke, A.E., et al., *Genetic studies of body mass index yield new insights for obesity biology.* Nature, 2015. 518(7538): p. 197-206.
57. Forsyth, A.K., P.G. Williams, and F.P. Deane, *Nutrition status of primary care patients with depression and anxiety.* Australian Journal of Primary Health, 2012. 18(2): p. 172-176.
58. Ruusunen, A., et al., *Dietary patterns are associated with the prevalence of elevated depressive symptoms and the risk of getting a hospital discharge diagnosis of depression in middle-aged or older Finnish men.* J Affect Disord, 2014. 159: p. 1-6.
59. Simopoulos, A.P., *Evolutionary aspects of diet, the omega-6/omega-3 ratio and genetic variation: nutritional implications for chronic diseases.* Biomedicine & Pharmacotherapy, 2006. 60(9): p. 502-507.
60. Jacka, F.N., et al., *Association of Western and traditional diets with depression and anxiety in women.* American journal of psychiatry, 2010. 167(3): p. 305-311.
61. Gibson-Smith, D., et al., *Diet quality in persons with and without depressive and anxiety disorders.* Journal of Psychiatric Research, 2018. 106: p. 1-7.
62. Castanon, N., G. Luheshi, and S. Laye, *Role of neuroinflammation in the emotional and cognitive alterations displayed by animal models of obesity.* Front Neurosci, 2015. 9(229): p. 229.

63. León-Pedroza, J.I., et al., *Low-grade systemic inflammation and the development of metabolic diseases: From the molecular evidence to the clinical practice.* Cirugía y Cirujanos (English Edition), 2015. 83(6): p. 543-551.
64. León-Pedroza, J.I., et al., *[Low-grade systemic inflammation and the development of metabolic diseases: from the molecular evidence to the clinical practice].* Cir Cir, 2015. 83(6): p. 543-51.
65. Dinh, K.M., et al., *Low-grade inflammation is negatively associated with physical Health-Related Quality of Life in healthy individuals: Results from The Danish Blood Donor Study (DBDS).* 2019. 14(3): p. e0214468.
66. Milaneschi, Y., et al., *Depression and obesity: evidence of shared biological mechanisms.* 2019. 24(1): p. 18-33.
67. de Wit, L., et al., *Depression and obesity: a meta-analysis of community-based studies.* Psychiatry Res, 2010. 178(2): p. 230-5.
68. Shelton, R.C. and A.H. Miller, *Inflammation in depression: is adiposity a cause?* Dialogues in clinical neuroscience, 2011. 13(1): p. 41-53.
69. Duivis, H.E., et al., *Differential association of somatic and cognitive symptoms of depression and anxiety with inflammation: findings from the Netherlands Study of Depression and Anxiety (NESDA).* Psychoneuroendocrinology, 2013. 38(9): p. 1573-85.
70. Glaus, J., et al., *Associations between mood, anxiety or substance use disorders and inflammatory markers after adjustment for multiple covariates in a population-based study.* J Psychiatr Res, 2014. 58: p. 36-45.
71. Valkanova, V., K.P. Ebmeier, and C.L. Allan, *CRP, IL-6 and depression: a systematic review and meta-analysis of longitudinal studies.* J Affect Disord, 2013. 150(3): p. 736-44.
72. Liukkonen, T., et al., *The association between anxiety and C-reactive protein (CRP) levels: Results from the Northern Finland 1966 Birth Cohort Study.* European Psychiatry, 2011. 26(6): p. 363-369.
73. Buckman, L.B., et al., *Obesity induced by a high-fat diet is associated with increased immune cell entry into the central nervous system.* 2014. 35: p. 33-42.
74. Schachter, J., et al., *Effects of obesity on depression: A role for inflammation and the gut microbiota.* Brain, Behavior, and Immunity, 2018. 69: p. 1-8.
75. Capuron, L., et al., *Relationship between adiposity, emotional status and eating behavior in obese women: role of inflammation.* 2011. 41(7): p. 1517.
76. Dinel, A.-L., et al., *Lipopolysaccharide-induced brain activation of the indoleamine 2, 3-dioxygenase and depressive-like behavior are impaired in a mouse model of metabolic syndrome.* 2014. 40: p. 48-59.
77. Lawrence, C.B., et al., *Obese mice exhibit an altered behavioural and inflammatory response to lipopolysaccharide.* 2012. 5(5): p. 649-659.

78. Saltiel, A.R. and J.M. Olefsky, *Inflammatory mechanisms linking obesity and metabolic disease.* The Journal of Clinical Investigation, 2017. 127(1): p. 1-4.
79. Singh, A.K. and Y. Jiang, *How does peripheral lipopolysaccharide induce gene expression in the brain of rats?* Toxicology, 2004. 201(1-3): p. 197-207.
80. Zatterale, F., et al., *Chronic Adipose Tissue Inflammation Linking Obesity to Insulin Resistance and Type 2 Diabetes.* 2020. 10(1607).
81. Penninx, B.W., et al., *Understanding the somatic consequences of depression: biological mechanisms and the role of depression symptom profile.* BMC medicine, 2013. 11(1): p. 129.
82. Wellen, K.E. and G.S. Hotamisligil, *Obesity-induced inflammatory changes in adipose tissue.* The Journal of clinical investigation, 2003. 112(12): p. 1785-1788.
83. Capuron, L., et al., *Depressive symptoms and metabolic syndrome: is inflammation the underlying link?* Biol Psychiatry, 2008. 64(10): p. 896-900.
84. Liu, Y., et al., *High-fat diet triggers obesity-related early infiltration of macrophages into adipose tissue and transient reduction of blood monocyte count.* Molecular Immunology, 2020. 117: p. 139-146.
85. Santos, S., A. Oliveira, and C.J.N.r. Lopes, *Systematic review of saturated fatty acids on inflammation and circulating levels of adipokines.* 2013. 33(9): p. 687-695.
86. Kaufman, F.R. and J.J.P.D. Shaw, *Type 2 diabetes in youth: rates, antecedents, treatment, problems and prevention.* 2007. 8: p. 4-6.
87. Choi, J., L. Joseph, and L. Pilote, *Obesity and C-reactive protein in various populations: a systematic review and meta-analysis.* 2013. 14(3): p. 232-244.
88. Demers, G., et al., *Fish oil supplementation alleviates metabolic and anxiodepressive effects of diet-induced obesity and associated changes in brain lipid composition in mice.* 2020: p. 1-10.
89. Chamberlain, S.R., et al., *Treatment-resistant depression and peripheral C-reactive protein.* 2019. 214(1): p. 11-19.
90. Zunszain, P.A., et al., *Glucocorticoids, cytokines and brain abnormalities in depression.* Prog Neuropsychopharmacol Biol Psychiatry, 2011. 35(3): p. 722-9.
91. Castanon, N., G. Luheshi, and S. Layé, *Role of neuroinflammation in the emotional and cognitive alterations displayed by animal models of obesity.* Frontiers in neuroscience, 2015. 9: p. 229-229.
92. Dey, A., et al., *Glucocorticoid sensitization of microglia in a genetic mouse model of obesity and diabetes.* J Neuroimmunol, 2014. 269(1-2): p. 20-27.
93. Alshehri, T., et al., *The association between overall and abdominal adiposity and depressive mood: a cross-sectional analysis in 6459 participants.* 2019. 110: p. 104429.
94. Pace, T.W. and A.H.J.A.o.t.N.Y.A.o.S. Miller, *Cytokines and glucocorticoid receptor signaling: relevance to major depression.* 2009. 1179: p. 86.

95. Wester, V.L., et al., *Long-term cortisol levels measured in scalp hair of obese patients.* 2014. 22(9): p. 1956-1958.
96. Pariante, C.M. and S.L.J.T.i.n. Lightman, *The HPA axis in major depression: classical theories and new developments.* 2008. 31(9): p. 464-468.
97. Schmaal, L., et al., *Cortical abnormalities in adults and adolescents with major depression based on brain scans from 20 cohorts worldwide in the ENIGMA Major Depressive Disorder Working Group.* 2017. 22(6): p. 900-909.
98. Maes, M., et al., *The new '5-HT'hypothesis of depression: cell-mediated immune activation induces indoleamine 2, 3-dioxygenase, which leads to lower plasma tryptophan and an increased synthesis of detrimental tryptophan catabolites (TRYCATs), both of which contribute to the onset of depression.* 2011. 35(3): p. 702-721.
99. Kunugi, H., et al., *Interface between hypothalamic-pituitary-adrenal axis and brain-derived neurotrophic factor in depression.* 2010. 64(5): p. 447-459.
100. Singhal, G. and B.T.J.F.i.c.n. Baune, *Microglia: an interface between the loss of neuroplasticity and depression.* 2017. 11: p. 270.
101. Dinel, A.-L., et al., *Lipopolysaccharide-induced brain activation of the indoleamine 2,3-dioxygenase and depressive-like behavior are impaired in a mouse model of metabolic syndrome.* Psychoneuroendocrinology, 2014. 40: p. 48-59.
102. Brites, D. and A. Fernandes, *Neuroinflammation and Depression: Microglia Activation, Extracellular Microvesicles and microRNA Dysregulation.* Front Cell Neurosci, 2015. 9: p. 476.
103. Ménard, C., et al., *Immune and Neuroendocrine Mechanisms of Stress Vulnerability and Resilience.* Neuropsychopharmacology, 2017. 42(1): p. 62-80.
104. Kelly, J.R., et al., *Breaking down the barriers: the gut microbiome, intestinal permeability and stress-related psychiatric disorders.* Frontiers in cellular neuroscience, 2015. 9: p. 392-392.
105. Castanon, N., G. Luheshi, and S. Laye, *Role of neuroinflammation in the emotional and cognitive alterations displayed by animal models of obesity.* Front Neurosci, 2015. 9: p. 229.
106. Owen, L. and B.J.P.o.t.N.S. Corfe, *The role of diet and nutrition on mental health and wellbeing.* 2017. 76(4): p. 425-426.
107. Noble, E.E., T.M. Hsu, and S.E. Kanoski, *Gut to Brain Dysbiosis: Mechanisms Linking Western Diet Consumption, the Microbiome, and Cognitive Impairment.* Front Behav Neurosci, 2017. 11: p. 9.
108. Cani, P.D. and A. Everard, *Talking microbes: When gut bacteria interact with diet and host organs.* Mol Nutr Food Res, 2016. 60(1): p. 58-66.
109. Moreira, A.P., et al., *Influence of a high-fat diet on gut microbiota, intestinal permeability and metabolic endotoxaemia.* Br J Nutr, 2012. 108(5): p. 801-9.

110. Henry, C.J., et al., *Minocycline attenuates lipopolysaccharide (LPS)-induced neuroinflammation, sickness behavior, and anhedonia.* Journal of Neuroinflammation, 2008. 5(1): p. 15.
111. Pendyala, S., J.M. Walker, and P.R.J.G. Holt, *A high-fat diet is associated with endotoxemia that originates from the gut.* 2012. 142(5): p. 1100-1101. e2.
112. Cani, P.D., et al., *Changes in gut microbiota control metabolic endotoxemia-induced inflammation in high-fat diet-induced obesity and diabetes in mice.* Diabetes, 2008. 57(6): p. 1470-81.
113. Kim, K.-A., et al., *High fat diet-induced gut microbiota exacerbates inflammation and obesity in mice via the TLR4 signaling pathway.* 2012. 7(10): p. e47713.
114. Shi, H., et al., *TLR4 links innate immunity and fatty acid-induced insulin resistance.* J Clin Invest, 2006. 116(11): p. 3015-25.
115. Winer, D.A., et al., *Immunologic impact of the intestine in metabolic disease.* J Clin Invest, 2017. 127(1): p. 33-42.
116. Stevens, B.R., et al., *Increased human intestinal barrier permeability plasma biomarkers zonulin and FABP2 correlated with plasma LPS and altered gut microbiome in anxiety or depression.* Gut, 2018. 67(8): p. 1555-1557.
117. Kim, J.S., B.J.P. Claire, and behavior, *Diet, gut microbiota composition and feeding behavior.* 2018. 192: p. 177-181.
118. Erny, D., et al., *Host microbiota constantly control maturation and function of microglia in the CNS.* Nature Neuroscience, 2015. 18(7): p. 965-977.
119. Werry, E.L., et al., *Recent Developments in TSPO PET Imaging as A Biomarker of Neuroinflammation in Neurodegenerative Disorders.* International journal of molecular sciences, 2019. 20(13): p. 3161.
120. Sochocka, M., B.S. Diniz, and J. Leszek, *Inflammatory Response in the CNS: Friend or Foe?* Mol Neurobiol, 2017. 54(10): p. 8071-8089.
121. DiSabato, D.J., N. Quan, and J.P. Godbout, *Neuroinflammation: the devil is in the details.* J Neurochem, 2016. 139 Suppl 2(Suppl 2): p. 136-153.
122. Hasegawa-Ishii, S., et al., *Endotoxemia-induced cytokine-mediated responses of hippocampal astrocytes transmitted by cells of the brain-immune interface.* Scientific reports, 2016. 6: p. 25457-25457.
123. Heneka, M.T., et al., *Neuroinflammation in Alzheimer's disease.* Lancet Neurol, 2015. 14(4): p. 388-405.
124. Troubat, R., et al., *Neuroinflammation and depression: A review.* Eur J Neurosci, 2020.
125. Kempuraj, D., et al., *Neuroinflammation Induces Neurodegeneration.* Journal of neurology, neurosurgery and spine, 2016. 1(1): p. 1003.
126. Calder, P.C., *n-3 fatty acids, inflammation and immunity: new mechanisms to explain old actions.* Proc Nutr Soc, 2013. 72(3): p. 326-36.

127. Kabba, J.A., et al., *Microglia: housekeeper of the central nervous system.* Cellular and molecular neurobiology, 2018. 38(1): p. 53-71.
128. Button, E.B., et al., *Microglial cell activation increases saturated and decreases monounsaturated fatty acid content, but both lipid species are proinflammatory.* Lipids, 2014. 49(4): p. 305-16.
129. De Souza, C.T., et al., *Consumption of a fat-rich diet activates a proinflammatory response and induces insulin resistance in the hypothalamus.* Endocrinology, 2005. 146(10): p. 4192-4199.
130. Enriori, P.J., et al., *Diet-induced obesity causes severe but reversible leptin resistance in arcuate melanocortin neurons.* Cell metabolism, 2007. 5(3): p. 181-194.
131. Seong, J., et al., *Hypothalamic inflammation and obesity: a mechanistic review.* Arch Pharm Res, 2019. 42(5): p. 383-392.
132. Velloso, L. and M.J.I.j.o.o. Schwartz, *Altered hypothalamic function in diet-induced obesity.* 2011. 35(12): p. 1455-1465.
133. Pan, W.W. and M.G.J.N.R.N. Myers Jr, *Leptin and the maintenance of elevated body weight.* 2018. 19(2): p. 95.
134. Koch, C., et al., *High-fat diet induces leptin resistance in leptin-deficient mice.* 2014. 26(2): p. 58-67.
135. Kanoski, S.E., et al., *The effects of a high-energy diet on hippocampal function and blood-brain barrier integrity in the rat.* 2010. 21(1): p. 207-219.
136. Ohashi, K., et al., *Role of anti-inflammatory adipokines in obesity-related diseases.* 2014. 25(7): p. 348-355.
137. Wesselingh, R., et al., *Innate Immunity in the Central Nervous System: A Missing Piece of the Autoimmune Encephalitis Puzzle?* Frontiers in immunology, 2019. 10: p. 2066-2066.
138. Russo, S.J. and E.J. Nestler, *The brain reward circuitry in mood disorders.* Nature Reviews Neuroscience, 2013. 14(9): p. 609-625.
139. Ferrario, C.R., et al., *Homeostasis Meets Motivation in the Battle to Control Food Intake.* 2016. 36(45): p. 11469-11481.
140. Klawonn, A.M. and R.C. Malenka, *Nucleus Accumbens Modulation in Reward and Aversion.* Cold Spring Harbor symposia on quantitative biology, 2018. 83: p. 119-129.
141. Sharma, S. and S.J.I.j.o.o. Fulton, *Diet-induced obesity promotes depressive-like behaviour that is associated with neural adaptations in brain reward circuitry.* 2013. 37(3): p. 382-389.
142. Hryhorczuk, C., et al., *Dampened Mesolimbic Dopamine Function and Signaling by Saturated but not Monounsaturated Dietary Lipids.* Neuropsychopharmacology, 2016. 41(3): p. 811-821.
143. Lim, B.K., et al., *Anhedonia requires MC4R-mediated synaptic adaptations in nucleus accumbens.* 2012. 487(7406): p. 183-189.

144. Francis, T.C. and M.K. Lobo, *Emerging Role for Nucleus Accumbens Medium Spiny Neuron Subtypes in Depression.* Biol Psychiatry, 2017. 81(8): p. 645-653.
145. Russo, S.J. and E.J. Nestler, *The brain reward circuitry in mood disorders.* Nat Rev Neurosci, 2013. 14(9): p. 609-25.
146. Derman, R.C. and C.R. Ferrario, *Enhanced incentive motivation in obesity-prone rats is mediated by NAc core CP-AMPARs.* Neuropharmacology, 2018. 131: p. 326-336.
147. Décarie-Spain, L., et al., *Nucleus accumbens inflammation mediates anxiodepressive behavior and compulsive sucrose seeking elicited by saturated dietary fat.* Molecular metabolism, 2018. 10: p. 1-13.
148. Williams, Z.M., *Good vibrations with deep brain stimulation.* Nat Neurosci, 2015. 18(5): p. 618-9.
149. Sousa, C., K. Biber, and A. Michelucci, *Cellular and Molecular Characterization of Microglia: A Unique Immune Cell Population.* Frontiers in immunology, 2017. 8: p. 198-198.
150. Mosser, C.A., et al., *Microglia in CNS development: Shaping the brain for the future.* Prog Neurobiol, 2017. 149-150: p. 1-20.
151. Ginhoux, F., et al., *Origin and differentiation of microglia.* Front Cell Neurosci, 2013. 7: p. 45.
152. Perry, V.H. and J. Teeling, *Microglia and macrophages of the central nervous system: the contribution of microglia priming and systemic inflammation to chronic neurodegeneration.* Semin Immunopathol, 2013. 35(5): p. 601-12.
153. Ginhoux, F. and M. Prinz, *Origin of microglia: current concepts and past controversies.* Cold Spring Harbor perspectives in biology, 2015. 7(8): p. a020537.
154. Fatoba, O., T. Itokazu, and T. Yamashita, *Microglia as therapeutic target in central nervous system disorders.* Journal of Pharmacological Sciences, 2020. 144(3): p. 102-118.
155. Timmerman, R., S.M. Burm, and J.J. Bajramovic, *An Overview of in vitro Methods to Study Microglia.* Frontiers in Cellular Neuroscience, 2018. 12(242).
156. Garaschuk, O. and A. Verkhratsky, *Microglia: The Neural Cells of Nonneural Origin.* Methods Mol Biol, 2019. 2034: p. 3-11.
157. Burda, J.E. and M.V. Sofroniew, *Reactive gliosis and the multicellular response to CNS damage and disease.* Neuron, 2014. 81(2): p. 229-48.
158. Cianciulli, A., et al., *Microglia Mediated Neuroinflammation: Focus on PI3K Modulation.* Biomolecules, 2020. 10(1): p. 137.
159. Yasuhisa, A., et al., *Amyloid β-induced Mesenteric Inflammation in an Alzheimer's Disease Transgenic Mouse Model.* Current Alzheimer Research, 2020. 17(1): p. 52-59.
160. Crews, F.T. and R.P. Vetreno, *Mechanisms of neuroimmune gene induction in alcoholism.* Psychopharmacology, 2016. 233(9): p. 1543-1557.

161. Cherry, J.D., J.A. Olschowka, and M.K. O'Banion, *Neuroinflammation and M2 microglia: the good, the bad, and the inflamed.* J Neuroinflammation, 2014. 11: p. 98.
162. Singhal, G. and B.T. Baune, *Microglia: an interface between the loss of neuroplasticity and depression.* Frontiers in cellular neuroscience, 2017. 11: p. 270.
163. Biber, K., J. Vinet, and H.W. Boddeke, *Neuron-microglia signaling: chemokines as versatile messengers.* J Neuroimmunol, 2008. 198(1-2): p. 69-74.
164. Zhang, L., J. Zhang, and Z. You, *Switching of the Microglial Activation Phenotype Is a Possible Treatment for Depression Disorder.* Frontiers in Cellular Neuroscience, 2018. 12(306).
165. Shen, Z., X. Bao, and R.J.F.i.a.n. Wang, *Clinical PET imaging of microglial activation: implications for microglial therapeutics in Alzheimer's disease.* 2018. 10: p. 314.
166. Salvi, V., et al., *Role of Atypical Chemokine Receptors in Microglial Activation and Polarization.* Frontiers in aging neuroscience, 2017. 9: p. 148-148.
167. Takahashi, K., C.D. Rochford, and H. Neumann, *Clearance of apoptotic neurons without inflammation by microglial triggering receptor expressed on myeloid cells-2.* The Journal of experimental medicine, 2005. 201(4): p. 647-657.
168. Laffer, B., et al., *Loss of IL-10 Promotes Differentiation of Microglia to a M1 Phenotype.* Frontiers in cellular neuroscience, 2019. 13: p. 430-430.
169. Du, L., et al., *Role of Microglia in Neurological Disorders and Their Potentials as a Therapeutic Target.* Mol Neurobiol, 2017. 54(10): p. 7567-7584.
170. Yirmiya, R., N. Rimmerman, and R. Reshef, *Depression as a microglial disease.* Trends Neurosci, 2015. 38(10): p. 637-658.
171. Puig, K.L., et al., *Amyloid precursor protein and proinflammatory changes are regulated in brain and adipose tissue in a murine model of high fat diet-induced obesity.* PLoS One, 2012. 7(1): p. e30378.
172. Cope, E.C., et al., *Microglia Play an Active Role in Obesity-Associated Cognitive Decline.* The Journal of neuroscience : the official journal of the Society for Neuroscience, 2018. 38(41): p. 8889-8904.
173. Wang, Z., et al., *Saturated fatty acids activate microglia via Toll-like receptor 4/NF-κB signalling.* Br J Nutr, 2012. 107(2): p. 229-41.
174. Shastri, A., D.M. Bonifati, and U. Kishore, *Innate immunity and neuroinflammation.* Mediators of inflammation, 2013. 2013: p. 342931-342931.
175. Camara, M.L., et al., *Effects of centrally administered etanercept on behavior, microglia, and astrocytes in mice following a peripheral immune challenge.* Neuropsychopharmacology, 2015. 40(2): p. 502-12.
176. Wang, Y.-L., et al., *Microglial activation mediates chronic mild stress-induced depressive- and anxiety-like behavior in adult rats.* Journal of neuroinflammation, 2018. 15(1): p. 21-21.

177. Torres-Platas, S.G., et al., *Evidence for increased microglial priming and macrophage recruitment in the dorsal anterior cingulate white matter of depressed suicides.* 2014. 42: p. 50-59.
178. Schmidtner, A.K., et al., *Minocycline alters behavior, microglia and the gut microbiome in a trait-anxiety-dependent manner.* Translational Psychiatry, 2019. 9(1): p. 223.
179. Tynan, R.J., et al., *A comparative examination of the anti-inflammatory effects of SSRI and SNRI antidepressants on LPS stimulated microglia.* Brain Behav Immun, 2012. 26(3): p. 469-79.
180. Kim, H.J., et al., *Deficient autophagy in microglia impairs synaptic pruning and causes social behavioral defects.* Mol Psychiatry, 2017. 22(11): p. 1576-1584.
181. Köhler, O., et al., *Effect of Anti-inflammatory Treatment on Depression, Depressive Symptoms, and Adverse Effects: A Systematic Review and Meta-analysis of Randomized Clinical Trials.* JAMA Psychiatry, 2014. 71(12): p. 1381-1391.
182. Gaikwad, S., C. Naveen, and R. Agrawal-Rajput, *Toll-like receptor-4 antagonism mediates benefits during neuroinflammation.* Neural regeneration research, 2016. 11(4): p. 552-553.
183. Deczkowska, A., I. Amit, and M. Schwartz, *Microglial immune checkpoint mechanisms.* Nature Neuroscience, 2018. 21(6): p. 779-786.
184. Fiebich, B.L., et al., *Role of Microglia TLRs in Neurodegeneration.* Frontiers in cellular neuroscience, 2018. 12: p. 329-329.
185. Cheng, Y., et al., *Stress-induced neuroinflammation is mediated by GSK3-dependent TLR4 signaling that promotes susceptibility to depression-like behavior.* Brain, behavior, and immunity, 2016. 53: p. 207-222.
186. Rahimifard, M., et al., *Targeting the TLR4 signaling pathway by polyphenols: a novel therapeutic strategy for neuroinflammation.* 2017. 36: p. 11-19.
187. Hines, D.J., et al., *Prevention of LPS-induced microglia activation, cytokine production and sickness behavior with TLR4 receptor interfering peptides.* PLoS One, 2013. 8(3): p. e60388.
188. Zhou, H., C.J. Urso, and V. Jadeja, *Saturated Fatty Acids in Obesity-Associated Inflammation.* Journal of inflammation research, 2020. 13: p. 1-14.
189. Davis, J.E., et al., *Tlr-4 deficiency selectively protects against obesity induced by diets high in saturated fat.* Obesity (Silver Spring), 2008. 16(6): p. 1248-55.
190. Gaikwad, S. and R. Agrawal-Rajput, *Lipopolysaccharide from Rhodobacter sphaeroides Attenuates Microglia-Mediated Inflammation and Phagocytosis and Directs Regulatory T Cell Response.* Int J Inflam, 2015. 2015: p. 361326.
191. Subedi, L., et al., *Equol, a Dietary Daidzein Gut Metabolite Attenuates Microglial Activation and Potentiates Neuroprotection In Vitro.* Nutrients, 2017. 9(3): p. 207.

192. Valdearcos, M., et al., *Microglia dictate the impact of saturated fat consumption on hypothalamic inflammation and neuronal function.* 2014. 9(6): p. 2124-2138.
193. Mendes, N.F., et al., *Hypothalamic Microglial Activation in Obesity: A Mini-Review.* 2018. 12(846).
194. Thaler, J.P., et al., *Obesity is associated with hypothalamic injury in rodents and humans.* 2012. 122(1): p. 153-162.
195. Seong, K.-J., et al., *Epigallocatechin-3-gallate rescues LPS-impaired adult hippocampal neurogenesis through suppressing the TLR4-NF-κB signaling pathway in mice.* 2016. 20(1): p. 41-51.
196. Alshammari, M.A., et al., *Systemic TNF-α blockade attenuates anxiety and depressive-like behaviors in db/db mice through downregulation of inflammatory signaling in peripheral immune cells.* Saudi Pharmaceutical Journal, 2020. 28(5): p. 621-629.
197. Finucane, O.M., et al., *Monounsaturated fatty acid–enriched high-fat diets impede adipose NLRP3 inflammasome–mediated IL-1β secretion and insulin resistance despite obesity.* Diabetes, 2015. 64(6): p. 2116-2128.
198. Duewell, P., et al., *NLRP3 inflammasomes are required for atherogenesis and activated by cholesterol crystals.* Nature, 2010. 464(7293): p. 1357-61.
199. Xu, Y., et al., *NLRP3 inflammasome activation mediates estrogen deficiency-induced depression- and anxiety-like behavior and hippocampal inflammation in mice.* Brain Behav Immun, 2016. 56: p. 175-86.
200. Gao, H., et al., *Anti-depressant-like effect of atractylenolide I in a mouse model of depression induced by chronic unpredictable mild stress.* Exp Ther Med, 2018. 15(2): p. 1574-1579.
201. Scheiblich, H., et al., *Activation of the NLRP3 inflammasome in microglia: the role of ceramide.* J Neurochem, 2017. 143(5): p. 534-550.
202. Wu, K.K.-L., S.W.-M. Cheung, and K.K.-Y. Cheng, *NLRP3 Inflammasome Activation in Adipose Tissues and Its Implications on Metabolic Diseases.* International Journal of Molecular Sciences, 2020. 21(11): p. 4184.
203. Rheinheimer, J., et al., *Current role of the NLRP3 inflammasome on obesity and insulin resistance: A systematic review.* Metabolism, 2017. 74: p. 1-9.
204. Kursawe, R., et al., *A role of the inflammasome in the low storage capacity of the abdominal subcutaneous adipose tissue in obese adolescents.* Diabetes, 2016. 65(3): p. 610-618.
205. Unamuno, X., et al., *NLRP3 inflammasome blockade reduces adipose tissue inflammation and extracellular matrix remodeling.* Cellular & Molecular Immunology, 2019.

206. Yang, J.W., et al., *3-(Naphthalen-2-yl(propoxy)methyl)azetidine hydrochloride attenuates NLRP3 inflammasome-mediated signaling pathway in lipopolysaccharide-stimulated BV2 microglial cells.* Biochem Biophys Res Commun, 2018. 495(1): p. 151-156.
207. Pan, Y., et al., *Microglial NLRP3 inflammasome activation mediates IL-1β-related inflammation in prefrontal cortex of depressive rats.* Brain, Behavior, and Immunity, 2014. 41: p. 90-100.
208. Zhang, Y., et al., *NLRP3 Inflammasome Mediates Chronic Mild Stress-Induced Depression in Mice via Neuroinflammation.* Int J Neuropsychopharmacol, 2015. 18(8).
209. Alcocer-Gomez, E., et al., *NLRP3 inflammasome is activated in mononuclear blood cells from patients with major depressive disorder.* Brain Behav Immun, 2014. 36: p. 111-7.
210. Zhao, Z., et al., *A novel role of NLRP3-generated IL-1β in the acute-chronic transition of peripheral lipopolysaccharide-elicited neuroinflammation: implications for sepsis-associated neurodegeneration.* Journal of Neuroinflammation, 2020. 17(1): p. 64.
211. Quan, N., M. Whiteside, and M. Herkenham, *Time course and localization patterns of interleukin-1beta messenger RNA expression in brain and pituitary after peripheral administration of lipopolysaccharide.* Neuroscience, 1998. 83(1): p. 281-93.
212. Gustin, A., et al., *NLRP3 Inflammasome Is Expressed and Functional in Mouse Brain Microglia but Not in Astrocytes.* PLoS One, 2015. 10(6): p. e0130624.
213. Ślusarczyk, J., et al., *Targeting the NLRP3 inflammasome-related pathways via tianeptine treatment-suppressed microglia polarization to the M1 phenotype in lipopolysaccharide-stimulated cultures.* International journal of molecular sciences, 2018. 19(7): p. 1965.
214. He, W., et al., *Microglial NLRP3 inflammasome activation mediates IL-1β release and contributes to central sensitization in a recurrent nitroglycerin-induced migraine model.* J Neuroinflammation, 2019. 16(1): p. 78.
215. Butler, M.J., et al., *Fatty food, fatty acids, and microglial priming in the adult and aged hippocampus and amygdala.* Brain, Behavior, and Immunity, 2020. 89: p. 145-158.
216. Koletzko, B., et al., *Current Information and Asian Perspectives on Long-Chain Polyunsaturated Fatty Acids in Pregnancy, Lactation, and Infancy: Systematic Review and Practice Recommendations from an Early Nutrition Academy Workshop.* Annals of Nutrition and Metabolism, 2014. 65(1): p. 49-80.
217. Bernstein, A.M., et al., *Major dietary protein sources and risk of coronary heart disease in women.* Circulation, 2010. 122(9): p. 876-83.
218. Falomir-Lockhart, L.J., et al., *Fatty Acid Signaling Mechanisms in Neural Cells: Fatty Acid Receptors.* 2019. 13(162).
219. Chianese, R., et al., *Impact of Dietary Fats on Brain Functions.* Current neuropharmacology, 2018. 16(7): p. 1059-1085.
220. Agriculture, U.D.o., *Profiling food consumption in America*. 2003, US Department of Agriculture Washington, DC.

221. Alvarez-Alvarez, I., et al., *Mediterranean diet, physical activity and their combined effect on all-cause mortality: The Seguimiento Universidad de Navarra (SUN) cohort.* Prev Med, 2018. 106: p. 45-52.
222. Schwingshackl, L., J. Morze, and G. Hoffmann, *Mediterranean diet and health status: Active ingredients and pharmacological mechanisms.* British Journal of Pharmacology, 2020. 177(6): p. 1241-1257.
223. Zhang, R., et al., *Associations of n-3, n-6 Fatty Acids Intakes and n-6:n-3 Ratio with the Risk of Depressive Symptoms: NHANES 2009-2016.* Nutrients, 2020. 12(1): p. 240.
224. Liao, Y., et al., *Efficacy of omega-3 PUFAs in depression: A meta-analysis.* Translational Psychiatry, 2019. 9(1): p. 190.
225. Kris-Etherton, P.M., et al., *Position of the American Dietetic Association and Dietitians of Canada: dietary fatty acids.* J Am Diet Assoc, 2007. 107(9): p. 1599-611.
226. Garcia-Aloy, M., et al., *Biomarkers of food intake for nuts and vegetable oils: an extensive literature search.* Genes & Nutrition, 2019. 14(1): p. 7.
227. Hajeyah, A.A., et al., *The Biosynthesis of Enzymatically Oxidized Lipids.* 2020. 11(910).
228. Saini, R.K. and Y.-S. Keum, *Omega-3 and omega-6 polyunsaturated fatty acids: Dietary sources, metabolism, and significance—A review.* Life sciences, 2018. 203: p. 255-267.
229. McNamara, R.K., et al., *Omega-3 fatty acid deficiency increases constitutive pro-inflammatory cytokine production in rats: relationship with central serotonin turnover.* Prostaglandins Leukot Essent Fatty Acids, 2010. 83(4-6): p. 185-91.
230. Hussain, G., et al., *Fatting the brain: a brief of recent research.* 2013. 7: p. 144.
231. Bousquet, M., F. Calon, and F. Cicchetti, *Impact of omega-3 fatty acids in Parkinson's disease.* Ageing Res Rev, 2011. 10(4): p. 453-63.
232. Zhang, W., et al., *Fatty acid transporting proteins: Roles in brain development, aging, and stroke.* Prostaglandins, leukotrienes, and essential fatty acids, 2018. 136: p. 35-45.
233. Layé, S., et al., *Anti-Inflammatory Effects of Omega-3 Fatty Acids in the Brain: Physiological Mechanisms and Relevance to Pharmacology.* Pharmacological Reviews, 2018. 70(1): p. 12-38.
234. Silva-Santi, D., et al., *Liver fatty acid composition and inflammation in mice fed with high-carbohydrate diet or high-fat diet.* Nutrients, 2016. 8(11): p. 682.
235. Simopoulos, A.P., *An Increase in the Omega-6/Omega-3 Fatty Acid Ratio Increases the Risk for Obesity.* Nutrients, 2016. 8(3): p. 128-128.
236. Ma, J., et al., *Short-and long-term repeatability of fatty acid composition of human plasma phospholipids and cholesterol esters. The Atherosclerosis Risk in Communities (ARIC) Study Investigators.* The American journal of clinical nutrition, 1995. 62(3): p. 572-578.
237. Bazinet, R.P. and S. Layé, *Polyunsaturated fatty acids and their metabolites in brain function and disease.* Nature Reviews Neuroscience, 2014. 15(12): p. 771-785.

238. Wiktorowska-Owczarek, A., M. Berezińska, and J.Z. Nowak, *PUFAs: Structures, Metabolism and Functions.* Adv Clin Exp Med, 2015. 24(6): p. 931-41.
239. Wall, R., et al., *Fatty acids from fish: the anti-inflammatory potential of long-chain omega-3 fatty acids.* Nutrition Reviews, 2010. 68(5): p. 280-289.
240. Colombo, S.M., et al., *Projected declines in global DHA availability for human consumption as a result of global warming.* Ambio, 2020. 49(4): p. 865-880.
241. Boer, M., M.-L. Wetten, and L. Pruimboom, *Chronic inflammatory diseases are stimulated by current lifestyle: How diet, stress levels and medication prevent our body from recovering.* Nutrition & metabolism, 2012. 9: p. 32.
242. Psaltopoulou, T., et al., *Mediterranean diet, stroke, cognitive impairment, and depression: a meta-analysis.* Annals of neurology, 2013. 74(4): p. 580-591.
243. Sánchez-Villegas, A., et al., *The Association Between the Mediterranean Lifestyle and Depression.* Clinical Psychological Science, 2016. 4(6): p. 1085-1093.
244. Shafiei, F., et al., *Adherence to the Mediterranean diet and risk of depression: a systematic review and updated meta-analysis of observational studies.* Nutrition Reviews, 2019. 77(4): p. 230-239.
245. Sadeghi, O., et al., *Adherence to Mediterranean dietary pattern is inversely associated with depression, anxiety and psychological distress.* Nutritional Neuroscience, 2019: p. 1-12.
246. Lai, J.S., et al., *A systematic review and meta-analysis of dietary patterns and depression in community-dwelling adults.* The American journal of clinical nutrition, 2014. 99(1): p. 181-197.
247. Li, F., X. Liu, and D. Zhang, *Fish consumption and risk of depression: a meta-analysis.* J Epidemiol Community Health, 2016. 70(3): p. 299-304.
248. Bozzatello, P., et al., *Supplementation with Omega-3 Fatty Acids in Psychiatric Disorders: A Review of Literature Data.* J Clin Med, 2016. 5(8): p. 67.
249. Jahangard, L., et al., *Influence of adjuvant omega-3-polyunsaturated fatty acids on depression, sleep, and emotion regulation among outpatients with major depressive disorders - Results from a double-blind, randomized and placebo-controlled clinical trial.* J Psychiatr Res, 2018. 107: p. 48-56.
250. Lespérance, F., et al., *The efficacy of omega-3 supplementation for major depression: a randomized controlled trial.* Journal of Clinical Psychiatry, 2011. 72(8): p. 1054.
251. Su, K.-P., et al., *Omega-3 fatty acids in major depressive disorder: a preliminary double-blind, placebo-controlled trial.* European Neuropsychopharmacology, 2003. 13(4): p. 267-271.
252. Mischoulon, D., et al., *A double-blind, randomized controlled clinical trial comparing eicosapentaenoic acid versus docosahexaenoic acid for depression.* The Journal of clinical psychiatry, 2015. 76(1): p. 54-61.

253. Guu, T.W., et al., *International Society for Nutritional Psychiatry Research Practice Guidelines for Omega-3 Fatty Acids in the Treatment of Major Depressive Disorder.* Psychother Psychosom, 2019. 88(5): p. 263-273.
254. Hallahan, B., et al., *Efficacy of omega-3 highly unsaturated fatty acids in the treatment of depression.* Br J Psychiatry, 2016. 209(3): p. 192-201.
255. Marangell, L.B., et al., *A double-blind, placebo-controlled study of the omega-3 fatty acid docosahexaenoic acid in the treatment of major depression.* Am J Psychiatry, 2003. 160(5): p. 996-8.
256. Ghasemi Fard, S., et al., *How does high DHA fish oil affect health? A systematic review of evidence.* Crit Rev Food Sci Nutr, 2019. 59(11): p. 1684-1727.
257. Nemets, B., Z. Stahl, and R.H. Belmaker, *Addition of omega-3 fatty acid to maintenance medication treatment for recurrent unipolar depressive disorder.* Am J Psychiatry, 2002. 159(3): p. 477-9.
258. Rapaport, M.H., et al., *Inflammation as a predictive biomarker for response to omega-3 fatty acids in major depressive disorder: a proof-of-concept study.* Molecular psychiatry, 2016. 21(1): p. 71-79.
259. Albracht-Schulte, K., et al., *Omega-3 fatty acids in obesity and metabolic syndrome: a mechanistic update.* The Journal of Nutritional Biochemistry, 2018. 58: p. 1-16.
260. Schilperoort, M., et al., *The GPR120 agonist TUG-891 promotes metabolic health by stimulating mitochondrial respiration in brown fat.* EMBO molecular medicine, 2018. 10(3): p. e8047.
261. Huang, C.-W., et al., *Role of n-3 Polyunsaturated Fatty Acids in Ameliorating the Obesity-Induced Metabolic Syndrome in Animal Models and Humans.* International journal of molecular sciences, 2016. 17(10): p. 1689.
262. Wen, L., et al., *Polarization of Microglia to the M2 Phenotype in a Peroxisome Proliferator-Activated Receptor Gamma–Dependent Manner Attenuates Axonal Injury Induced by Traumatic Brain Injury in Mice.* Journal of Neurotrauma, 2018. 35(19): p. 2330-2340.
263. Talukdar, S., et al., *GPR120 is an omega-3 fatty acid receptor mediating potent anti-inflammatory and insulin-sensitizing effects.* 2010. 142(5): p. 687-698.
264. Tian, Y., et al., *Resolvin D2 recovers neural injury by suppressing inflammatory mediators expression in lipopolysaccharide-induced Parkinson's disease rat model.* 2015. 460(3): p. 799-805.
265. Souza, P.R., et al., *Enriched Marine Oil Supplements Increase Peripheral Blood Specialized Pro-Resolving Mediators Concentrations and Reprogram Host Immune Responses.* 2020. 126(1): p. 75-90.

266. Ishihara, T., M. Yoshida, and M. Arita, *Omega-3 fatty acid-derived mediators that control inflammation and tissue homeostasis.* International Immunology, 2019. 31(9): p. 559-567.
267. Sriram, K. and P.A. Insel, *G Protein-Coupled Receptors as Targets for Approved Drugs: How Many Targets and How Many Drugs?* Molecular pharmacology, 2018. 93(4): p. 251-258.
268. Vassilatis, D.K., et al., *The G protein-coupled receptor repertoires of human and mouse.* Proceedings of the National Academy of Sciences of the United States of America, 2003. 100(8): p. 4903-4908.
269. Wise, H., *The roles played by highly truncated splice variants of G protein-coupled receptors.* Journal of molecular signaling, 2012. 7(1): p. 13-13.
270. Moran, B.M., P.R. Flatt, and A.M. McKillop, *G protein-coupled receptors: signalling and regulation by lipid agonists for improved glucose homoeostasis.* Acta Diabetologica, 2016. 53(2): p. 177-188.
271. Davies, M.N., et al., *GPCRTree: online hierarchical classification of GPCR function.* 2008. 1(1): p. 1-5.
272. Nordström, K.J., et al., *The Secretin GPCRs descended from the family of Adhesion GPCRs.* 2009. 26(1): p. 71-84.
273. Hotamisligil, G.S. and D.A. Bernlohr, *Metabolic functions of FABPs--mechanisms and therapeutic implications.* Nature reviews. Endocrinology, 2015. 11(10): p. 592-605.
274. Yonezawa, T., et al., *Free fatty acids-sensing G protein-coupled receptors in drug targeting and therapeutics.* Current medicinal chemistry, 2013. 20(31): p. 3855-3871.
275. Hirasawa, A., et al., *Free fatty acid receptors and drug discovery.* Biological and Pharmaceutical Bulletin, 2008. 31(10): p. 1847-1851.
276. Kimura, I., et al., *Free Fatty Acid Receptors in Health and Disease.* Physiol Rev, 2020. 100(1): p. 171-210.
277. Son, S.-E., N.-J. Kim, and D.-S. Im, *Development of Free Fatty Acid Receptor 4 (FFA4/GPR120) Agonists in Health Science.* Biomolecules & therapeutics, 2021. 29(1): p. 22-30.
278. Falomir-Lockhart, L.J., et al., *Fatty Acid Signaling Mechanisms in Neural Cells: Fatty Acid Receptors.* Frontiers in cellular neuroscience, 2019. 13: p. 162-162.
279. Oh, D.Y., et al., *GPR120 is an omega-3 fatty acid receptor mediating potent anti-inflammatory and insulin-sensitizing effects.* Cell, 2010. 142(5): p. 687-98.
280. Dragano, N.R.V., et al., *Polyunsaturated fatty acid receptors, GPR40 and GPR120, are expressed in the hypothalamus and control energy homeostasis and inflammation.* J Neuroinflammation, 2017. 14(1): p. 91.
281. Talukdar, S., et al., *GPR120 is an omega-3 fatty acid receptor mediating potent anti-inflammatory and insulin-sensitizing effects.* Cell, 2010. 142(5): p. 687-698.

282. Dátilo, M.N., et al., *Omega-3 from Flaxseed Oil Protects Obese Mice Against Diabetic Retinopathy Through GPR120 Receptor.* Scientific reports, 2018. 8(1): p. 14318-14318.
283. Hirasawa, A., et al., *Free fatty acids regulate gut incretin glucagon-like peptide-1 secretion through GPR120.* Nat Med, 2005. 11(1): p. 90-4.
284. Oliveira, V., et al., *Diets Containing α-Linolenic (ω3) or Oleic (ω9) Fatty Acids Rescues Obese Mice From Insulin Resistance.* Endocrinology, 2015. 156(11): p. 4033-46.
285. Cintra, D.E., et al., *Unsaturated Fatty Acids Revert Diet-Induced Hypothalamic Inflammation in Obesity.* PLOS ONE, 2012. 7(1): p. e30571.
286. Li, X., et al., *Endogenously generated omega-3 fatty acids attenuate vascular inflammation and neointimal hyperplasia by interaction with free fatty acid receptor 4 in mice.* Journal of the American Heart Association, 2015. 4(4): p. e001856.
287. Fredriksson, R., et al., *Seven evolutionarily conserved human rhodopsin G protein-coupled receptors lacking close relatives.* 2003. 554(3): p. 381-388.
288. Mo, X.L., et al., *Free fatty acid receptor GPR120 and pathogenesis of obesity and type 2 diabetes mellitus.* Prog Mol Biol Transl Sci, 2013. 114: p. 251-76.
289. Ichimura, A., et al., *Dysfunction of lipid sensor GPR120 leads to obesity in both mouse and human.* Nature, 2012. 483(7389): p. 350-4.
290. Milligan, G., et al., *Characterizing pharmacological ligands to study the long-chain fatty acid receptors GPR40/FFA1 and GPR120/FFA4.* British journal of pharmacology, 2015. 172(13): p. 3254-3265.
291. Moniri, N.H., *Free-fatty acid receptor-4 (GPR120): Cellular and molecular function and its role in metabolic disorders.* Biochem Pharmacol, 2016. 110-111: p. 1-15.
292. Burns, R.N. and N.H. Moniri, *Agonism with the omega-3 fatty acids alpha-linolenic acid and docosahexaenoic acid mediates phosphorylation of both the short and long isoforms of the human GPR120 receptor.* Biochem Biophys Res Commun, 2010. 396(4): p. 1030-5.
293. Watson, S.J., A.J. Brown, and N.D. Holliday, *Differential signaling by splice variants of the human free fatty acid receptor GPR120.* Mol Pharmacol, 2012. 81(5): p. 631-42.
294. Ulven, T. and E. Christiansen, *Dietary fatty acids and their potential for controlling metabolic diseases through activation of FFA4/GPR120.* Annual Review of Nutrition, 2015. 35: p. 239-263.
295. Senatorov, I.S., et al., *Carboxy-Terminal Phosphoregulation of the Long Splice Isoform of Free-Fatty Acid Receptor-4 Mediates β-Arrestin Recruitment and Signaling to ERK1/2.* 2020. 97(5): p. 304-313.
296. Kishikawa, A., et al., *Docosahexaenoic Acid Inhibits Inflammation-Induced Osteoclast Formation and Bone Resorption in vivo Through GPR120 by Inhibiting TNF-α Production in Macrophages and Directly Inhibiting Osteoclast Formation.* Frontiers in Endocrinology, 2019. 10(157).

297. Ichimura, A., et al., *Dysfunction of lipid sensor GPR120 leads to obesity in both mouse and human.* Nature, 2012. 483(7389): p. 350-354.
298. Engelstoft, M.S., et al., *Seven transmembrane G protein-coupled receptor repertoire of gastric ghrelin cells.* Mol Metab, 2013. 2(4): p. 376-92.
299. Oh, D.Y., et al., *A Gpr120-selective agonist improves insulin resistance and chronic inflammation in obese mice.* Nat Med, 2014. 20(8): p. 942-7.
300. Hara, T., et al., *Role of free fatty acid receptors in the regulation of energy metabolism.* Biochim Biophys Acta, 2014. 1841(9): p. 1292-300.
301. Auguste, S., et al., *Central Agonism of GPR120 Acutely Inhibits Food Intake and Food Reward and Chronically Suppresses Anxiety-Like Behavior in Mice.* The international journal of neuropsychopharmacology, 2016. 19(7): p. pyw014.
302. Guo, C., et al., *Anti-neuroinflammatory effect of Sophoraflavanone G from Sophora alopecuroides in LPS-activated BV2 microglia by MAPK, JAK/STAT and Nrf2/HO-1 signaling pathways.* Phytomedicine, 2016. 23(13): p. 1629-1637.
303. Choi, M.-J., et al., *Anti-inflammatory mechanism of galangin in lipopolysaccharide-stimulated microglia: Critical role of PPAR-γ signaling pathway.* Biochemical Pharmacology, 2017. 144: p. 120-131.
304. McArdle, M., et al., *Mechanisms of Obesity-Induced Inflammation and Insulin Resistance: Insights into the Emerging Role of Nutritional Strategies.* 2013. 4(52).
305. Głombik, K., et al., *Brain Metabolic Alterations in Rats Showing Depression-Like and Obesity Phenotypes.* Neurotoxicity Research, 2020. 37(2): p. 406-424.
306. Kumar, N., et al., *15-Lipoxygenase metabolites of α-linolenic acid, [13-(S)-HPOTrE and 13-(S)-HOTrE], mediate anti-inflammatory effects by inactivating NLRP3 inflammasome.* Scientific Reports, 2016. 6(1): p. 31649.
307. Yan, Y., et al., *Omega-3 fatty acids prevent inflammation and metabolic disorder through inhibition of NLRP3 inflammasome activation.* Immunity, 2013. 38(6): p. 1154-63.
308. Suckow, A.T., et al., *Alteration of the glucagon axis in GPR120 (FFAR4) knockout mice: a role for GPR120 in glucagon secretion.* J Biol Chem, 2014. 289(22): p. 15751-63.
309. López-Ferreras, L., et al., *The supramammillary nucleus controls anxiety-like behavior; key role of GLP-1R.* Psychoneuroendocrinology, 2020. 119: p. 104720.
310. Kappe, C., et al., *GLP-1 secretion by microglial cells and decreased CNS expression in obesity.* 2012. 9(1): p. 276.
311. Wang, X.-F., et al., *Endogenous glucagon-like peptide-1 suppresses high-fat food intake by reducing synaptic drive onto mesolimbic dopamine neurons.* 2015. 12(5): p. 726-733.
312. Müller, T., et al., *Anti-obesity therapy: from rainbow pills to polyagonists.* 2018. 70(4): p. 712-746.
313. Zheng, H., et al., *Chronic suppression of glucagon-like peptide-1 receptor (GLP1R) mRNA translation in the rat bed nucleus of the stria terminalis reduces anxiety-like behavior*

*and stress-induced hypophagia, but prolongs stress-induced elevation of plasma corticosterone.* 2019. 39(14): p. 2649-2663.

314. Onoviran, O.F., et al., *Effects of glucagon-like peptide 1 receptor agonists on comorbidities in older patients with diabetes mellitus.* 2019. 10: p. 2040622319862691.
315. Drucker, D.J. and M.A.J.T.L. Nauck, *The incretin system: glucagon-like peptide-1 receptor agonists and dipeptidyl peptidase-4 inhibitors in type 2 diabetes.* 2006. 368(9548): p. 1696-1705.
316. Wellhauser, L. and D.D. Belsham, *Activation of the omega-3 fatty acid receptor GPR120 mediates anti-inflammatory actions in immortalized hypothalamic neurons.* J Neuroinflammation, 2014. 11: p. 60.
317. Han, L., et al., *Immunomodulatory Activity of Docosahexenoic Acid on RAW264.7 Cells Activation through GPR120-Mediated Signaling Pathway.* J Agric Food Chem, 2018. 66(4): p. 926-934.
318. Han, L., et al., *The immunomodulatory activity and mechanism of docosahexenoic acid (DHA) on immunosuppressive mice models.* Food Funct, 2018. 9(6): p. 3254-3263.
319. Han, L., et al., *Eicosapentaenoic Acid (EPA) Induced Macrophages Activation through GPR120-Mediated Raf-ERK1/2-IKKβ-NF-κB p65 Signaling Pathways.* Nutrients, 2017. 9(9): p. 937.
320. Ren, Z., et al., *Activation of the Omega-3 Fatty Acid Receptor GPR120 Protects against Focal Cerebral Ischemic Injury by Preventing Inflammation and Apoptosis in Mice.* J Immunol, 2019. 202(3): p. 747-759.
321. Talukdar, S., J.M. Olefsky, and O. Osborn, *Targeting GPR120 and other fatty acid-sensing GPCRs ameliorates insulin resistance and inflammatory diseases.* Trends in pharmacological sciences, 2011. 32(9): p. 543-550.
322. Mo, Z., et al., *Eicosapentaenoic acid prevents inflammation induced by acute cerebral infarction through inhibition of NLRP3 inflammasome activation.* Life Sci, 2020. 242: p. 117133.
323. Lee, K.R., Y. Midgette, and R. Shah, *Fish Oil Derived Omega 3 Fatty Acids Suppress Adipose NLRP3 Inflammasome Signaling in Human Obesity.* Journal of the Endocrine Society, 2018. 3(3): p. 504-515.
324. Williams-Bey, Y., et al., *Omega-3 free fatty acids suppress macrophage inflammasome activation by inhibiting NF-κB activation and enhancing autophagy.* PLoS One, 2014. 9(6): p. e97957.
325. Lin, C., et al., *Omega-3 fatty acids regulate NLRP3 inflammasome activation and prevent behavior deficits after traumatic brain injury.* Exp Neurol, 2017. 290: p. 115-122.
326. Chunchai, T., N. Chattipakorn, and S.C. Chattipakorn, *The possible factors affecting microglial activation in cases of obesity with cognitive dysfunction.* Metabolic Brain Disease, 2018. 33(3): p. 615-635.

327. Sivagnanam, V., X. Zhu, and L.C. Schlichter, *Dominance of E. coli phagocytosis over LPS in the inflammatory response of microglia.* J Neuroimmunol, 2010. 227(1-2): p. 111-9.
328. Lively, S. and L.C. Schlichter, *The microglial activation state regulates migration and roles of matrix-dissolving enzymes for invasion.* J Neuroinflammation, 2013. 10: p. 75.
329. Siemsen, B.M., et al., *Chronic intermittent ethanol and lipopolysaccharide exposure differentially alter Iba1-derived microglia morphology in the prelimbic cortex and nucleus accumbens core of male Long-Evans rats.* n/a(n/a).
330. Oh, D.Y., et al., *GPR120 is an omega-3 fatty acid receptor mediating potent anti-inflammatory and insulin-sensitizing effects.* Cell, 2010. 142(5): p. 687-698.
331. Grosso, G., et al., *Role of omega-3 fatty acids in the treatment of depressive disorders: a comprehensive meta-analysis of randomized clinical trials.* PLoS One, 2014. 9(5): p. e96905.
332. Dantzer, R., et al., *From inflammation to sickness and depression: when the immune system subjugates the brain.* Nat Rev Neurosci, 2008. 9(1): p. 46-56.
333. Daniels, M.J.D. and D. Brough, *Unconventional Pathways of Secretion Contribute to Inflammation.* International journal of molecular sciences, 2017. 18(1): p. 102.
334. Burm, S.M., et al., *Inflammasome-Induced IL-1β Secretion in Microglia Is Characterized by Delayed Kinetics and Is Only Partially Dependent on Inflammatory Caspases.* 2015. 35(2): p. 678-687.
335. Heras-Sandoval, D., J. Pedraza-Chaverri, and J.M. Pérez-Rojas, *Role of docosahexaenoic acid in the modulation of glial cells in Alzheimer's disease.* J Neuroinflammation, 2016. 13(1): p. 61.
336. Oh, D.Y., et al., *A Gpr120-selective agonist improves insulin resistance and chronic inflammation in obese mice.* Nature medicine, 2014. 20(8): p. 942-947.
337. Wannick, M., et al., *Oral administration of the selective GPR120/FFA4 agonist compound A is not effective in alleviating tissue inflammation in mouse models of prototypical autoimmune diseases.* Pharmacology research & perspectives, 2018. 6(6): p. e00438-e00438.
338. Lu, Y., et al., *Spinal injection of docosahexaenoic acid attenuates carrageenan-induced inflammatory pain through inhibition of microglia-mediated neuroinflammation in the spinal cord.* Neuroscience, 2013. 241: p. 22-31.
339. Rey, C., et al., *Resolvin D1 and E1 promote resolution of inflammation in microglial cells in vitro.* 2016. 55: p. 249-259.
340. Heras-Sandoval, D., J. Pedraza-Chaverri, and J.M.J.J.o.n. Pérez-Rojas, *Role of docosahexaenoic acid in the modulation of glial cells in Alzheimer's disease.* 2016. 13(1): p. 1-13.
341. Kong, W., et al., *Docosahexaenoic acid prevents dendritic cell maturation and in vitro and in vivo expression of the IL-12 cytokine family.* Lipids Health Dis, 2010. 9: p. 12.

342. Inoue, T., et al., *Omega-3 polyunsaturated fatty acids suppress the inflammatory responses of lipopolysaccharide-stimulated mouse microglia by activating SIRT1 pathways.* Biochimica et Biophysica Acta (BBA) - Molecular and Cell Biology of Lipids, 2017. 1862(5): p. 552-560.
343. Brás, J.P., et al., *TNF-alpha-induced microglia activation requires miR-342: impact on NF-kB signaling and neurotoxicity.* Cell Death & Disease, 2020. 11(6): p. 415.
344. Kong, X., et al., *TNF-α regulates microglial activation via the NF-κB signaling pathway in systemic lupus erythematosus with depression.* International Journal of Biological Macromolecules, 2019. 125: p. 892-900.
345. Wang, J., et al., *Microglia activation induced by serum of SLE patients.* Journal of Neuroimmunology, 2017. 310: p. 135-142.
346. Ye, X., et al., *Lipopolysaccharide induces neuroinflammation in microglia by activating the MTOR pathway and downregulating Vps34 to inhibit autophagosome formation.* Journal of neuroinflammation, 2020. 17(1): p. 18-18.
347. Ji, J., et al., *Antagonizing peroxisome proliferator-activated receptor γ facilitates M1-to-M2 shift of microglia by enhancing autophagy via the LKB1-AMPK signaling pathway.* Aging Cell, 2018. 17(4): p. e12774.
348. Lai, M., et al., *The NLRP3-Caspase 1 Inflammasome Negatively Regulates Autophagy via TLR4-TRIF in Prion Peptide-Infected Microglia.* 2018. 10(116).
349. Yalagala, P.C., et al., *Dietary lysophosphatidylcholine-EPA enriches both EPA and DHA in the brain: potential treatment for depression.* 2019. 60(3): p. 566-578.
350. Kelly, L., et al., *The polyunsaturated fatty acids, EPA and DPA exert a protective effect in the hippocampus of the aged rat.* Neurobiology of Aging, 2011. 32(12): p. 2318.e1-2318.e15.
351. Li, L., et al., *Resolvin D1 promotes the interleukin-4-induced alternative activation in BV-2 microglial cells.* 2014. 11(1): p. 72.
352. Gao, B., et al., *Eicosapentaenoic acid attenuates dexamethasome-induced apoptosis by inducing adaptive autophagy via GPR120 in murine bone marrow-derived mesenchymal stem cells.* Cell Death & Disease, 2016. 7(5): p. e2235-e2235.
353. Peng, Z., et al., *EPA is More Effective than DHA to Improve Depression-Like Behavior, Glia Cell Dysfunction and Hippcampal Apoptosis Signaling in a Chronic Stress-Induced Rat Model of Depression.* Int J Mol Sci, 2020. 21(5).
354. Carey, A.N., et al., *Walnut-Associated Fatty Acids Inhibit LPS-Induced Activation of BV-2 Microglia.* Inflammation, 2020. 43(1): p. 241-250.
355. Oh, Y.T., et al., *Oleic acid reduces lipopolysaccharide-induced expression of iNOS and COX-2 in BV2 murine microglial cells: Possible involvement of reactive oxygen species, p38 MAPK, and IKK/NF-κB signaling pathways.* Neuroscience Letters, 2009. 464(2): p. 93-97.

356. Khatchadourian, A., et al., *Dynamics and regulation of lipid droplet formation in lipopolysaccharide (LPS)-stimulated microglia.* Biochim Biophys Acta, 2012. 1821(4): p. 607-17.
357. Bailey, A.P., et al., *Antioxidant Role for Lipid Droplets in a Stem Cell Niche of Drosophila.* Cell, 2015. 163(2): p. 340-53.
358. Rohwedder, A., et al., *Lipid droplet formation in response to oleic acid in Huh-7 cells is mediated by the fatty acid receptor FFAR4.* 2014. 127: p. 3104 - 3115.
359. Chausse, B., et al., *Distinct metabolic patterns during microglial remodeling by oleate and palmitate.* Bioscience reports, 2019. 39(4): p. BSR20190072.
360. Lobo-Silva, D., et al., *Balancing the immune response in the brain: IL-10 and its regulation.* J Neuroinflammation, 2016. 13(1): p. 297.
361. Hutchins, A.P., Y. Takahashi, and D. Miranda-Saavedra, *Genomic analysis of LPS-stimulated myeloid cells identifies a common pro-inflammatory response but divergent IL-10 anti-inflammatory responses.* Sci Rep, 2015. 5: p. 9100.
362. Ledeboer, A., et al., *Expression and regulation of interleukin-10 and interleukin-10 receptor in rat astroglial and microglial cells.* 2002. 16(7): p. 1175-1185.
363. Mizuno, T., et al., *Production of interleukin-10 by mouse glial cells in culture.* 1994. 205(3): p. 1907-1915.
364. Richwine, A.F., et al., *Cognitive deficits in interleukin-10-deficient mice after peripheral injection of lipopolysaccharide.* 2009. 23(6): p. 794-802.
365. Das, A., et al., *RNA sequencing reveals resistance of TLR4 ligand-activated microglial cells to inflammation mediated by the selective jumonji H3K27 demethylase inhibitor.* Sci Rep, 2017. 7(1): p. 6554.
366. Szczepanik, A.M., et al., *IL-4, IL-10 and IL-13 modulate A beta(1--42)-induced cytokine and chemokine production in primary murine microglia and a human monocyte cell line.* J Neuroimmunol, 2001. 113(1): p. 49-62.
367. Milligan, E.D., et al., *Spinal interleukin-10 therapy to treat peripheral neuropathic pain.* 2012. 15(6): p. 520-526.
368. Ji, R.-R., et al., *p38 MAPK activation by NGF in primary sensory neurons after inflammation increases TRPV1 levels and maintains heat hyperalgesia.* 2002. 36(1): p. 57-68.
369. Moore, K.W., et al., *Interleukin-10 and the interleukin-10 receptor.* 2001. 19(1): p. 683-765.
370. Laffer, B., et al., *Loss of IL-10 Promotes Differentiation of Microglia to a M1 Phenotype.* 2019. 13(430).
371. Lively, S. and L.C. Schlichter, *Microglia Responses to Pro-inflammatory Stimuli (LPS, IFNγ+TNFα) and Reprogramming by Resolving Cytokines (IL-4, IL-10).* Frontiers in cellular neuroscience, 2018. 12: p. 215-215.

372. Penna, E., et al., *Neurodevelopmental Disorders: Effect of High-Fat Diet on Synaptic Plasticity and Mitochondrial Functions.* 2020. 10(11): p. 805.
373. Taylor, R.A. and L.H. Sansing, *Microglial responses after ischemic stroke and intracerebral hemorrhage.* Clinical & developmental immunology, 2013. 2013: p. 746068-746068.
374. Hovens, I.B., C. Nyakas, and R.G. Schoemaker, *A novel method for evaluating microglial activation using ionized calcium-binding adaptor protein-1 staining: cell body to cell size ratio.* Neuroimmunology and Neuroinflammation, 2014. 1: p. 82-88.
375. Biesmans, S., et al., *Systemic immune activation leads to neuroinflammation and sickness behavior in mice.* Mediators Inflamm, 2013. 2013: p. 271359.
376. Martin, S.A., et al., *Voluntary wheel running does not affect lipopolysaccharide-induced depressive-like behavior in young adult and aged mice.* Neuroimmunomodulation, 2014. 21(1): p. 52-63.
377. Zhao, J., et al., *Neuroinflammation induced by lipopolysaccharide causes cognitive impairment in mice.* Scientific Reports, 2019. 9(1): p. 5790.
378. Huang, H.-T., et al., *Intermittent peripheral exposure to lipopolysaccharide induces exploratory behavior in mice and regulates brain glial activity in obese mice.* Journal of neuroinflammation, 2020. 17(1): p. 163-163.
379. O'Connor, J.C., et al., *Lipopolysaccharide-induced depressive-like behavior is mediated by indoleamine 2,3-dioxygenase activation in mice.* Molecular psychiatry, 2009. 14(5): p. 511-522.
380. Galic, M., K. Riazi, and Q. Pittman, *Cytokines and brain excitability.* Frontiers in neuroendocrinology, 2011. 33: p. 116-25.
381. Allam-Ndoul, B., et al., *Effect of different concentrations of omega-3 fatty acids on stimulated THP-1 macrophages.* Genes & Nutrition, 2017. 12(1): p. 7.