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Fish oil supplementation alleviates metabolic, angiogenic and neurolipid consequences of a saturated high-fat diet

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Conflict of interest

The authors declare no conflict of interest.

Abstract

Objective: Dietary omega-3 (n-3) polyunsaturated fatty acids can improve both metabolic and mood impairments by relieving inflammation. Obesity significantly elevates the odds of developing mood disorders such as anxiety and depression, and chronic consumption of a saturated high-fat diet (HFD) elicits anxiodepressive behavior in a manner linked to metabolic dysfunction and neuroinflammation in mice. Despite these findings, the effects of n-3 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 control corn oil or fish oil (FO) enriched with equal amounts of docosahexanoic (DHA) and eicosapentaenoic acids (EPA). Food intake and body weight were measured throughout while additional metabolic parameters and anxiety and despair behaviors (elevated-plus maze, light-dark box and forced swim test) were evaluated during the final week of supplementation. Forebrain lipid composition and markers of reactive gliosis were assessed by gas chromatography mass spectrometry and real-time PCR, respectively.

Results: Five weeks of FO supplementation corrected glucose intolerance and inhibited hyperphagia in HFD-induced obese mice without affecting fat mass. FO supplementation also defended against anxiety- and despair responses to HFD. Brain lipids, particularly polyunsaturated fatty acids, were altered by HFD and largely reversed by FO. Gene expression markers of astrogliosis and microglia activation were diminished by FO supplementation.

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

Keywords: diet-induced obesity; omega-3 fatty acids; depression; anxiety; prediabetes; brain lipidomics

Introduction

Obesity is a multifactorial health problem that confers a major economic burden worldwide. While often associated with heightened risk for cardiovascular and metabolic diseases¹⁻⁴, obesity also significantly increases the incidence of mood disorders⁵⁻⁷. Anxiety and depressive disorders impair quality of life, motivation and occupational functioning, and these consequences diminish treatment adherence to further enhance metabolic dysfunction and associated complications⁸. As metabolic and mood deficits exacerbate the threat of obesity there is an imminent need to identify suitable therapeutic interventions.

Obesity is often characterized by a state of low-grade inflammation. Several lines of evidence suggest that enhanced immune activation, especially that concurrent with visceral obesity, contributes to metabolic and mood deficits, including insulin resistance and anxiodepressive behaviors. Excess consumption of saturated fats, in particular, is known to aggravate metabolic dysfunction in obesity, in part by generating physiological responses that favour deposition of pro-inflammatory visceral adipose tissue⁹⁻¹¹. We recently reported that a saturated (but not monounsaturated) high-fat diet (HFD) that enhances visceral obesity, peripheral inflammation and glucose intolerance triggers anxiodepressive behavior via NFκB-mediated neuroinflammatory processes in mice¹². These findings along with clinical and epidemiological data linking poor diet quality (including excess saturated fat), metabolic dysfunction and depressive symptomology^{10,11,13,14} and the protective effects of a Mediterranean diet¹⁵ suggest that the type and amount of dietary lipids contribute to the development of mood disorders in obese individuals.

Dietary omega-3 fatty acids (n-3), such as eicosapentaenoic (EPA) and docosahexaenoic acids (DHA), can reduce cerebral inflammation (REF). In turn, n-3 dietary deficiency and associated decreases in brain n-3 levels can stimulate neuroinflammation and concomitantly potentiate anxiety- and depressive-like behaviors in rodents¹⁶⁻¹⁸. In a consistent manner, n-3 supplementation (principally in EPA) effectively improves depressive symptoms in people suffering from major depressive disorder¹⁹.

Despite the strong link between diet, obesity and mood impairments, the impact of FO supplementation in the context of obesity and ongoing consumption of a diet high in saturated fat on metabolic and emotional endpoints has not been fully explored. In the present study, we show that daily supplementation of fish oil (FO) that contains similar amounts of DHA and EPA, initiated following the onset of diet-induced obesity, improves negative metabolic and mood corollaries of a saturated HFD in a manner linked to increased anti-inflammatory neurolipids and reduced gliosis.

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 seven days prior to initiation of experiments. Mice were decapitated under isoflurane anesthesia. Brains and blood samples were harvested and stored at -80°C.

Diets and Fish Oil Supplementation

Mice (n=12/group) 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 (from the 16th to the 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: high-fat diet + control (HFD^{Ctrl}); high-fat diet + fish oil (HFD^{FO}); chow + control (chow^{Ctrl}); chow + fish oil (chow^{FO}). The amount of FO administered (0.7 mg/kg) was based on the

American Dietetic Association and *Dietitians of Canada* recommendations for humans of 500 mg of combined DHA and EPA per day²⁰. Assuming an average human body weight of 60kg, this recommended human dose of 8.33 mg/kg was converted for mice by dividing by 12.3 (according to published standards²¹) to provide 0.7 mg/kg of combined DHA and EPA. At an average body weight of 30g, each mouse received a daily gavage of 80µl 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 during the last week of the diet protocol. For the glucose tolerance test, mice were fasted for four hours prior to intraperitoneal (IP) injection of glucose (2 g/kg of body weight). Blood glucose was measured at 0, 15, 30, 60 and 120 minutes post-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 hours in the Comprehensive Lab Animal Monitoring System (CLAMS; Columbus Instruments) after a 1-day habituation period to cages that were set at 22°C.

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 antibodies and reference standards contained in R&D Systems (Minneapolis, MN, USA) Duokits enzyme-linked immunosorbent assay (ELISA) kits according to the manufacturer's protocol. C reactive protein (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 reported²². 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 travelled were measured over a period of five minutes.

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 next to a dark compartment 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. The number of entries and time spent in the lit compartment were measured for five minutes.

Forced Swim Test

The forced swim test was used as a measure of behavioral despair as described in detail previously²². Mice are placed in a beaker of water (24°C) for 6 minutes. Velocity and locomotor capacity were evaluated during the first two minutes while despair-related mobility was measured during the last four minutes.

Brain Lipid Quantification

Forebrains (n=12 per group) were used for quantitative profiling of fatty acids, both free and bound to triglycerides and phospholipids, by gas chromatography-mass spectrometry using previously described methods²³⁻²⁵. In brief, samples containing 25 mg of pulverized f tissue were incubated overnight at 4°C in a solution of chloroform/methanol (2:1) containing 0.004% butylated hydroxytoluene (BHT), 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 x 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+NH₃]⁺ ion by selective ion monitoring and concentrations were calculated using standard curves and isotope-labeled internal standards.

Quantitative PCR

Forebrain tissue samples were processed for mRNA extraction with TRIzol following the manufacturer's instructions and cDNA synthesis. Quantitative gene expression was measured from 1:10 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.

Iba-1 forward: GGATTTGCAGGGAGGAAAAG, reverse: TGGGATCATCGAGGAATTG; GFAP forward: AACGACTATCGCCGCAACTG, reverse: CTCTTCCTGTTTCGCGCATTTG; Cyclophilin forward: GCTTTTCGCCGCTTGCTGCA, reverse: TGCAAACAGCTCGAAGGAGACGC. Relative

gene expression was calculated using the $\Delta\Delta\text{CT}$ method using cyclophilin as the housekeeping gene. Each PCR reaction was performed in triplicate.

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 Tukey pairwise post-tests were used to evaluate pairwise comparisons as described in the figure legends. All data are presented as mean \pm SEM. $p < 0.05$ was set as criterion for statistical significance.

RESULTS

Fish oil supplementation attenuates metabolic disturbances induced by a saturated high-fat diet

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 Figure 1A, body weights of HFD mice significantly increased relative to control mice (diet effect; $F_{(3,38)} = 24.74$; $p < 0.0001$); 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 to HFD^{Ctrl} mice by the 5th week of supplementation (Fig. 1A), fat and lean mass were unchanged (Fig. 1B). Calorie 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^{FO} mice by the 5th week (supplementation effect; $F_{(3,38)} = 88.42$; $p < 0.0001$; 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 were not affected by supplementation or diet (Fig. 1E). As expected, HFD increased energy expenditure; however, FO did not influence this parameter in either diet group (Fig. 1F,G). The HFD caused glucose intolerance which was fully corrected by FO: glucose excursion curves for HFD^{FO} mice were similar to Chow^{Ctrl} mice (supplementation effect; $F_{(3,36)} = 9.601$; $p < 0.0001$; Fig. 1H). Mice consuming the HFD were hyperinsulinemic (Fig. 1I) and had higher plasma CRP levels (Fig. 1J), but FO did not decrease fed-state plasma insulin or CRP levels (HFD effect: $F_{(1,32)} = 15.14$; $p = 0.0005$).

Obesity-induced anxiety and despair is alleviated by fish oil supplementation

We next set out to determine the influence of diet-induced obesity with or without FO supplementation on anxiety and depressive-like behaviour using three behavioral tasks. In the elevated-plus maze (EPM), chronic high-fat feeding increased signs of anxiety as reflected by reduced open arm entries (diet effect; $F_{(1,35)} = 7.946$; $p = 0.0079$; Fig. 2A) and proportion of time spent in the open arms for HFD^{Ctrl} mice as compared to Chow^{Ctrl} mice (diet effect; $F_{(1,35)} = 7.315$; $p = 0.0105$; Fig. 2A). FO removed this difference between groups: open arm entries (supplementation effect; $F_{(1,35)} = 2.215$; $p = 0.1456$; Fig.

2A) and time (supplementation effect; $F_{(1,35)} = 0.4955$; $p=0.4861$; Fig. 2A) between Chow^{Ctrl} and HFD^{FO} groups were not significantly different. HFD also decreased distance travelled in the EPM (diet effect; $F_{(1,34)} = 5.203$; $p=0.0289$; Fig. 2A), an effect reversed by FO (supplementation effect; $F_{(1,34)} = 3.684$; $p=0.0634$; Fig. 2A).

In a second test of anxiety, the light/dark box (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; $F_{(1,36)} = 10.98$; $p=0.0021$; Fig. 2B). In contrast, the HFD^{FO} group had similar entries (supplementation effect; $F_{(1,35)} = 4.268$; $p=0.0463$; Fig. 2B) and lit compartment time as compared to the Chow^{Ctrl} group (supplementation effect; $F_{(1,36)} = 0.05502$; $p=0.8159$; Fig. 2B).

In the forced swim test (FST), HFD enhanced behavioral despair: Immobility time was increased in HFD^{Ctrl} mice as compared to Chow^{Ctrl} mice (diet effect; $F_{(1,36)} = 9.987$; $p=0.0032$; Fig. 2C). On the other hand, immobility time was similar between HFD^{FO} and Chow^{Ctrl} mice (supplementation effect; $F_{(1,36)} = 0.7648$; $p=0.3876$; Fig. 2C). As an index of locomotor capacity, swim velocity was comparable across the four groups.

Saturated high fat feeding and fish oil supplementation modulate brain fatty acid content

To evaluate the impact of an FO supplementation on brain lipid composition, we performed quantitative profiling of forebrain fatty acids. As shown in Figure 3A, the total amount of all fatty acids combined was not modified by HFD; however, FO elevated total content (supplementation effect: $F_{(1, 36)} = 4.15$; $p=.04$). On the other hand, the HFD increased the proportion of total saturated fatty acids (SFA) (Fig. 3B; diet effect: $F_{(1, 35)} = 5,576$; $p=.023$) while decreasing total PUFA (Fig. 3C; diet effect; $F_{(1, 34)} = 16,63$; $p=.0003$) whereas FO did not alter these parameters. As illustrated in Figure 3D, individual PUFA were significantly modified by HFD, such as EPA (diet effect: $F_{(1, 29)} = 7,714$; $p=.009$), DGLA (diet effect: $F_{(1, 37)} = 49,74$; $p<.0001$) and LA (diet effect; $F_{(1,27)} = 15.07$; $p=0.0006$). HFD elicited a non-significant trend

for reduced DHA levels ($F_{(1, 27)} = 3,316$; $p=.08$). N-3 fatty acids, EPA and docosapentaenoic acid (DPA) (but not DHA), were increased by FO supplementation in both the chow and HFD groups (Fig. 3D). In contrast, arachidonic acid (AA) was decreased by FO in both chow and HFD mice. DGLA, another n-6 that competes with AA to ultimately inhibit the production of AA-derived eicosanoids, was increased by FO. Detectable saturated and monounsaturated fatty acid species are presented in Supplemental Table 2.

Fish oil supplementation dampens reactive gliosis

As a final step, we measured glial fibrillary acidic protein (GFAP) and ionized calcium binding adaptor molecule 1 (Iba-1) mRNA levels as markers of forebrain astrogliosis and microglia activation, respectively. As shown in Figure 4, while HFD did not significantly alter GFAP and Iba-1 pan-forebrain expression, FO supplementation reduced GFAP (supplementation effect; $F_{(1,37)} = 10.44$; $p=0.003$) and decreased Iba-1 levels in HFD^{FO} relative HFD^{Ctrl} mice.

DISCUSSION

The last several decades have witnessed an increase in the consumption of saturated and n-6 polyunsaturated fatty acids to the detriment of n-3 fatty acids, a change in dietary pattern that is posited to contribute to heightened immune activity and increased susceptibility to metabolic and psychiatric conditions²⁶. Indeed, both body mass index²⁷ and depression rates²⁸ are inversely related to circulating n-3 fatty acid levels, one of several findings connecting n-3 intake to the regulation of energy balance and mood. As metabolic impairments arising from poor dietary lifestyle and obesity development increase the risk of anxiety and depression (REFS), the present work determined the impact on of FO supplementation in a diet-induced obesity setting on both metabolic and mood outcomes. In addition to restoring glycemic control and attenuating hyperphagia of obese mice consuming a saturated HFD, FO supplementation was effective at protecting against anxiety and despair-like behavioral responses to the HFD while altering forebrain levels of select PUFA and attenuating signs of brain gliosis, changes that collectively suggest reduced neuroinflammation.

Although several reports assessed the protective effects of n-3 in the development of obesity using diets varying in nutritional composition (e.g.,²⁹⁻³¹), the effects of FO supplementation imitated after obesity has developed and during ongoing consumption of a saturated HFD on metabolic and emotional endpoints has not been investigated³². Moreover, to our knowledge no study has employed an administration protocol that is comparable to taking a FO supplement (gavage) with n-3 doses intended to biologically simulate 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 fatty acids that are more potent than n-3 alpha-linolenic acid which has a limited ability to convert to longer chain n-3 fatty acids³⁷.

In accordance with observations here, previous studies demonstrated protective effects of n-3 on HFD-induced glucose intolerance mice^{36, 38, 39,40}. However, these results were obtained in conditions where body weight and fat mass deviated with n-3 dietary intervention. Here we demonstrate that daily

FO supplementation defends against the deleterious effects of diet-induced obesity on glucose tolerance and anxiodepressive behavior despite similar body composition and energy expenditure in HFD mice with or without FO supplementation. While food intake was moderately reduced in the HFD^{FO} group, fat mass was unchanged. Thus, the benefits of FO on glucose homeostasis are unlikely to be purely secondary to catabolic actions of n-3, a result that is in agreement with recent studies^{33,35}. Improved glucose tolerance by FO did not coincide with changes in insulin levels. These findings suggest that FO supplementation may improve glucose tolerance independent of modulating insulin sensitivity. These findings are in contrast with studies showing that FO³³ or partial substitution of dietary fat by flaxseed oil³⁵ in high fat-fed obese mice improves insulin sensitivity. This discrepancy may be related to differences in n-3 type, doses 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 adolescents⁴¹ and overweight adults⁴².

Several mechanisms have been proposed to explain the increased risk of anxiety and depression in individuals with obesity, including immune activation, impaired hypothalamic-pituitary-adrenal axis activity and neuroendocrine dysfunction⁹. We and other have reported on the effects of a saturated HFD to trigger anxiodepressive behaviors⁴³. Consistently, here we show increased anxiety-like behavior and indices of despair by HFD. Although HFD^{Ctrl} mice exhibited reduced distance traveled in the elevated-plus maze we believe this reflects decreased exploratory behavior (increased anxiety) by HFD rather than blunted locomotor capacity for several reasons: (1) absence of changes in total dark and light cycle ambulatory activity; (2) similar swimming velocity in the forced swim task; and (3) the reduced distance travelled in HFD^{Ctrl} mice in the elevated-plus maze was reversed by FO. Furthermore, the protective effect of FO on HFD-induced anxiodepressive behavior is supported by similar results in the light-dark box and forced swim tasks.

Despite the abundance of DHA in the brain, central DHA levels remains relatively over the short-term in response to dietary interventions⁴⁴⁻⁴⁶, findings consistent with our lipidomic results showing a no

impact of 5-week FO supplementation. Indeed, longer n-3 nutritional interventions have been shown to elevate brain DHA⁴⁷ and we observed a trend for reduced DHA with the 20-week HFD regimen in the present study. EPA levels, on the other hand, fluctuate more rapidly and according to ongoing nutritional status. EPA and DPA concentrations were significantly reduced by HFD whereas FO supplementation substantially increased EPA and DPA levels, especially in HFD mice. Of importance, EPA rather than DHA supplementation ameliorates major depressive disorder symptomology in randomized controlled trials¹⁹ and improves rodent depressive behavior⁴⁹ and the deleterious effect of central interleukin-1 β injection⁴⁸. Two n-6 fatty acids, AA and DGLA, were also considerably modified by FO supplementation in an opposing manner. DGLA was reduced by HFD and potentiated by FO supplementation in both chow and HFD groups. Closely related n-3 and n-6 fatty acids such as AA and DGLA act as competing substrates for the same enzymes. Although found in only trace amounts in the brain, DGLA yields anti-inflammatory eicosanoids and competes with AA for cyclooxygenase and lipoxygenase, inhibiting the production of AA-derived eicosanoids (REF). Thus, reduced brain DGLA by HFD and reversal by FO supplementation may participate towards dampening neuroimmune activity and mitigation of anxiety and despair. Finally, while the palm oil diet we used substantially modifies plasma palmitate levels (REF), brain palmitate content was not affected by HFD in the current study. This contrasts results of Morselli et al.⁵⁰ of reduced central palmitate by HFD, a discrepancy that may be due to the different duration and nutritional composition of the diets used between studies.

The pro-inflammatory state triggered by a high-fat diet is coincident with reactive gliosis in certain brain regions^{43,46}. Increased Iba-1 and GFAP markers reflect reactive gliosis, a neuroimmune response to invasion or injury. Although expression of Iba-1 and GFAP were not elevated by chronic high fat diet, these two markers were suppressed by FO supplementation. The lack of HFD-induced reactive gliosis in our study is likely due to the use of whole forebrain samples and not specific brain regions (such as the striatum) that may be more sensitive to obesity-induced neuroinflammation^{34,43}.

Together, our results demonstrate that in the context of poor dietary and metabolic conditions, FO supplementation is sufficient to reverse glucose intolerance, anxiodepressive behaviors and to enhance anti-inflammatory PUFA in the brain. How does FO supplementation offset metabolic and mood dysfunction by diet-induced obesity? In view of increased brain n-3 levels following supplementation, we speculate that enhanced n-3 signaling may be partly involved. N-3 fatty acids can activate GPR120⁵¹, a G protein-coupled receptor that is implicated in the anti-inflammatory effects of n-3 in the periphery, including the insulin-sensitizing effect of n-3 intake³³. Such a possibility is consistent with our previous observations of the anxiolytic actions of central pharmacological GPR120 activation⁵². In parallel or alternatively, n-3 are known to be agonists and activators of nuclear peroxisome proliferator activated receptor gamma (PPAR γ), a receptor that when activated in adipocytes promotes the expression of genes involved in glucose metabolism, thus improving glucose tolerance^{53,54}. Also, the cellular mechanism used by enzymatic and non-enzymatic derivatives of n-3 fatty acids, which have been the subject of recent publications demonstrating their cardio and neuroprotective effects, remains largely unknown. Future research will be required to determine if either of these mechanisms or their combined actions could be involved in the protective effects of FO supplementation.

REFERENCES

1. Rimm E, Stampfer M. Body size and fat distribution as predictors of coronary heart disease among middle-aged and older US men. *Am J Epidemiol*. 1995;144(12):1143-1150. <http://aje.oxfordjournals.org/content/141/12/1117.short>.
2. Harris MI, Flegal KM, Cowie CC, et al. Prevalence of Diabetes, Impaired Fasting Glucose, and Impaired Glucose Tolerance in U.S. Adults. *Diabetes Care*. 1998;21(4):518-524. doi:10.4158/EP.12.4.358
3. Grundy SM. Obesity, metabolic syndrome, and cardiovascular disease. *J Clin Endocrinol Metab*. 2004;89(6):2595-2600. doi:10.1210/jc.2004-0372
4. Dixon JB. The effect of obesity on health outcomes. *Mol Cell Endocrinol*. 2010;316(2):104-108. doi:10.1016/j.mce.2009.07.008
5. Garipey 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(3):407-419. doi:10.1038/ijo.2009.252
6. Faith MS, Butryn M, Wadden TA, Fabricatore A, Nguyen AM, Heymsfield SB. Evidence for prospective associations among depression and obesity in population-based studies. *Obes Rev*. 2011;12(501):438-453. doi:10.1111/j.1467-789X.2010.00843.x
7. 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(6):e0157240. doi:10.1371/journal.pone.0157240
8. Luppino FS, De Wit LM, Bouvy PF, et al. Overweight, Obesity, and Depression A Systematic Review and Meta-analysis of Longitudinal Studies.
9. Davis JE, Gabler NK, Walker-Daniels J, Spurlock ME. Tlr-4 deficiency selectively protects against obesity induced by diets high in saturated fat. *Obesity*. 2008;16(6):1248-1255. doi:10.1038/oby.2008.210
10. Lai JS, Oldmeadow C, Hure AJ, et al. Inflammation mediates the association between fatty acid intake and depression in older men and women. *Nutr Res*. 2016;36(3):234-245. doi:10.1016/j.nutres.2015.11.017
11. Rogero MM, Calder PC. Obesity, inflammation, toll-like receptor 4 and fatty acids. *Nutrients*. 2018;10(4):1-19. doi:10.3390/nu10040432
12. Décarie-Spain L, Sharma S, Hryhorczuk C, et al. Nucleus accumbens inflammation mediates anxiodepressive behavior and compulsive sucrose seeking elicited by saturated dietary fat. *Mol Metab*. 2018;10:1-13. doi:10.1016/j.molmet.2018.01.018
13. Beydoun MA, Fanelli Kuczmarski MT, Beydoun HA, Rostant OS, Evans MK, Zonderman AB. Associations of the ratios of n-3 to n-6 dietary fatty acids with longitudinal changes in depressive symptoms among us women. *Am J Epidemiol*. 2015;181(9):691-705. doi:10.1093/aje/kwu334
14. Q3 Q, Tolkien K, Bradburn S, Murgatroyd C. Meta-analyses An anti-inflammatory diet as a potential intervention for depressive disorders: A systematic review and meta-analysis. *Clin Nutr*. 2018;(November):1-8. doi:10.1016/j.clnu.2018.11.007

15. Lassale C, Batty GD, Baghdadli A, et al. Healthy dietary indices and risk of depressive outcomes: a systematic review and meta-analysis of observational studies. *Mol Psychiatry*. 2018;(September). doi:10.1038/s41380-018-0237-8
16. Auguste S, Sharma S, Fisette A, et al. Perinatal deficiency in dietary omega-3 fatty acids potentiates sucrose reward and diet-induced obesity in mice. *Int J Dev Neurosci*. 2018;64(February 2017):8-13. doi:10.1016/j.ijdevneu.2017.09.003
17. Carrié I, Clément M, De Javel D, Francès H, Bourre J-M. Phospholipid supplementation reverses behavioral and biochemical alterations induced by n-3 polyunsaturated fatty acid deficiency in mice. *J Lipid Res*. 2000;41. <http://www.jlr.org.proxy3.library.mcgill.ca/content/41/3/473.full.pdf>. Accessed November 27, 2017.
18. Fernandes MF, Mutch DM, Leri F. The relationship between fatty acids and different depression-related brain regions, and their potential role as biomarkers of response to antidepressants. *Nutrients*. 2017;9(3). doi:10.3390/nu9030298
19. Grosso G, Pajak A, Marventano S, 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). doi:10.1371/journal.pone.0096905
20. Position of the American Dietetic Association and Dietitians of Canada: Dietary Fatty Acids. *J Am Diet Assoc*. 2007;107(9):1599.e1-1599.e15. doi:10.1016/j.jada.2007.07.024
21. Nair A, Jacob S. A simple practice guide for dose conversion between animals and human. *J Basic Clin Pharm*. 2016;7(2):27. doi:10.4103/0976-0105.177703
22. 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. doi:10.1038/ijo.2012.48
23. Gelinas R, Thompson-Legault J, Bouchard B, et al. Prolonged QT interval and lipid alterations beyond β -oxidation in very long-chain acyl-CoA dehydrogenase null mouse hearts. *AJP Hear Circ Physiol*. 2011;301(3):H813-H823. doi:10.1152/ajpheart.01275.2010
24. 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 Diet*. 2015;28(6):646-658. doi:10.1111/jhn.12272
25. Thompson Legault J, Strittmatter L, Tardif J, et al. A Metabolic Signature of Mitochondrial Dysfunction Revealed through a Monogenic Form of Leigh Syndrome. *Cell Rep*. 2015;13(5):981-989. doi:10.1016/j.celrep.2015.09.054
26. Simopoulos AP. An increase in the Omega-6/Omega-3 fatty acid ratio increases the risk for obesity. *Nutrients*. 2016;8(3):1-17. doi:10.3390/nu8030128
27. Howe P, Buckley J. Metabolic Health Benefits of Long-Chain Omega-3 Polyunsaturated Fatty Acids. *Mil Med*. 2014;179(11S):138-143. doi:10.7205/MILMED-D-14-00154
28. Thesing CS, Bot M, Milaneschi Y, Giltay EJ, Penninx BWJH. Omega-3 and omega-6 fatty acid levels in depressive and anxiety disorders. *Psychoneuroendocrinology*. 2018;87(May 2017):53-62. doi:10.1016/j.psyneuen.2017.10.005
29. Parrish CC, Pathy DA, Angel A. Dietary Fish Oils Limit Adipose Tissue Hypertrophy in Rats. *Metabolism*. 1990;39(3):217-219.

30. Hainault I, Carlotti M, Hajdouch E, Guichard C, Lavau M. Fish Oil in a High Lard Diet Prevents Obesity, Hyperlipemia, and Adipocyte Insulin Resistance in Rats. *Ann N Y Acad Sci.* 1993;683(1):98-101. doi:10.1111/j.1749-6632.1993.tb35696.x
31. 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 - Regul Integr Comp Physiol.* 1993;264(6):R1111--R1118. <http://ajpregu.physiology.org/content/264/6/R1111>.
32. 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(1):35-44. doi:10.1111/j.1463-1326.2004.00312.x
33. Oh DY, Talukdar S, Bae EJ, et al. GPR120 Is an Omega-3 Fatty Acid Receptor Mediating Potent Anti-inflammatory and Insulin-Sensitizing Effects. *Cell.* 2010. doi:10.1016/j.cell.2010.07.041
34. de Mello AH, Schraiber RDB, Goldim MPDS, et al. Omega-3 Fatty Acids Attenuate Brain Alterations in High-Fat Diet-Induced Obesity Model. *Molecular Neurobiology.* <https://link-springer-com.proxy3.library.mcgill.ca/content/pdf/10.1007%2Fs12035-018-1097-6.pdf>. Published 2018. Accessed June 13, 2018.
35. Dátilo MN, Sant'Ana MR, Formigari GP, et al. Omega-3 from Flaxseed Oil Protects Obese Mice Against Diabetic Retinopathy Through GPR120 Receptor. *Sci Rep.* 2018;8(1):1-13. doi:10.1038/s41598-018-32553-5
36. Oliveira V, Marinho R, Vitorino D, et al. Diets containing α -linolenic (ω 3) or oleic (ω 9) fatty acids rescues obese mice from insulin resistance. *Endocrinology.* 2015;156(11):4033-4046. doi:10.1210/en.2014-1880
37. Burdge GC, Calder PC. Dietary α -linolenic acid and health-related outcomes: a metabolic perspective. *Nutr Res Rev.* 2006;19(01):26. doi:10.1079/NRR2005113
38. Tschöp MH, Speakman JR, Arch JRS, et al. A guide to analysis of mouse energy metabolism. *Nat Methods.* 2011;9(1):57-63. doi:10.1038/nmeth.1806
39. Kalupahana N, Claycombe K. Eicosapentaenoic acid prevents and reverses insulin resistance in high-fat diet-induced obese mice via modulation of adipose tissue inflammation. *J ...* 2010;1915-1922. doi:10.3945/jn.110.125732.1915
40. Kasbi Chadli F, Andre A, Prieur X, 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(9):1305-1315. doi:10.1017/S0007114511004387
41. López-Alarcón M, Inda-Icaza P, Márquez-Maldonado MC, et al. A randomized control trial of the impact of LCPUFA- ω 3 supplementation on body weight and insulin resistance in pubertal children with obesity. *Pediatr Obes.* 2018;(November):e12499. doi:10.1111/ijpo.12499
42. Helland A, Bratlie M, Hagen I V., 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(10):1368-1378. doi:10.1017/S0007114517001234
43. Décarie-Spain L, Sharma S, Hryhorczuk C, et al. Nucleus accumbens inflammation mediates

anxiodepressive behavior and compulsive sucrose seeking elicited by saturated dietary fat. *Mol Metab.* 2018;10:1-13. doi:10.1016/j.molmet.2018.01.018

44. Bourre J, Dăcemoț O, Pascal G, et al. Nutrient Requirements and Interactions Dietary α -Linolenic Acid at 1 . 3 g / kg Maintains Maximal Docosahexaenoic Acid Concentration in Brain , Heart and Liver of Adult Rats1. 1993;(July 1992):1313-1319.
45. Mohrhauer H. and Holman R.T. Alteration of the fatty acid composition of brain lipids by varying levels of dietary essential fatty acids. *J Neurochem.* 1963;10:523-530.
http://chemport.cas.org/cgi-bin/sdcgi?APP=cp_scifinder&SERVICE=STN&CLI=scifinder&SID=388839-1088463628-103&FID=REDISPLAY&LANG=english&R=328246&DLP-REFERER=&DLP=1.
46. Thaler JP, Yi CX, Schur EA, et al. Obesity is associated with hypothalamic injury in rodents and humans. *J Clin Invest.* 2012. doi:10.1172/JCI59660
47. Naliwaiko K, Araújo RLF, Da Fonseca R V., et al. Effects of fish oil on the central nervous system: A new potential antidepressant? *Nutr Neurosci.* 2004;7(2):91-99.
doi:10.1080/10284150410001704525
48. Song C, Manku MS, Horrobin DF. Long-chain polyunsaturated fatty acids modulate interleukin-1 β -induced changes in behavior, monoaminergic neurotransmitters, and brain inflammation in rats. *J Nutr.* 2008;138(5):954-963. doi:138/5/954 [pii]
49. 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(1):46-56. doi:10.1038/nrn2297
50. Morselli E, Fuente-Martin E, Finan B, et al. Hypothalamic PGC-1 α Protects Against High-Fat Diet Exposure by Regulating ER α . *CellReports.* 2014;9:633-645.
doi:10.1016/j.celrep.2014.09.025
51. Talukdar S, Olefsky JM, Osborn O. Targeting GPR120 and other fatty acid-sensing GPCRs ameliorates insulin resistance and inflammatory diseases. *Trends Pharmacol Sci.* 2011;32(9):543-550. doi:10.1016/j.tips.2011.04.004
52. Auguste S, Fiset A, Fernandes MF, 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):1-10. doi:10.1093/ijnp/pyw014
53. Martinez-Fernandez L, Laiglesia LM, Huerta AE, Martinez JA, Moreno-Aliaga MJ. Omega-3 fatty acids and adipose tissue function in obesity and metabolic syndrome. *Prostaglandins Other Lipid Mediat.* 2015;121:1-18. doi:10.1016/j.prostaglandins.2015.07.003
54. Belchior T, Paschoal VA, Magdalon J, et al. Omega-3 fatty acids protect from diet-induced obesity, glucose intolerance, and adipose tissue inflammation through PPAR γ -dependent and PPAR γ -independent actions. *Mol Nutr Food Res.* 2015;59(5):957-967.
doi:10.1002/mnfr.201400914

Figure Legends

Figure 1. Fish oil supplementation remedies diet-induced glucose intolerance and hyperphagia without changing 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) 24h 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 group mean \pm SEM; Two-way ANOVA, Tukey post hoc; * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, **** $p \leq 0.0001$. N=9-12/condition.

Figure 2. Fish oil supplementation alleviates anxiety- and despair-like behavior by a high-fat diet

(A) Number of entries in the open arms, percent of open-arms time and total distance travelled in the elevated-plus maze. (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 group mean \pm SEM; Two-way ANOVA, Tukey post hoc; * $p \leq 0.05$, ** $p \leq 0.01$. N=9-12/condition.

Figure 3. Forebrain fatty acid composition is altered by high-fat feeding and supplementation

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 concentration of fatty acids \pm SEM; Two-way ANOVA, Tukey post hoc; * $p \leq 0.05$, **** $p \leq 0.0001$. N=4-12/condition.

Figure 4. forebrain markers of gliosis Fish oil supplementation inhibits

(A) Relative gene expression of glial fibrillary acidic protein (GFAP) and (B) ionized calcium-binding adapter molecule 1 (Iba-1) of whole brain sample measured by RT-PCR. Values are expressed as group mean \pm SEM; fold change vs chow^{Ctrl} for all genes; Two-way ANOVA, Tukey post hoc; * $p \leq 0.05$. N=9-12/condition.

Figure 1

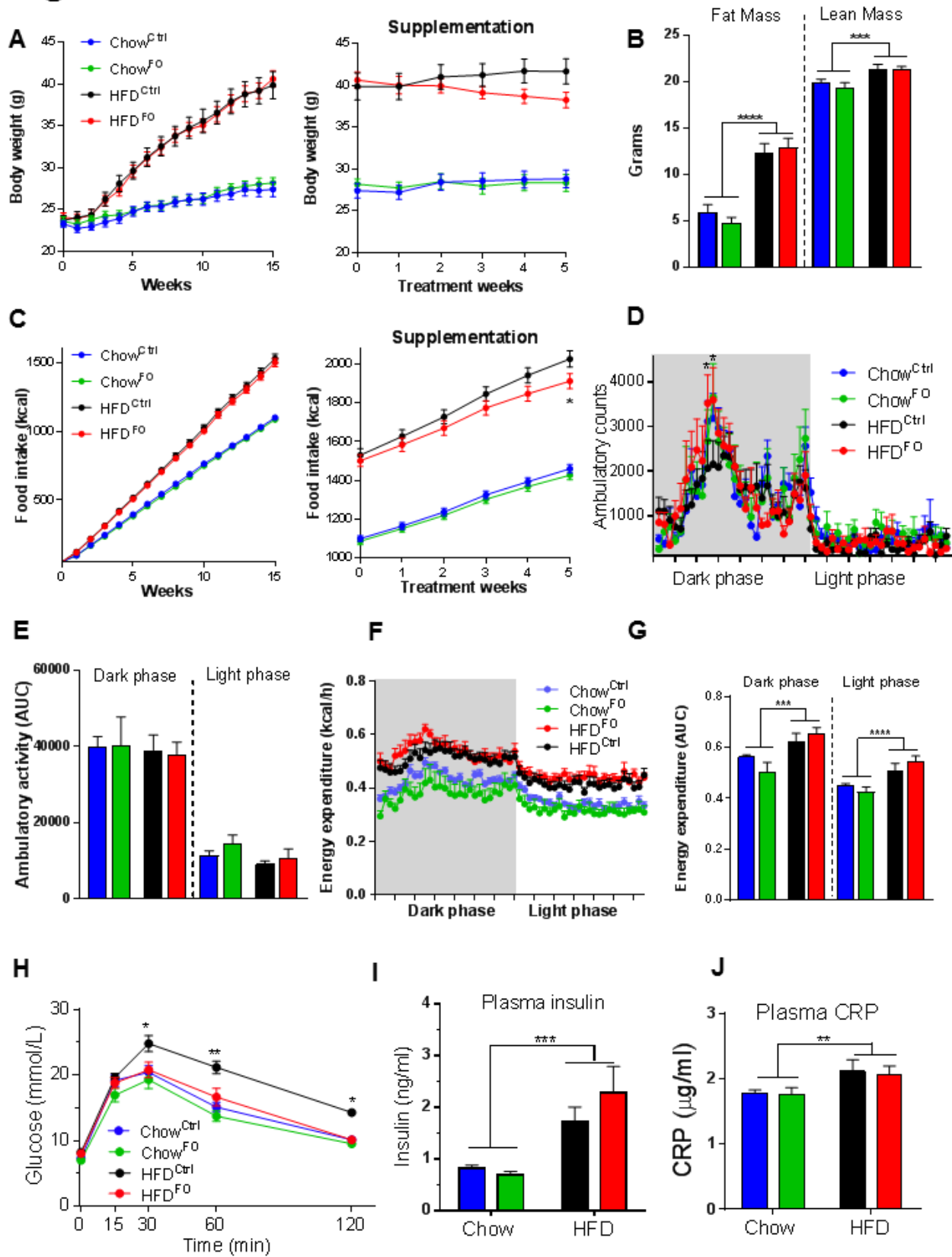
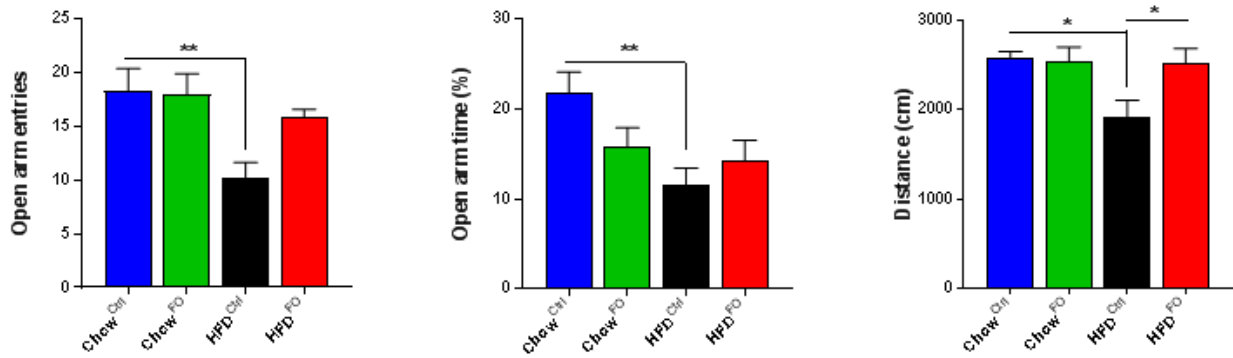


Figure 2

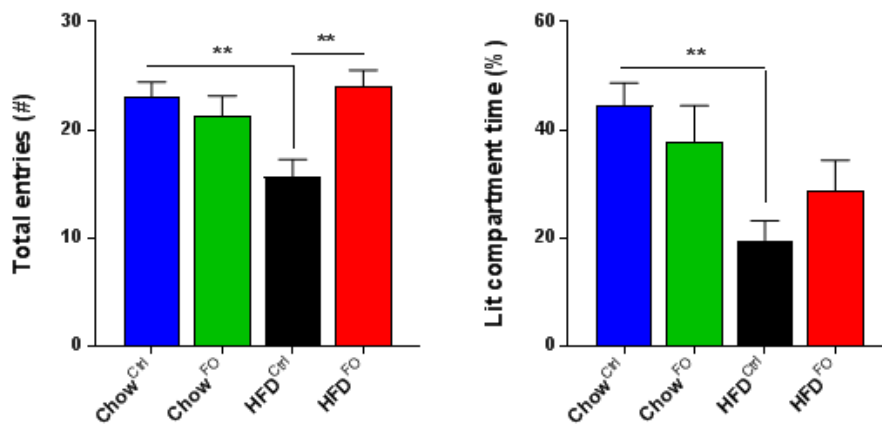
A

Elevated-Plus Maze



B

Light/Dark Box



C

Forced Swim Test

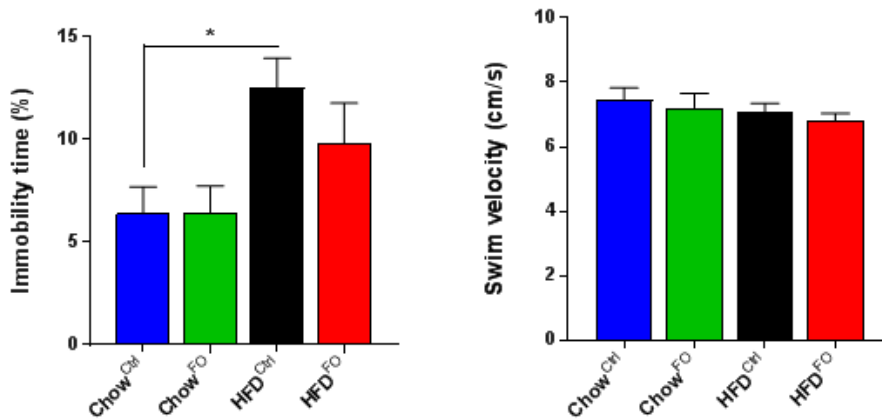
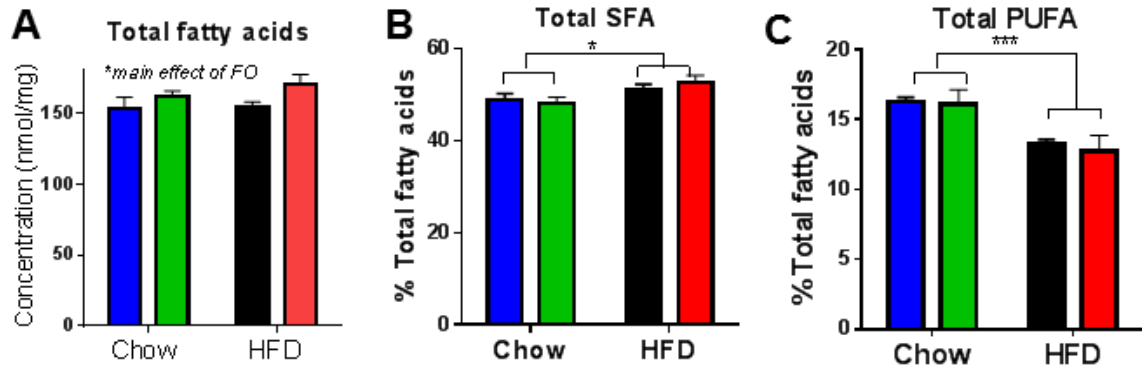


Figure 3



Forebrain polyunsaturated fatty acid profile

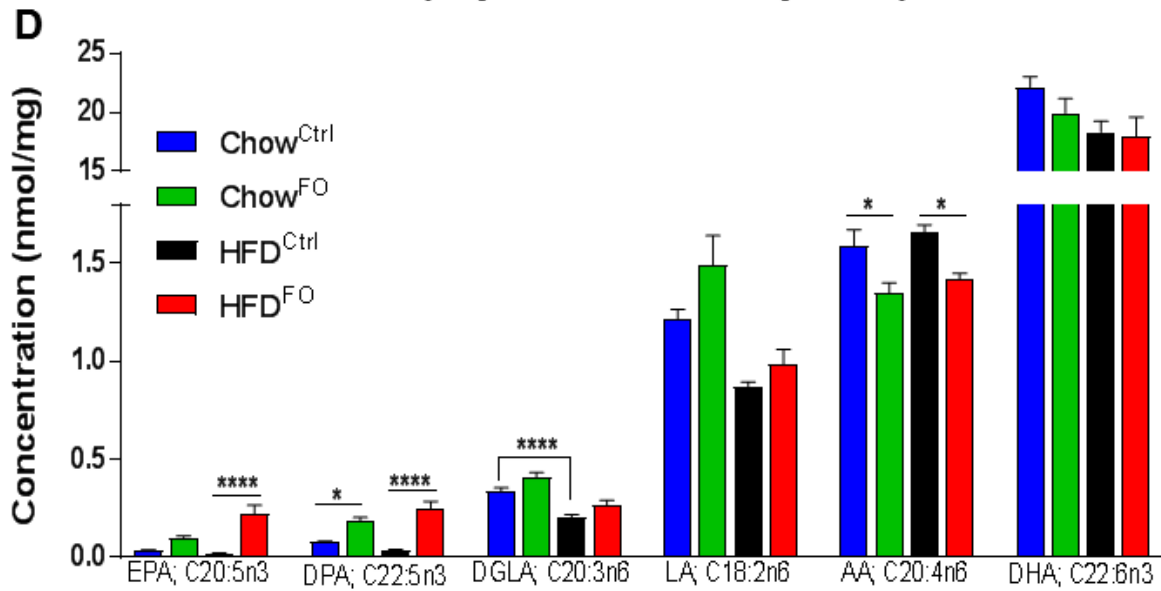


Figure 4

