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Albert Amran March 26, 2017

Diet-Induced Regional and Sex-Dependent Changes in Markers of Neuroinflammation

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Abstract

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A common hallmark of many neurodegenerative diseases (ND) is chronic activation of the immune response in the central nervous system (CNS). The immune system is thought to be dysregulated in ND patients, leading to a pathological cascade of inflammation and apoptosis that is unable to be properly controlled. Through the identification of risk factors, researchers have begun to focus on what may precipitate dysregulation of the immune response in ND patients. Alzheimer's Disease (AD) has been shown to be more common in patients with Metabolic Syndrome (MetS), prompting researchers to focus on how factors in MetS could influence the immune system. In this study, we explore how an inflammatory high-fat high-fructose (HFHF) diet capable of generating MetS-like conditions impacts the transcription of several immune factors in the prefrontal cortices and hippocampi of mice. Previous studies have focused on the influence of diet on the hypothalamus, the brain's center of metabolic control, and excluded other regions of the brain that are not directly involved with dietary intake. Here we show that diet generates transcriptional restriction of immune factors in the hippocampi of male mice, and that the prefrontal cortex is relatively resistant to a HFHF diet. Additionally, sex differences in the transcription of immune factors of mice fed a control diet were non-existent in mice fed a HFHF diet. These findings demonstrate that no significant dysregulation of the immune response was observed in response to a chronic inflammatory diet. Furthermore, female hippocampi were relatively resistant to the effects of the diet. The results of this study add to a growing body of evidence that diet is able to influence the immune response of the brain.

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Table of Contents

Introduction

Neurodegenerative diseases of the central nervous system such as Alzheimer's Disease (AD), Parkinson's Disease, and Multiple Sclerosis are characterized by a gradual loss of neuronal populations and function, as well as immune activation of the CNS.[1] Of all neurodegenerative diseases, Alzheimer's is the largest contributor to the prevalence of dementia, accounting for two out of every three dementia cases.[2] AD's lack of cure and late clinical presentation have made it difficult to treat, but by identifying risk factors associated with the disease, researchers have made it possible focus efforts on conditions associated with at-risk populations. Recently, epidemiological studies have concluded that patients with metabolic syndrome (MetS), obesity, and type-2 diabetes (T2D) are at a greater risk for developing AD. By studying how these conditions may associate with other identified risk factors to contribute to the genesis of AD, we may be able to focus further research to prevent the onset of AD in the first place.

Aging is the most prominent risk factor for the onset of AD by far, increasing a patient's chances to have AD around 0.5% per year for adults aged 65-70 and a 4.8% increase per year for adults aged 85+.[3] This data is confounded by the difficulty in diagnosing AD early on, and the even more difficult prospect of determining the exact age of onset.[4] Despite the obvious challenges that AD offers to the timing of clinical diagnoses, the extent of aging clearly has a measurable effect on the incidence of AD in elderly populations.[4]

While aging remains the clearest risk factor of AD, it is rather uninformative as a cause. Aging has a too broad an effect on human physiology to investigate, which prompted research into linking individual AD hallmark pathologies to certain organ systems. Furthermore, approximately 5-10% of non-familial AD cases occur in patients below the age of 65 which demonstrates that aging is most likely not the direct cause of AD,[5] but rather may precipitate the conditions necessary for AD to develop. Therefore, scientists have looked to how other risk factors could generate AD, and how aging may play a role in strengthening their effect.

Of the many pathological hallmarks of AD, chronic activation of the immune response in the CNS has become the most promising research target. In fact, many neurodegenerative diseases are thought to be driven by age-associated chronic inflammatory dysregulation in CNS, and potential therapies lie in prevention, inhibition, or delaying the onset of age-associated

chronic neuroinflammation.[6] Aging's effect on the immune system is well-documented. Immunosenescence, or the impairment of immune function in aged systems, results in increased susceptibility to infections or weaker responses to vaccinations.[7, 8] However, peripheral levels of circulating cytokines are increased in elderly patients,[9] and together with evidence suggesting that increased levels of serum cytokines can worsen cognitive decline in AD patients,[10] strongly links the aged immune system to the hallmark pathologies of AD. Furthermore, higher-than-average amounts of circulating inflammatory factors are also typical of other diseases including Type II Diabetes and atherosclerosis.[11] Both disorders are preceded by Metabolic Syndrome (MetS), suggesting that similar increased baseline levels of cytokines are found in both aged immune systems and the immune systems of patients with metabolic disorders.

MetS refers to a collection of risk factors for diseases such as heart disease, stroke, and diabetes. Symptoms of MetS can include obesity, but more generally refers to high blood pressure, high triglyceride levels, low HDL cholesterol, and high blood sugar. A 2006 population study concluded that there was a 2.5-fold chance for AD to be detected in patients with MetS compared to patients without MetS.[12] Since then, researchers have focused on how the chronic low-grade inflammation detected in both MetS patients and animal models of MetS can affect the CNS over time. One of the ways to study the effects of MetS in animals has been through the use of high-fat or high-fructose diets. These diets induce conditions usually referred to as pre-MetS, and over time begin to generate systemic low-grade inflammation, weight gain, and insulin resistance.[13]

With the rising consumption rates of diets rich in fats and sugars among western societies,[14] it is now more important than ever to determine what links between unhealthy diet and the generation of MetS may have to health outcomes later in life. By introducing a similar diet to those that have been shown to induce MetS-like states in mice, we hypothesize that a high-fat high-fructose (HFHF) diet will induce dysregulation of the CNS immune response and result increased inflammatory activity in the brains of mice.

Experimental Approach

The gene panel we are using to analyze the immune profile of the mouse brains can account for several types of immune responses as well as serve as a barometer for general immune activity in the brain. Different facets of immune activity are represented in our gene panel, and their relevance is discussed below:

The fractalkine receptor (FKNr) or CX3C chemokine receptor 1 (CX3CR1), along with its chemokine ligand, FKN or chemokine (C-X3-C motif) ligand 1 (CX3CL1), are involved in the protection of cells in the event of injury and recruitment of immune cells.[15] Found on immune cells both in the CNS and periphery, FKNr-mediating signaling can prolong cellular survival as well, resulting in often conflicting theories as to the role of fractalkine and its receptor in the protection of neurons. In AD models, knock-outs of CX3CR1 prevented neuronal death, perhaps by limiting the degree to which microglia could become activated.[16] However, overexpression of fractalkine in mice inhibited diet-induced inflammation of the hypothalamus.[17] Changes in FKNr expression has been shown to be antagonistically controlled by different arms of the immune system. Typically, cytokines promoting Th1 type immune responses such as IFN-γ will promote expression of FKNr while cytokines associated with the Th2 response such as IL-4 will inhibit it.[18]

TGF-β, or transforming growth factor, has been shown to be essential for the suppression of inflammation. However, it does so by promoting the induction of FOXP3-positive T-regulatory cells which inhibits all aspects of the immune response. This can include blocking differentiation of effector T-cells, production of effector cytokines, and proliferation of innate immune cells.[19] The regulatory cells induced by TGF-β in the absence of Interleukin-6 (IL-6) are part of the Th17 immune response, which acts antagonistically to suppress both the Th1 and Th2 responses. Thus, we would expect the transcription of FKNr and TGF-β to change inversely depending on the immune response generated by the diet. TGF-β, when expressed simultaneously with IL-6, can potentially lead to a different type of inflammation. Cells that

differentiate when exposed to a combination of IL-6 and TGF-β are self-reactive, and are currently targets of research seeking to elucidate the etiology of autoimmune disease.[20]

CD45, also known as leukocyte common antigen, is present on all immune cells.[21] CD45 is a transmembrane tyrosine phosphatase necessary to promote antigen-receptor signaling. Increased expression of CD45 has been tightly linked to higher activation states in microglia, suggesting that the expression of this protein is positively correlated with the activation of the immune response.[22]

Tumor Necrosis Factor (TNF) and IL-6 are both signals associated with a pro-inflammatory response. Serum TNF, along with IL-6, have both been observed to change in metabolic syndrome.[23] Serum lipocalin-2 (LCN2) positively correlates with metabolic syndrome as well.[24] Originally identified as a bacteriostatic produced by neutrophils, LCN2 is now known to be produced by a wide variety of cells such as neurons and astrocytes in response to an array of inflammatory insults.[25]

C-C chemokine receptor 2 (CCR2) and its ligand CCL2 have been implicated as one of the major influences on a cell's ability to travel. CCR2 knockout mice had an accumulation of CD11b+ Ly6C+ cells (inflammatory monocyte markers) in the bone marrow, where they are produced but were unable to travel out.[26] All monocytes require CCR2 to exit the bone marrow from which they originate, and either keep expressing CCR2 or down-regulate it based on the cell's role and up-regulate once the cell needs to traffic into tissue. Because CCR2 is necessary for monocyte trafficking, it is the most important target to demonstrate the migration of circulating monocytes to key areas under different conditions. Detecting an increase in *Ccl2* transcription signals that innate immune cell recruitment from the periphery is taking place.

To assess sex differences in the diet-induced inflammatory response that could be clinically-relevant, we have included female mice as well as males in our study. The role of sex hormones as a risk factor in Alzheimer's Disease remains controversial. Studies have argued both in favor and against sex's effect on the incidence of AD.[27] An interesting trend has emerged in epidemiological studies originating in Europe and Asia suggesting that women were more commonly affected by AD, whereas studies conducted in the US have found that there is no effect of sex on the incidence of AD. One of the largest studies examining this took place in

Rochester, MN and followed a cohort of fourteen-thousand patients and found no effect of sex.[28] However, there are physiological arguments in favor of sex's role in the development of chronic immune dysregulation because both male and female sex hormones have demonstrated effects on the immune response *in vitro* and *in vivo*.[27] Because we are trying to establish how diet may cause dysregulation of CNS inflammation, it is important to observe how sex may determine the nature of a this specific diet-induced immune response.

Methods

Mice

Each cohort consisted of sixteen inbred C57BL/6J mice ordered from the Jackson Laboratory (stock no. 000664). The C57BL/6J strain has been used in most diet studies, and has been known to have a different immune response than other mouse strains.[29] Thus, it was prudent to ensure as little variability across studies as possible by using the BL/6 strain. At the beginning of each experiment, mice were eight weeks old. Each cohort consisted of eight males and eight females. Mice were group housed for the duration of the experiment. At the end of the experiment, mice were anaesthetized with isofluorane and then underwent cervical dislocation before other protocols were followed to collect samples.

Diet Manipulation

For the duration of the experiment, mice had *ad libitum* access to regular drinking water, and *ad libitum* access to either CD (control diet) or high-fat high-fructose diet (HFHF). The CD diet (4% fat diet #7001, Harlan-Teklad, Madison, WI) is a purified diet (made without grains) in order to match the general consistency of the HFHF diet and to eliminate possible confounding related to anti- or pro-inflammatory factors. CD chow consisted of 4.4% fat, 39.5% carbohydrates, and 25.2% protein, totaling 12.6 kJ/g. HFHF chow consisted of 21.2% fat (61.85% saturated fat, 27.3% monounsaturated fat, and 4.7% polyunsaturated fat), 48.5% carbohydrates, and 4.7% protein, totaling 18.8 kJ/g. Mice were fed either CD or HFHF for 5 weeks and measurements of food intake were recorded. After 5 weeks, mice were sacrificed and the brains, fat, plasma, livers, and intestines were collected.

Quantitative Polymerase Chain Reaction (qPCR)

Mouse tissue was homogenized using a TissueLyser II (Qiagen), a metal bead, and TRIzol® Reagent (Invitrogen). To extract RNA, homogenized tissue was run through Qiagen QIAshredder™ columns and then processed according to the manufacturer's instructions using Qiagen's RNeasy mini protocol (Valencia, CA, USA) for animal tissue. A DNase treatment (DNase I; Invitrogen) was included to secure additional purity. Total RNA yield was determined by

absorbance at 260 nm and purity was determined by 260/280 nm ratio using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Inc.). RNA was reverse transcribed with 2 µg of normalized total RNA from each sample using the QuantiTect® Reverse Transcription Kit (Qiagen). Quantitative qPCR was performed using an ABI Prism 7900 HT Fast Real-time PCR System (Applied Biosystems Inc., Foster City, CA). Each sample was run in triplicate as a 10 µL reaction consisting of 25 ng cDNA, approximately 5.7 µL SYBER green PCR Master mix (Power SYBER Green; Applied Biosystems), and 150 nM of each forward and reverse PCR primer.

Acute Neroinflammation model

Three female two-month old C57BL/6J mice, were injected intraperitoneally with LPS (5mg/kg, Sigma-Aldrich, St. Louis, MO) derived from *Escherichia coli* clone: 0111:B4, Lot#: 014M4018V. Mice were left with water and food for six hours prior to sacrifice. This model is widely used to model acute neuroinflammation.[30] These mice were compared with the HFHF-diet fed female mice from cohort 2 because their normalization values were the most closely matched.

Statistical analysis

Sex hormones have significant effects on the immune response, but there is a high degree of variability as to what extent.[31] Thus, the effect of diet on the neuroinflammatory response is expected to vary with sex. RNA levels of common inflammatory mediators were measured in mice fed the Control Diet (CD) for five weeks to establish a baseline for the HFHF diet group as well as to measure sex differences in baseline immune activity.

Cohorts were analyzed separately as two experiments. A student's t-test was conducted to compare the average of each group's mean within each cohort. Normal distribution of the samples was assumed as sample sizes were too small to conduct tests for normality. CT (cycles to threshold) values belonging to the prefrontal cortex were normalized to an arithmetic average of *TATABP*'s and *GAPDH*'s CT values. Hippocampus CT values were normalized to only the *TATABP* CT values.

Results

All groups have an *n* of 4

Key: smaller dCT values indicate higher levels of RNA. Bolded values are significantly different compared to their opposite-sex counterparts. P-values less than or equal to 0.05, 0.01, 0.001, and 0.0001 are indicated by asterisks of *, **, ***, and **** respectively.

M=Males
F=Females

Prefrontal Cortex (PFC)
dCT values (normalized)

Gene	M-Cohort 1	F-Cohort 1	M-Cohort 2	F-Cohort 2
Fknr	3.689 ± 0.085	4.092 ± 0.160	**5.005 ± 0.134** ***	**3.605 ± 0.148** ***
Tgf-β	5.035 ± 0.027	5.104 ± 0.113	**5.303 ± 0.080** **	**4.630 ± 0.128** **
Cd45	6.468 ± 0.063	6.564 ± 0.141	**5.525 ± 0.111** *	**5.915 ± 0.107** *
Tnf	11.40 ± 0.242	11.61 ± 0.259	12.23 ± 0.295	12.36 ± 0.103
Lcn2	7.581 ± 0.247	7.458 ± 0.432	**4.303 ± 1.301** *	**8.464 ± 0.497** *
Ccl2	9.178 ± 1.015	10.11 ± 0.4150	10.98 ± 0.174	11.07 ± 0.210
Il-6	12.11 ± 0.245	12.07 ± 0.104	12.22 ± 0.125	12.44 ± 0.220
Cyclophilin	1.582 ± 0.027	1.486 ± 0.053	1.187 ± 0.045	1.048 ± 0.109

Table 1: Sex differences of murine PFCs in RNA transcription at baseline (Control Diet). Sex differences in baseline cytokine transcription were observed only in cohort 2. Significant differences in the mRNA responsible for fractalkine receptors, *Tgf*- β, and Lipocalin-2 were observed only in cohort 2 but not cohort 1. In the prefrontal cortices of cohort 2, male mice at baseline had less FKNr and *Tgf*-β mRNA while females had less Lipocalin-2. No other changes were observed.

Hippocampus (HPC)
dCT values (normalized)

Gene	M-Cohort 1	F-Cohort 1	M-Cohort 2	F-Cohort 2
Fknr	**-0.105 ± 0.082** **	**0.407 ± 0.104** **	0.011 ± 0.042	0.118 ± 0.093
Tgf-β	1.117 ± 0.085	1.368 ± 0.093	1.341 ± 0.130	1.388 ± 0.095
Cd45	3.054 ± 0.102	2.913 ± 0.131	3.009 ± 0.108	3.024 ± 0.116
Tnf	8.912 ± 0.118	8.705 ± 0.238	8.793 ± 0.399	9.153 ± 0.135
Lcn2	5.786 ± 0.240	5.248 ± 0.684	**0.856 ± 1.045** **	**6.561 ± 0.302** **
Ccl2	7.584 ± 0.318	7.966 ± 0.305	7.866 ± 0.382	8.306 ± 0.123
Gapdh	-7.094 ± 0.070	-6.875 ± 0.088	-6.953 ± 0.016	-6.900 ± 0.059
Il-6	8.309 ± 0.173	8.550 ± 0.06829	8.099 ± 0.1238	8.092 ± 0.1438

Table 2: Sex differences of murine HPCs in RNA transcription at baseline (Control Diet). Hippocampal fractalkine receptor appeared to be more highly transcribed in the males of cohort 1 and *Lcn2* was observed to be more highly transcribed in the males of cohort 2. No other significant changes were observed.

PFC
dCT values (normalized)

Gene	M-Cohort 1	F-Cohort 1	M-Cohort 2	F-Cohort 2
Fknr	3.638 ± 0.1112	3.839 ± 0.099	5.022 ± 0.093	4.801 ± 0.138
Tgf-β	5.017 ± 0.1950	5.136 ± 0.078	5.347 ± 0.118	5.192 ± 0.041
Cd45	6.210 ± 0.09995	6.277 ± 0.053	5.637 ± 0.080	5.810 ± 0.026
Tnf	11.16 ± 0.7066	11.22 ± 0.363	12.57 ± 0.155	12.15 ± 0.226
Lcn2	6.793 ± 0.3621	7.948 ± 0.536	8.219 ± 0.097	9.057 ± 0.392
Ccl2	8.846 ± 1.349	10.33 ± 0.745	10.70 ± 0.188	10.25 ± 0.260
Il-6	11.55 ± 0.095	11.94 ± 0.168	**12.30 ± 0.145** *	**12.81 ± 0.143***
Cyclophilin	1.441 ± 0.057	1.478 ± 0.080	1.273 ± 0.062	1.162 ± 0.056

Table 3: Mouse PFCs' sex differences in RNA transcription when given High-Fat High-Fructose (HFHF) diet. When analyzing sex differences in mice given HFHF diet, the trends observed at baseline disappeared in cohort 2. However, *Il-6* transcripts were found in higher amounts in males.

HPC
dCT values (normalized)

Gene	M-Cohort 1	F-Cohort 1	M-Cohort 2	F-Cohort 2
Fknr	0.279 ± 0.090	0.070 ± 0.123	0.193 ± 0.056	0.268 ± 0.077
Tgf-β	1.435 ± 0.097	1.369 ± 0.106	1.360 ± 0.077	1.419 ± 0.059
Cd45	**3.383 ± 0.076** *	**2.852 ± 0.176** *	3.165 ± 0.101	2.973 ± 0.119
Tnf	8.381 ± 0.378	8.527 ± 0.378	9.566 ± 0.213	9.152 ± 0.146
Lcn2	5.365 ± 0.541	5.581 ± 0.287	5.890 ± 0.557	6.854 ± 0.293
Ccl2	7.286 ± 0.800	7.417 ± 0.284	7.993 ± 0.291	7.745 ± 0.210
Gapdh	-6.797 ± 0.082	-6.819 ± 0.133	-6.662 ± 0.083	-6.740 ± 0.066
Il-6	8.446 ± 0.1116	8.397 ± 0.148	8.295 ± 0.196	8.513 ± 0.276

Table 4: Mouse HPCs' sex differences in RNA transcription when given High-Fat High-Fructose (HFHF) diet. Statistically significant differences in the hippocampi of mice given HFHF diet were observed in the fractalkine receptor mRNA of Cohort 1 and the mRNA of Lipocalin-2 of cohort 2.

PFC (normalized)

Gene	Diet	M-Cohort 1	F-Cohort 1	M-Cohort 2	F-Cohort 2
Fknr	CD	3.689 ± 0.085	4.092 ± 0.160	5.005 ± 0.134	**3.605 ± 0.148** **
	HFHF	3.638 ± 0.111	3.839 ± 0.099	5.022 ± 0.093	**4.801 ± 0.138** **
Tgf-β	CD	5.035 ± 0.027	5.104 ± 0.113	5.303 ± 0.080	**4.630 ± 0.128** **
	HFHF	5.017 ± 0.195	5.347 ± 0.118	5.136 ± 0.078	**5.192 ± 0.041** **
Cd45	CD	6.468 ± 0.063	6.564 ± 0.141	5.525 ± 0.111	5.915 ± 0.107
	HFHF	6.210 ± 0.010	6.277 ± 0.053	5.637 ± 0.080	5.810 ± 0.026
Tnf	CD	11.40 ± 0.242	11.61 ± 0.259	12.23 ± 0.295	12.36 ± 0.103
	HFHF	11.16 ± 0.707	11.22 ± 0.363	12.57 ± 0.155	12.15 ± 0.226
Lcn2	CD	7.581 ± 0.247	7.458 ± 0.432	**4.303 ± 1.301** *	8.464 ± 0.497
	HFHF	6.793 ± 0.362	7.948 ± 0.536	**8.219 ± 0.097** *	9.057 ± 0.392
Ccl2	CD	9.178 ± 1.015	10.11 ± 0.415	10.98 ± 0.174	**11.07 ± 0.210** *
	HFHF	8.846 ± 1.349	10.33 ± 0.745	10.70 ± 0.188	**10.25 ± 0.260** *
Il-6	CD	12.11 ± 0.245	12.07 ± 0.104	12.22 ± 0.125	12.44 ± 0.220
	HFHF	11.55 ± 0.095	11.94 ± 0.168	12.30 ± 0.145	12.81 ± 0.143
Cyclophilin	CD	1.582 ± 0.027	1.486 ± 0.053	1.187 ± 0.045	1.048 ± 0.109
	HFHF	1.441 ± 0.056	1.478 ± 0.080	1.273 ± 0.062	1.162 ± 0.056

Table 5: HFHF diet induces immune restricted changes in female PFC but not males. Diet induced increases in Cohort 2 females' *Ccl2* mRNA levels and decreases in their Fractalkine receptor and *Tgf*-β mRNA in the PFC. All other groups were unaffected with the exception of the decreased *Lcn2* mRNA of the cohort 2 males. dCT values displayed above. Red boxes surround groups where diet induced a decrease in transcription. Green boxes surround groups where diet induced an increase in transcription.

HPC (normalized)

Gene	Diet	M-Cohort 1	F-Cohort 1	M-Cohort 2	F-Cohort 2
Fknr	CD	**-0.105 ± 0.082** *	0.407 ± 0.104	**0.011 ± 0.042** *	0.118 ± 0.093
	HFHF	**0.279± 0.090** *	0.070 ± 0.123	**0.193 ± 0.056** *	0.268 ± 0.077
Tgf-β	CD	**1.117 ± 0.085** *	1.368 ± 0.093	**1.341 ± 0.130** *	1.388 ± 0.095
	HFHF	**1.435 ± 0.097** *	1.369 ± 0.106	**2.605 ± 0.487** *	1.419 ± 0.059
Cd45	CD	**3.054 ± 0.102** *	2.913 ± 0.131	3.009 ± 0.108	3.024 ± 0.116
	HFHF	**3.383 ± 0.076** *	2.852 ± 0.176	3.165 ± 0.101	2.973 ± 0.119
Tnf	CD	8.912 ± 0.118	8.705 ± 0.238	8.793 ± 0.399	9.153 ± 0.135
	HFHF	8.381 ± 0.378	8.527 ± 0.377	9.566 ± 0.213	9.152 ± 0.148
Lcn2	CD	5.786 ± 0.240	5.248 ± 0.684	**0.856 ± 1.045** *	6.561 ± 0.302
	HFHF	5.365 ± 0.541	5.581 ± 0.287	**5.890 ± 0.557** *	6.854 ± 0.293
Ccl2	CD	7.584 ± 0.318	7.966 ± 0.305	7.866 ± 0.382	8.306 ± 0.126
	HFHF	7.286 ± 0.800	7.417 ± 0.284	7.993 ± 0.291	7.745 ± 0.210
Il-6	CD	8.309 ± 0.173	8.550 ± 0.068	8.099 ± 0.124	8.092 ± 0.144
	HFHF	8.446 ± 0.117	8.397 ± 0.148	8.295 ± 0.196	8.513 ± 0.276
Gapdh	CD	**-7.094 ± 0.070** *	-6.875 ± 0.088	**-6.953 ± 0.016** *	-6.900 ± 0.059
	HFHF	**-6.797 ± 0.082** *	-6.819 ± 0.133	**-6.662 ± 0.083** *	-6.740 ± 0.066

Table 6: HFHF diet induces immune-restricted characteristics in male hippocampi but not in females. Reductions were observed in both groups of males in FKNr, TGF-β, and GAPDH. Reductions in CD45 and LCN2 mRNA were observed in Cohort 1 and Cohort 2, respectively. dCT values displayed above. Red boxes surround groups where diet induced a decrease in transcription. Green boxes surround groups where diet induced an increase in transcription.

Acute Neuroinflammatory Profiles

Prefrontal Cortex

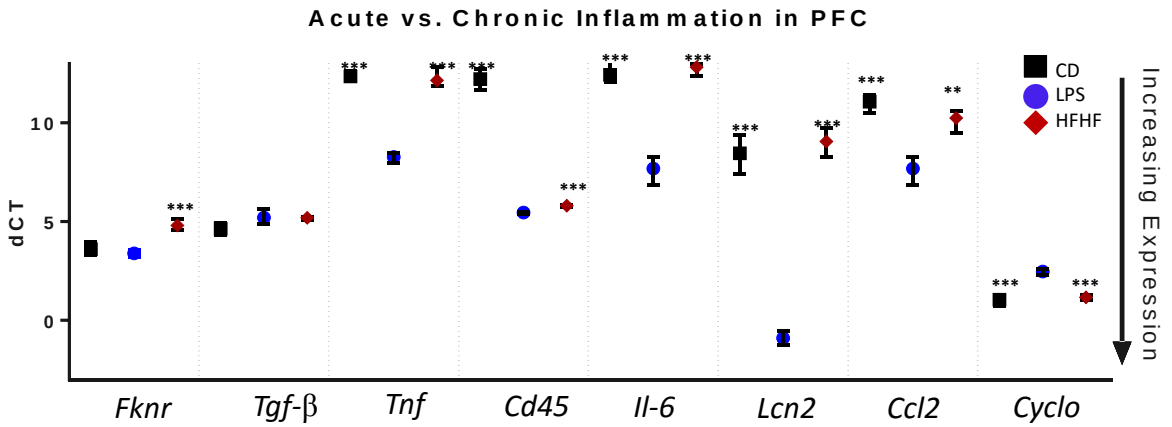

Figure 1: LPS injection leads to higher transcription of almost all inflammatory markers in our gene panel relative to HFHF diet models. Acute inflammation led to a significant increase in all markers' mRNA except *Cyclophilin* and *Tgf*-β relative to chronic inflammation induced by the diet model. *Cyclophilin* decreased while *Tgf*-β was not any different. Asterisks signify statistical differences relative to the LPS acute neuroinflammatory model.

Hippocampus

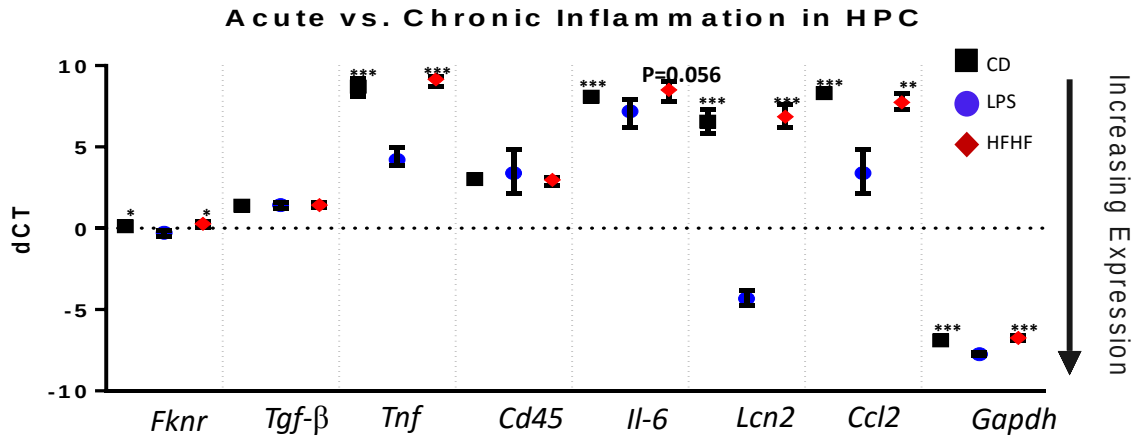

Figure 2: LPS injection leads to higher transcription of less markers than in the PFC. Overall, higher transcription levels were observed in all markers except *Tgf*-β and *Cd45. Il-6* levels of the LPS-injected mice were almost significantly increased (p=0.056) compared to the HFHF-diet mice. Asterisks signify statistical differences relative to the LPS acute neuroinflammatory model.

Discussion

Sex differences with Control Diet

As discussed earlier, evidences suggest that sex hormones can have a profound effect on the immune response. Different diets, can introduce additional variable to the gender associated immune outcomes. It has been reported that male and female mice that have consumed high-fat diet experience different inflammatory profiles in the hypothalamus. More specifically, males develop inflammation in both the hypothalamus and periphery while females do not.[32] To establish baseline sex differences in cytokine transcription, we assessed the relatively inactive immune profiles of the mouse brain. In the prefrontal cortex of mice fed control diet (Table 1), we observed sex differences only in cohort 2. Of those, males had less *Tgf*-β, *Fknr*, and *Cd45* mRNA expression. In the context of sexual dimorphism of the immune response, this was unexpected. TGF-β and FKNr represent separate arms of the immune response that antagonistically control one another, and for them both to be decreased suggests an attempt to restrict inflammation. This conclusion is further supported by the decreased amount of *Cd45* mRNA in the pre-frontal cortex. We would expect this result in females, as estrogen has been shown to reduce circulating immune cells populations such as monocytes.[33] However, males on control diet had more *Lcn2* mRNA, suggesting that the male mice were reacting to something at baseline, or that *Lcn2* transcription is higher in males overall. Because these mice were on control diet with only sex as the varying condition, we hypothesized that perhaps the lack of significant amounts of fiber in the control diet may make males somewhat more inflammatory. Increased fiber intake has been shown to reduce inflammation in the gut,[34] and females have been shown to be less responsive, immunologically, to diet-induced inflammation.[32]

Interestingly, some sex differences in one region were found in both the PFC and HPC while others did not. Hippocampi of cohort 2 males also had a significantly higher amount of *Lcn2* mRNA than females, but did not display the same differences in *Cd45*, *Fknr*, or *Tgf*-β (Table 2). The opposite trend appeared in the hippocampi of cohort 1 males, with the males having more *Fknr* mRNA but no other differences.

HFHF diet induces inflammation in the periphery in males but not females

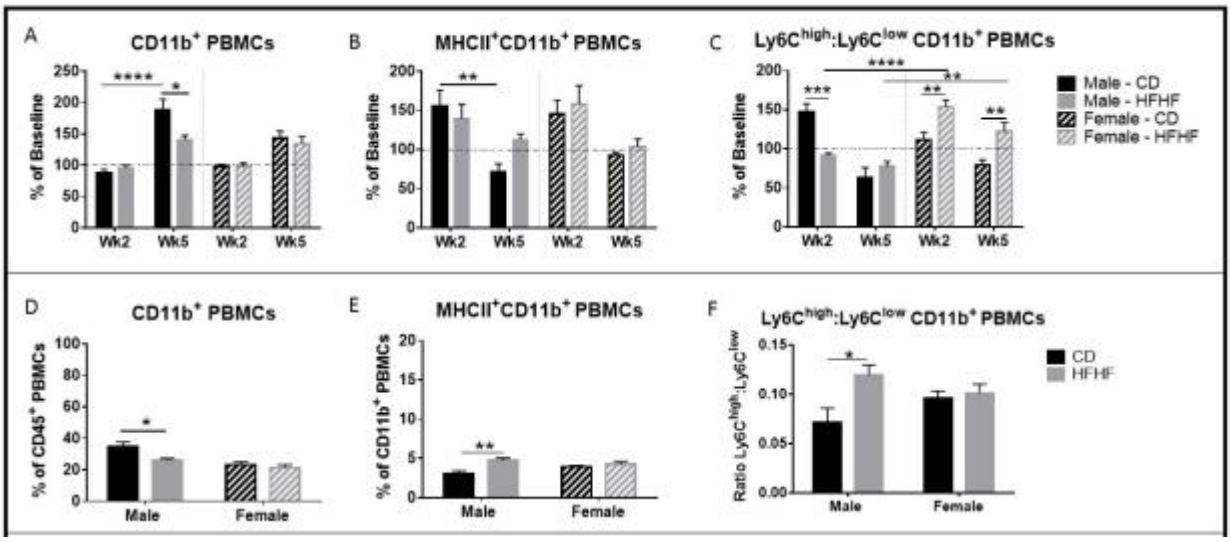

Figure taken from MacPherson et. al (in preparation for publication). The same mice whose brains are shown in this study's results section were assessed for the effects of HFHF diet on immune cell populations in the periphery using flow cytometry. That data is displayed above. After five weeks of HFHF diet, the ratio of Ly6Chi CD11b+ cells (activated monocytes) to Ly6Clo CD11b+ (not-activated monocytes) were significantly increased in males but not females (Graph F). A higher Ly6Chi:Ly6Clo ratio indicates increased innate immune activation, and here it was found only in male mice. Diet-induced peripheral inflammation produced in males but not females was consistent with other diet models.[35, 36] This was an important point to prove considering that our diet's fat content was less than that of other studies,[37] as well as the high amount of variability between different diet models.[38] If the diet had not been able to induce peripheral immune activation, we would have been unable to say if there was an inflammatory insult at all. The figure above provides evidence that there was peripheral immune activation after five weeks of HFHF diet.

Sex differences after HFHF diet

All sex-specific trends that were present in the prefrontal cortices in both control diet cohorts disappeared in the HFHF diet condition. Reasons that the trends will have disappeared with be discussed during the CD vs. HFHF comparison (Tables 5 and 6). IL-6, however, appears to be higher in males than in females when both were fed HFHF (Table 3). Increased IL-6 transcription levels in males in the absence of differences in other markers suggests an effect of sex hormones on the response to the diet. MetS has been correlated with lower than average levels of testosterone, and a high-fructose diet does induce lower circulating testosterone levels, even before the onset of MetS.[39] Other studies have found an inverse relationship between testosterone levels and soluble IL-6.[40] The diet, through the restriction of testosterone, would allow for increased IL-6 transcription relative to the females.

The differences from the prefrontal cortex do not mirror the hippocampus, which only displayed a difference in *Cd45* mRNA in cohort 1 HFHF mice (Table 4). Females given HFHF diet had higher expression of *Cd45* mRNA than males, suggesting an increased immune presence. The lack of changes elsewhere in the panel (Figure 4) as well as the absence of any diet-induced changes in the females of cohort 1 (Table 6) suggests that the decreased *Cd45* transcription occurred in male hippocampi, not females.

HFHF diet induced immune-restrictive changes in the Prefrontal Cortex of Females

No changes were observed in cohort 1 HFHF mice, males or females. However, in cohort two, several changes occurred (Figure 5). Decreased FKNr and TGF-β mRNA accompanied an increase in *Ccl2* transcription in females only. Females have been reported to be much better at controlling diet-induced metabolic symptoms as well as inflammation.[32, 36] These results suggest that a sort of immune-related transcription suppression is going on, as indicated by the drops in *Fknr* and *Tgf*-β mRNA. Both cytokines are involved with different arms of the immune response. Because FKNr and TGF-β often act in antagonism of one another, it is surprising to see both undergo restricted transcription. We speculate that this could also be a response to diet-induced inflammatory saturation of the PFC microenvironment, though a quantitative

analysis of protein levels between control and HFHF diet mice would be needed to ascertain if that hypothesis is correct. The increase in *Ccl2* transcription could signal an attempt to recruit peripheral immune cells to the PFC. While other diet studies have not studied *Ccl2* transcription in the PFC, other chronic inflammatory models have. CCL2-mediated peripheral immune traffic to the PFC has been demonstrated in models of social defeat, which have been theorized to become inflamed in response to chronic social anxiety.[41] Because studies examining innate immune recruitment to the CNS have generally concluded that it is beneficial,[42, 43, 44] we hypothesize that the PFC may be inducing immune recruitment and downregulating cytokines such as FKNr and TGF-β with the goal of downregulating inflammation.

The only change in the male prefrontal cortices occurred in cohort 2, and involved the decreased transcription of the bacteriostatic agent LCN2. This is unexpected because studies that had examinefd *Lcn2*'s transcription in the hippocampus reported the that diet actually increased levels of *Lcn2* mRNA.[37] However, our findings could be the result of a reduction in transcription after five weeks of the HFHF diet. At one point, *Lcn2* transcription may have been increased before dropping to below baseline levels. Our acute neuroinflammatory model demonstrates that *Lcn2* is more highly transcribed six hours after an immune insult (Figures 1 and 2).

HFHF diet induced immune-restrictive changes in male Hippocampi

Only males experienced any changes when given HFHF diet, an expected result given that females are less responsive to HFHF diet. Three changes were shared across both cohort 1 and 2 males' HPC. Drops in *Fknr*, *Tgf*-β, and *Gapdh* transcription were observed, suggesting as we had proposed earlier in the female PFCs that immune-related transcription was suppressed as a result of the HFHF diet. While historically used as a housekeeping gene, Glyceraldyhyde-3-phosphate dehydrogenase or GAPDH, has been shown to have several immune-related functions.[45] Among these includes the binding of GAPDH to TNF to modulate TNF expression by innate immune cells as well as the protective effect of injecting GAPDH to prevent sepsis-related injury.[46, 47] GAPDH has also been shown to inhibit production of pro-inflammatory cytokines that may trigger upregulation of FKNr.[47] Reductions of inflammatory as well as anti-

inflammatory agents suggests an overall reduction in immune activity, or rather immune suppression following the five-weeks of HFHF diet. The reduction of CD45 mRNA in cohort 1 males backs this hypothesis due to CD45's close association with the activation of immune cells, though the difference is not present in the cohort 2 males' HPCs. The cohort 2 males that experienced drops in LCN2 transcription in the PFC experienced a similar drop in the HPC.

Acute vs. Chronic models of Neuroinflammation

The pathway by which diet can induce inflammation has been theorized to involve the microbiome. Evidence for this theory includes the fact that diet-mediated cytokine production by adipocytes was demonstrated to require a microbiome *in vivo*, and that plated adipocytes washed with serum from mice given high-fat diet also began exhibiting inflammatory processes.[48] According to studies detailing the effects of diet on the intestinal microbiota, consumption of high-fat diets alters gut flora populations in such a way that the intestinal wall becomes permeable. High-fat high-fructose (HFHF) diets are able to do this by increasing the number of phosphorylated membrane transport proteins such as Claudin-2 (CLDN2), which leads to more permeable intestinal walls.[37] With a permeable gut, bacterial endotoxins produced by the microbiome can enter the rest of the body, triggering inflammation.[49] The mechanism for peripheral immune activation in response to high-fat diet in the periphery has been shown to be Toll-Like Receptor 4(TLR4)-mediated, suggesting that the inflammatory agent involved may be lipopolysaccharide (LPS), a common ligand of this receptor. LPS is commonly found in the cell walls of bacteria, further strengthening the case for the role of the leaky gut and host microbiota in driving diet-induced inflammation. TLR4 knock-out mice fed high-fat diet for 8 weeks did not experience the increased expression of pro-inflammatory markers as wild-type (WT) mice fed the same thing, and weighed 82% less than their WT counterparts.[50] TLR4 signaling thus may play a major role in diet-induced inflammation as well as weight gain.

Based on this information, modeling an acute version of diet-induced inflammation should include a ligand for the TLR4 receptor. LPS, a common bacterial endotoxin, is a Pathogen-Associated Molecular Pattern (PAMP) that triggers an innate immune response via TLR4.[51] LPS injected intraperitoneally results in inflammation of the central nervous system

within a few hours. The resulting cascade leads to the increased expression of pro-inflammatory mediators, such as TNF-α and IL-6. Figures 1 and 2 detail the effects of an LPS IP injection on the prefrontal cortex and hippocampus of female mice, effectively modeling an acute neuroinflammatory response. Comparing the influence of LPS-injections or HFHF diet-feeding on the markers included in our gene panel will be able to demonstrate the different neuroinflammatory profiles of acute and chronic inflammatory insults, respectively.

LPS injections resulted in a stronger immune response in both the PFC and HPC than the diet model. Certain gene transcripts that did change with the LPS injection, such as *Tnf* and *Il-6*, were not observed to change at all in the HFHF-model, at least in female mice. Because almost no sex differences in the gene panel were observed between males and females fed HFHF diet, we concluded that males and females exhibited were similar in their transcription of almost all inflammatory markers (Tables 1-4). Thus, the HFHF diet data in Figures 1 and 2 can represent males as well. As mentioned in the Methods section, HFHF females from cohort 2 were chosen to compare to the LPS injected females because their normalization values were the most closely matched.

In Figure 1, we see that all markers in the gene panel are more highly transcribed in the LPS model except for *Tgf*-β. Because our diet model did not observe any changes with *Tnf* and *Il-6*, we hypothesize that a different component of the diet model may be inhibiting the expression of TNF or IL-6 whereas inflammation derived solely from LPS did not have such hindrances. Furthermore, the trends we observed in the female HFHF mice PFCs were similar to those found in the LPS-injected mice. *Tgf*-β transcripts were not significantly different, suggesting that perhaps transcription of this particular anti-inflammatory pathway is restricted in the brain entirely, such that even strong acute immune insults do not alter transcription of the *Tgf*-β pathway. In this acute model, which simulates a bacterial infection, this makes sense because TGF-β production could lead to tolerance of the infectious agent. We hypothesized that restriction of *Tgf*-β transcription in the chronic inflammatory model would prevent escalation of immune activity of ongoing inflammation, and that this could be true in the context of a restriction in *Fknr* transcription, which is observed. In the acute inflammatory model, *Fknr* mRNA is increased relative to the chronic model but does not change relative to

the CD diet condition. This result was unexpected in the context of both inflammatory models. If *Fknr* transcription is indeed induced by pro-inflammatory cytokines, we should have observed a consequent increase in both inflammatory models. However, in the acute model, *Fknr* transcription is unchanged, and the chronic neuroinflammatory model is actually observed to have a reduction in *Fknr* mRNA. All other markers –except Cyclophilin– observed to be higher in the acute model are integral for or markers of immune activation, and thus suggest that the acute model generates a stronger immune response than the HFHF diet. Cyclophilin's role in immune or metabolic function is not well understood, and in humans is believed to have lost its original function. It belongs to a family of highly conserved family of proteins called immunophilins found in organisms ranging from plants to humans.[52] In Figure 1, we can see that the LPS model has a reduced amount of *Cyclophilin* mRNA relative to the diet model mouse PFCs.

All of these trends carry over to Figure 2 with the exception of *Cd45* and *Fknr*. CD45's mRNA was similar to that of the diet, suggesting that the activation status of CNS immune cells in diet models and acute inflammatory models are similar. Because we did not observe any changes in *Cd45* mRNA levels in female HPCs given HFHF, it is possible that hippocampal *Cd45* is unaffected in females given an LPS injection. The almost significantly different change in *Il-6* transcription is interesting, but would require a more highly powered assay to determine if hippocampal *Il-6* production was different from the HPCs of the HFHF diet mice. If it is not, we could be seeing how the HPC is more resistant to inflammatory insults than the PFC. *Fknr* displayed no change in the HFHF diet condition, but was increased in LPS-injected mice. This is likely different in males if we were to inject males with LPS and compare hippocampal *Fknr* transcription given that no changes in HPC transcripts were detected in the HFHF diet condition.

Diet-induced inflammation leads to increased inflammation in the hypothalamus and is thought to result in the generation of Met-S as well as T2D. Based on the results of the current study, however, we conclude that this does not happen in the PFCs and HPCs of two-month old mice after five weeks of HFHF feeding. In fact, we propose that the opposite is true.

Inflammation seems to be controlled in the context of our qPCR panel, with proliferative immune molecules reduced across many immune system branches.

In the hippocampus, the lack of an apparent effect on HFHF-fed females was expected. One study examining the effects of diet on hippocampal function have found that diet impaired memory formation in males but not females.[53] Though our study likewise found no changes in females, that does not mean that the immune system is not active in the female hippocampi. There are hundreds of other potential inflammatory mediators that could be involved in ensuring that the female hippocampus is not affected by HFHF diet, but of the few observed in this study, there were none that changed.

Male hippocampi underwent what we conclude to be a restriction of immune mediator transcription across the board. *Gapdh*, *Tgf-β*, and *Fknr* were reduced in both male HFHF cohorts, perhaps as a protective measure against the harmful effects of diet-induced inflammatory activity in the area. Downregulating these factors would prevent escalation of any immune activity. It has been shown that increased inflammatory activity in the hippocampus impairs hippocampal neurogenesis, and it is thought that this relationship may be responsible for cognitive deficits.[54] Any dysfunction in the ability to control the immune activity occurring in the HPC could allow for extensive impairment of memory formation.

Few studies using diet models have detected inflammation in the prefrontal cortex following high-fat diets.[55] Additionally, in our study, the only observed changes were in the female mice of cohort 2. While reductions in both *Fknr* and *Tgf*-β mRNA were consistent with other groups that appeared to respond to the diet, increased transcription of *Ccl2* was only reported in this one group. However, because *Ccl2* transcription was observed to increase in another study with a chronic inflammatory model, this assay bears repeating with a more strongly powered group of mice. *Ccl2* transcription signals recruitment of peripheral monocytes, suggesting that the PFC is reacting to the diet, at least in our cohort 2 females. An impairment in the ability to recruit immune cells or an inability to limit recruitment could result in unwanted exacerbation of inflammatory activity in the PFC.

AD and MetS are characterized by an inability to properly limit the extent to which the immune response activates. At baseline, both conditions are associated with higher than

normal levels of circulating inflammatory markers which signals poor control of the immune response. In older patients or patients with MetS, the mechanisms behind the reductions in *Fknr* and *Tgf*-β that were observed in this study could be impaired, resulting in unwanted proliferation of immune cell activity. In older patients especially, this could precipitate the beginning of the amyloid-β aggregation cascade observed in AD.[56] However, this is dependent on the ability of unhealthy diet to impair the immune regulatory mechanisms that we believe resulted in the restriction of immune markers we observed. Because we believe our study did not result in dysregulation of the CNS immune response, it will be necessary to experiment with how much and how long of a metabolic "insult" is necessary to dysregulate the immune response. Further insight into how diet and MetS could result in the regulatory impairment necessary to allow this unwanted proliferation to occur is the next step in determining the link between diet, MetS-like states, and chronic immune activation in the CNS. We believe our model was unable to fully impart MetS in our mice, and that they were still capable of regulating their immune responses after five-weeks on HFHF diet, even though peripheral inflammation was observed. In the future, increasing the duration of this experimental model would provide the MetS-mediated impairment of the immune response needed to observe how our HFHF diet interacts with a CNS immune response unable to properly regulate itself.

Literature Cited

1. Chen, W., Zhang, X. & Huang, W. Role of neuroinflammation in neurodegenerative diseases (Review). *Molecular medicine reports* **13**, 3391-3396 (2016).

2. Burns, A. & Iliffe, S. Alzheimer's disease. *BMJ (Clinical research ed.)* **338**, b158 (2009).

3. Brookmeyer, R., Gray, S. & Kawas, C. Projections of Alzheimer's disease in the United States and the public health impact of delaying disease onset. *American Journal of Public Health* **88**, 1337-1342 (1998).

4. Mayeux, R. & Stern, Y. Epidemiology of Alzheimer Disease. *Cold Spring Harbor perspectives in medicine* **2**.

5. Campion, D. *et al.* Early-onset autosomal dominant Alzheimer disease: prevalence, genetic heterogeneity, and mutation spectrum. *American Journal of Human Genetics* **65**, 664-670 (1999).

6. Ransohoff, R.M. How neuroinflammation contributes to neurodegeneration. *Science (New York, N.Y.)* **353**, 777-783 (2016).

7. Haralambieva, I.H. *et al.* The Impact of Immunosenescence on Humoral Immune Response Variation after Influenza A/H1N1 Vaccination in Older Subjects. *PloS one* **10** (2015).

8. Bruunsgaard, H., Pedersen, A.N., Schroll, M., Skinhøj, P. & Pedersen, B.K. Impaired production of proinflammatory cytokines in response to lipopolysaccharide (LPS) stimulation in elderly humans. *Clinical and Experimental Immunology* **118**, 235-241 (1999).

9. Sansoni, P. *et al.* The immune system in extreme longevity. *Experimental gerontology* **43**, 61-65 (2008).

10. Holmes, C. *et al.* Systemic inflammation and disease progression in Alzheimer disease. *Neurology* **73**, 768-774 (2009).

11. Esposito, K. *et al.* Inflammatory cytokine concentrations are acutely increased by hyperglycemia in humans: role of oxidative stress. *Circulation* **106**, 2067-2072 (2002).

12. Vanhanen, M. *et al.* Association of metabolic syndrome with Alzheimer disease: a population-based study. *Neurology* **67**, 843-847 (2006).

13. Posey, K.A. *et al.* Hypothalamic proinflammatory lipid accumulation, inflammation, and insulin resistance in rats fed a high-fat diet. *American Journal of Physiology - Endocrinology and Metabolism* **296**, E1003-1012 (2009).

14. Vos, M.B., Kimmons, J.E., Gillespie, C., Welsh, J. & Blanck, H.M. Dietary Fructose Consumption Among US Children and Adults: The Third National Health and Nutrition Examination Survey. *The Medscape Journal of Medicine* **10**, 160 (2008).

15. Sheridan, G.K. & Murphy, K.J. Neuron–glia crosstalk in health and disease: fractalkine and CX(3)CR1 take centre stage. *Open Biology* **3** (2013).

16. Fuhrmann, M. *et al.* Microglial Cx3cr1 knockout prevents neuron loss in a mouse model of Alzheimer's disease. *Nature neuroscience* **13**, 411-413 (2010).

17. Dorfman, M.D. *et al.* Sex differences in microglial CX3CR1 signalling determine obesity susceptibility in mice. *Nature Communications* **8** (2017).

18. Panek, C.A. *et al.* Differential expression of the fractalkine chemokine receptor (CX(3)CR1) in human monocytes during differentiation. *Cellular and Molecular Immunology* **12**, 669-680 (2015).

19. Yoshimura, A., Wakabayashi, Y. & Mori, T. Cellular and molecular basis for the regulation of inflammation by TGF-β. *Journal of Biochemistry* **147**, 781-792 (2010).

20. Rose-John, S. IL-6 Trans-Signaling via the Soluble IL-6 Receptor: Importance for the Pro-Inflammatory Activities of IL-6. *International Journal of Biological Sciences* **8**, 1237-1247 (2012).

21. Penninger, J.M., Irie-Sasaki, J., Sasaki, T. & Oliveira-dos-Santos, A.J. CD45: new jobs for an old acquaintance. *Nature immunology* **2**, 389-396 (2001).

22. Sedgwick, J.D. *et al.* Isolation and direct characterization of resident microglial cells from the normal and inflamed central nervous system. *Proceedings of the National Academy of Sciences of the United States of America* **88**, 7438-7442 (1991).

23. Stephens, J.W., Hurel, S.J., Lowe, G.D., Rumley, A. & Humphries, S.E. Association between plasma IL-6, the IL6 -174G>C gene variant and the metabolic syndrome in type 2 diabetes mellitus. *Molecular genetics and metabolism* **90**, 422-428 (2007).

24. Ni, J. *et al.* Serum lipocalin-2 levels positively correlate with coronary artery disease and metabolic syndrome. *Cardiovascular diabetology* **12**, 176 (2013).

25. Xing, C. *et al.* Neuronal production of lipocalin-2 as a help-me signal for glial activation. *Stroke* **45**, 2085-2092 (2014).

26. Serbina, N.V. & Pamer, E.G. Monocyte emigration from bone marrow during bacterial infection requires signals mediated by chemokine receptor CCR2. *Nature immunology* **7**, 311-317 (2006).

27. Pike, C.J. Sex and the development of Alzheimer's disease. *Journal of neuroscience research* **95**, 671-680 (2017).

28. Edland, S.D., Rocca, W.A., Petersen, R.C., Cha, R.H. & Kokmen, E. Dementia and Alzheimer disease incidence rates do not vary by sex in Rochester, Minn. *Arch Neurol* **59**, 1589-1593 (2002).

29. Devendra, D. *et al.* Differential immune response to B:9-23 insulin 1 and insulin 2 peptides in animal models of type 1 diabetes. *Journal of autoimmunity* **23**, 17-26 (2004).

30. Skelly, D.T., Hennessy, E., Dansereau, M.A. & Cunningham, C. A Systematic Analysis of the Peripheral and CNS Effects of Systemic LPS, IL-1B, TNF-α and IL-6 Challenges in C57BL/6 Mice. *PloS one* **8** (2013).

31. Roved, J., Westerdahl, H. & Hasselquist, D. Sex differences in immune responses: Hormonal effects, antagonistic selection, and evolutionary consequences. *Hormones and Behavior* **88**, 95-105 (2017).

32. Morselli, E., Criollo, A., Rodriguez-Navas, C. & Clegg, D.J. Chronic High Fat Diet Consumption Impairs Metabolic Health of Male Mice. *Inflammation and cell signaling* **1**, e561- (2014).

33. Bhatia, A., Sekhon, H.K. & Kaur, G. Sex Hormones and Immune Dimorphism. *The Scientific World Journal* **2014** (2014).

34. Zhang, Z. *et al.* Dietary Fiber Intake Regulates Intestinal Microflora and Inhibits Ovalbumin-Induced Allergic Airway Inflammation in a Mouse Model. *PloS one* **11** (2016).

35. Grove, K., Fried, S., Greenberg, A., Xiao, X. & Clegg, D. A microarray analysis of sexual dimorphism of adipose tissues in high-fat-diet-induced obese mice. *International journal of obesity (2005)* **34**, 989-1000 (2010).

36. Estrany, M.E., Proenza, A.M., Lladó, I. & Gianotti, M. Isocaloric intake of a high-fat diet modifies adiposity and lipid handling in a sex dependent manner in rats. *Lipids in Health and Disease* **10**, 52 (2011).

37. de Sousa Rodrigues, M. *et al.* Chronic psychological stress and high-fat high-fructose diet disrupt metabolic and inflammatory gene networks in the brain, liver, and gut and promote behavioral deficits in mice. *Brain, behavior, and immunity* **59**, 158-172 (2017).

38. Libbey, J.E. *et al.* The effects of diet on the severity of central nervous system disease: One part of lab-to-lab variability. *Nutrition (Burbank, Los Angeles County, Calif.)* **32**, 877-883 (2016).

39. Sakamuri, A. *et al.* Transient Decrease in Circulatory Testosterone and Homocysteine Precedes the Development of Metabolic Syndrome Features in Fructose-Fed Sprague Dawley Rats. *Journal of Nutrition and Metabolism* **2016** (2016).

40. Maggio, M. *et al.* Correlation between Testosterone and the Inflammatory Marker Soluble Interleukin-6 Receptor in Older Men. *The Journal of Clinical Endocrinology & Metabolism* **91**, 345-347 (2006).

41. Wohleb, E.S., Powell, N.D., Godbout, J.P. & Sheridan, J.F. Stress-Induced Recruitment of Bone Marrow-Derived Monocytes to the Brain Promotes Anxiety-Like Behavior. *The Journal of Neuroscience* **33**, 13820-13833 (2013).

42. Kipnis, J., Gadani, S. & Derecki, N.C. Pro-cognitive properties of T cells. *Nature reviews. Immunology* **12**, 663-669 (2012).

43. Raposo, C. & Schwartz, M. Glial scar and immune cell involvement in tissue remodeling and repair following acute CNS injuries. *Glia* **62**, 1895-1904 (2014).

44. Hickman, S.E. & El Khoury, J. Mechanisms of Mononuclear Phagocyte Recruitment in Alzheimer's Disease. **9**, 168-173 (2010).

45. Montero-Melendez, T. & Perretti, M. Gapdh Gene Expression Is Modulated by Inflammatory Arthritis and Is not Suitable for qPCR Normalization. *Inflammation* **37**, 1059-1069 (2014).

46. Millet, P., Vachharajani, V., McPhail, L., Yoza, B. & McCall, C.E. GAPDH Binding to TNF-alpha mRNA Contributes to Posttranscriptional Repression in Monocytes: A Novel Mechanism of Communication between Inflammation and Metabolism. *Journal of immunology (Baltimore, Md. : 1950)* **196**, 2541-2551 (2016).

47. Takaoka, Y. *et al.* Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) prevents lipopolysaccharide (LPS)-induced, sepsis-related severe acute lung injury in mice. *Scientific Reports* **4** (2014).

48. Caesar, R., Tremaroli, V., Kovatcheva-Datchary, P., Cani, P.D. & Backhed, F. Crosstalk between Gut Microbiota and Dietary Lipids Aggravates WAT Inflammation through TLR Signaling. *Cell metabolism* **22**, 658-668 (2015).

49. Campbell, S.C. *et al.* The Effect of Diet and Exercise on Intestinal Integrity and Microbial Diversity in Mice. *PloS one* **11** (2016).

50. Davis, J.E., Gabler, N.K., Walker-Daniels, J. & Spurlock, M.E. Tlr-4 deficiency selectively protects against obesity induced by diets high in saturated fat. *Obesity (Silver Spring, Md.)* **16**, 1248-1255 (2008).

51. Poltorak, A. *et al.* Defective LPS signaling in C3H/HeJ and C57BL/10ScCr mice: mutations in Tlr4 gene. *Science (New York, N.Y.)* **282**, 2085-2088 (1998).

52. Ferreira, P.A. & Orry, A. From Drosophila to humans: Reflections on the roles of the prolyl-isomerases and chaperones, cyclophilins, in cell function and disease. *Journal of neurogenetics* **26**, 132-143 (2012).

53. Underwood, E.L. & Thompson, L.T. High-fat diet impairs spatial memory and hippocampal intrinsic excitability and sex-dependently alters circulating insulin and hippocampal insulin sensitivity. *Biology of sex differences* **7**, 9 (2016).

54. Fujioka, H. & Akema, T. Lipopolysaccharide acutely inhibits proliferation of neural precursor cells in the dentate gyrus in adult rats. *Brain research* **1352**, 35-42 (2010).

55. Carlin, J.L., Grissom, N., Ying, Z., Gomez-Pinilla, F. & Reyes, T.M. Voluntary exercise blocks Western diet-induced gene expression of the chemokines CXCL10 and CCL2 in the prefrontal cortex. *Brain, behavior, and immunity* **58**, 82-90 (2016).

56. Kummer, M.P. *et al.* Nitration of tyrosine 10 critically enhances amyloid beta aggregation and plaque formation. *Neuron* **71**, 833-844 (2011).