Diet transition to a high-fat diet for 3 weeks reduces brain omega-3-fatty acid levels, alters BDNF signaling and induces anxiety & depression-like behavior in adult rats.

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Abstract:

Background: The consumption of diets high in calories and low in nutrient value is becoming increasingly common in the modern society, which can lead to metabolic disorders like diabetes and obesity, and potentially to psychiatric disorders. We have performed studies to assess how the shift from a healthy diet rich in omega-3 fatty acids to a diet rich in saturated fatty acid affects the substrates for brain plasticity and function, and anxiety and depression-like behavior. **Methods:** Pregnant rats were fed with omega-3 supplemented diet from their 2nd day of gestation period as well as their male pups for 12 weeks. Afterwards, the animals were randomly assigned to either a group fed on the same diet or a group fed on a high-fat diet (HFD) rich in saturated fats for 3 weeks. Anxiety and depression-like behaviors were assessed by using open field (OF) and elevated plus maze (EPM). Molecular assessments were performed in the frontal cortex and hippocampus as dysfunctions in these brain regions are main contributors towards depression, anxiety-like behavior and stress. **Results:** We found that the HFD increased vulnerability for anxiety and depression–like behavior, and that these modifications harmonized with changes in the anxiety-related neuropeptide Y (NPY)-1 receptor. The HFD reduced levels of brain-derived neurotrophic factor (BDNF), and the BDNF signaling receptor p*TrkB*, as well as the cyclic AMP response element binding protein (CREB), in these brain regions. Brain DHA contents were significantly associated with the levels of anxiety and depression–like behavior in these rats. **Conclusions:** These results suggest that the change in dietary lifestyle leading to alteration of dietary n3/n-6 fatty acids levels imposes a risk factor for anxiety-like behaviors. Dietary DHA might help for building cognitive reserve that can resist psychiatric disorders.

Introduction

Depression is about to edge out HIV/AIDS as the world's most significant health problem according to the World Health Organization. For Americans born a century ago, the chances of suffering any episode of major depression in the lifetime was only about 1 percent. Today, the lifetime incidence has increased almost 2000 times and is 19.2 percent (1). In turn, obesity has become a worldwide epidemic particularly in US, and a major cause for an increased risk of depressive disorders (2-4). Increased availability and excessive intake of energy-rich foods generally found in junk or fast foods is a significant factor contributing to obesity, and has made invasion in most cultures around the world. In spite of its poor health consequences, there is presently little information on how the diet switch to a high-fat diet that contributes to development of obesity heightens the risk for depression and mood disorders.

In turn, western diets that are high in saturated fat induce metabolic dysfunction and promote cognitive alterations (5-7). It is well known that diets rich in saturated fat increase oxidative stress in brain (8, 9), reduce neurogenesis (10, 11), enhance neuroinflammation (12) and exert anxiety-like behavior (13). Further recent evidences suggest that maternal high-fat diet consumption may have profound effects on the offspring's preference and consumption of high-fat high-sugar diets (14, 15). Contrary to what is known about the HF diet, recent clinical investigations have provided strong evidence that long chain omega-3 polyunsaturated fatty acids (PUFA) possess significant antidepressant activity(16). Indeed recent meta-analyses have reported a moderate effect size for omega-3 PUFA in depression comparable to that of conventional antidepressants, and reduced levels of omega-3 PUFA in the blood of patients with

depression(17, 18). Epidemiological observations report an inverse correlation between omega-3 PUFA intake and the development of depression (19, 20). In addition, a diet that is rich in omega-3 fatty acids is garnering appreciation for supporting cognitive processes in humans(21) and for up-regulating genes that are important for maintaining synaptic function and plasticity in rodents(22). The strong dichotomy between a HF diet and a PUFA diet implies that a switch from a PUFA diet to a HFD can have dramatic consequences for brain function; however, as far as we know, this question has not been addressed experimentally. Accordingly we have designed studies to determine the effects of this dietary switch on brain function and plasticity, which results are highly relevant for public health based on the increasingly common dietary changes related to industrialization and cultural migrations in our modern society.

We have focused these studies on brain-derived neurotrophic factors (BDNF) because of its described involvement on cognitive function and emotions (23-27), and its action supporting mechanisms of synaptic plasticity and neuronal excitability(28). Dietary deprivation of omega-3 fatty acids in rodents result in reduced BDNF levels in striatum (29) and frontal cortex(30) leading to reduced cAMP response element binding protein (CREB) transcription factor activation. On the other hand omega-3 supplementation in adult rats has been shown to increase hippocampal BDNF, and CREB levels which were associated with improved cognition (22). Accordingly, we designed this study to assess the effects of this dietary transition on plasticity related molecules in hippocampus & frontal cortex, which in turn may be responsible for underlying behavior alterations.

Methods and Materials

Experimental design

Female Sprague–Dawley rats were obtained on the 2nd day of pregnancy from Charles River (Portage, MI) weighing between 280 and 300 g were housed in cages and maintained in environmentally controlled rooms (22–24 °C) with a 12-h light/dark cycle. Pregnant females were fed an n-3 enriched fatty acid diet (DHA diet). Rats were maintained on this diet through gestation and lactation, and their pups were weaned to the same diet as their dams. Male pups were subjected to same diet as their dams for 15 weeks. The custom diet used was based on the composition of the American Institute of Nutrition diet and prepared commercially (Dyets, Bethlehem, PA) as previously described (31). However, several substitutions were made to produce an n-3 fatty acid enriched diet and this was achieved by adding a small amount of flaxseed oil and docosahexaenoic acid (Nordic Naturals, Inc. Watsonville, CA, USA) to the n-3 diet. These fats supply LNA and DHA, respectively, as their principal component. The total fat content in diet was 10 g/100 g of diet, and the amount of n-3 fatty acids in the n-3 diet was 3.8% of total fatty acids.

Diet transition

A total of 15 male rats were randomly selected for this study with a constraint that at least 2 rats from each litter were selected. On postnatal day (PND) 90 the male rats were randomly divided into two subgroups i.e. DHA (n=6) continued on same n-3 enriched diet and high-fat diet (HFD; n=9) provided with a custom diet high in saturated fatty acids that closely resembles to western

diet (D12079B, Research Diets, NJ USA). This HFD has 21% total fat but saturated fatty acid make 62.4% of this total fat. After 3 weeks of diet transition, the rats were subjected to a series of behavioral tests. A day after the last behavioral test the animals were killed by decapitation and the blood sample and fresh tissues including frontal cortex and hippocampus were dissected, frozen in dry ice and stored at −70 °C until use for biochemical analyses for both groups which are abbreviated throughout in this study as: DHA enriched diet (DHA) and high-fat diet (HFD). Experiments were performed in accordance with the United States National Institutes of Health Guide for the Care and Use of Laboratory Animals, and were approved by the University of California at Los Angeles Chancellor's Animal Research Committee. The suffering and number of animals used were minimized.

Open Field

After 3 weeks of diet transition, rats were tested in an open field. The open field consisted of 1.2-m-diameter circular tank with 60 cm walls. An inner circle, 80 cm in diameter was marked on the tank floor to serve as a central arena. Test began when each rat was placed in the middle of the central arena and allowed to explore the field for 10 min. The rat behavior was recorded by an overhead camera. Measurement included time spent in central arena, number of entries into central arena and the distance the rat travel using AnyMaze^{TM} video tracking software (San Diego instruments, San Diego, CA).

Elevated plus maze

The day after OF, rats were subjected to elevated plus maze (EPM) test. EPM assay was carried out according to the Walf and Frye protocol (32). Briefly, the EPM apparatus made of laminated wood consisted of 2 opposing open arms (10 X 50 cm) and 2 opposing closed arms (10 X 50 cm with 30 cm high walls). The maze was placed 60 cm above the floor. White curtains surrounded the maze and behavior was recorded by an overhead video camera. Each rat was placed in the middle of the maze facing the open arm that faced away from the experimenter, and a video camera recorded over a period of 5 min the time spend in each of the arms and the number of entries to each arm. A closed arm entry was counted when the rat placed all four paws in a closed arm. An open arm entry was recorded when the rat placed all four paws in an open arm and/or when the rat's hind-limbs were placed in the central area of the maze and both fore-limbs in an open arm while the head is protruding into the open arm. The ratio of open and closed entries to total arm entries was calculate to account for differences in general motor activity in the maze.

Fatty acid analysis

Fatty acid profiles were determined by using gas chromatography. The system consisted of model 5890A gas chromatograph (Hewlett Packard) and a model 7673A automatic, sampler and controller (Hewlett Packard). An Omegawax 250 column (30□m, 0.25-mm internal diameter, 0.25-μm film thickness; Sigma-aldrich) was used, with helium as the carrier gas. GC oven temperature was initially held at 50°C for 2 min and raised with a gradient of $2°Cmin^{-1}$ until 220°C and held for 30 min. The injector and detector were maintained at 250°C and 260°C, respectively. Tissues from middle cortex were grounded to powder under liquid nitrogen and subjected to extraction of total lipids. Fatty acid methylation was done by heating at 100°C for 1

hr with 14% boron tri-fluoride–methanol reagent. A 1µl sample of Fatty acid methyl esters (FAME) was injected in split injection mode with a 100:1 split ratio. Peaks of resolved fatty acid methyl esters were identified and quantified by comparison with standards (Supelco 37-component FAME Mix).

Western blot

Frontal cortex and hippocampal tissues were homogenized in a lysis buffer using published protocol (33). Levels of brain-derived neurotrophic factor (BDNF), Neuropeptide Y (NPY) 1, Phopho tyrosine kinase B (p*TrkB*), phopho cyclic AMP-response element binding protein (pCREB), p-synapsin, GAP-43 were analyzed by Western blot. Briefly, protein samples were separated by electrophoresis on a 10% (12.5 % for BDNF) polyacrylamide gel and electrotransferred to a PVDF or nitrocellulose membrane (Millipore, Bedford, MA). Non-specific binding sites were blocked in TBS 5% low-fat milk and 0.1% Tween-20 or 2% BSA. Membranes were rinsed in buffer (0.1% Tween-20 in TBS) and then incubated with anti-actin or anti-BDNF, p*TrkB*, (1:500; Santa Cruz Biotechnology, Santa Cruz, CA, USA), anti-pCREB(Ser133), anti-CREB, anti p-synapsin and anti-GAP-43 (1:1000; Millipore, Bedford, MA), NPY-1R (1:500; Alpha Diagnostics Intl.Inc. San Antonio, Texas) followed by anti-rabbit or anti goat or anti-mouse IgG horseradish peroxidase-conjugate (1:200,000; Santa Cruz Biotechnology). After rinsing with buffer, the immunocomplexes were visualized by chemiluminescence using the ECL plus kit (Amersham Pharmacia Biotech Inc., Piscataway, NJ, USA) for NPY1R, p*TrkB*, pCREB SuperSignal West femto kit (Thermo Scientific , Rockford, IL) for BDNF. Respective protein size was compared by using Bench mark pre-stained protein

ladder (Invitogen Technology, Carlsbad, CA). The film signals were digitally scanned and then quantified using ImajeJ software. Specific Protein sizes were chosen and quantified as β-actin (42 kDa), NPY-1 (39-42 kDa), BDNF (14 kDa), p*TrkB* (145 kDa), pCREB (43 kDa). Actin was used as an internal control for Western blot such that data were standardized according to actin values.

Statistical analysis

Data are presented as means and their standard errors. Data were analyzed using statistics software Graph pad 5 and unpaired two tailed t test was applied for the comparison between two groups. Criterion for significance was set to $p \leq 0.05$ in all comparisons.

Results

Diet transition to HFD leads to poor physiological consequences

In the present study animals subjected to diet transition on a HFD for 3 weeks gain significantly more body weight as compared to their counterparts continued a healthy DHA supplemented diet ($p < 0.001$; Figure 1A). The animals fed HFD for 3 weeks also showed significantly higher blood glucose, cholesterol, triglycerides ($p<0.05$) and higher uric acid ($p<0.01$). The results are shown in Table 1.

Effects of diet transition to HFD on anxiety-like behavior

Both group of animals either fed a HFD or DHA supplemented diet were tested for anxiety-like behaviors after 3 weeks of diet transition in open field and elevated plus maze. The HFD animals

showed a remarkably distinct behavior in open field as characterized by significantly less distance traveled ($p<0.001$; Figure 1B) compared to DHA fed animals. The animals switched to HFD made significantly less entries to the center of open field ($p<0.001$) and spent significantly less time in center of open field ($p<0.001$). The animals switched to HFD spent significantly less time in the open arms of elevated plus maze ($p<0.05$; Figure 1C-D) as compared to DHA fed animals. These results strongly suggest that switching to a saturated HFD increases anxiety and depressive-like behavior.

Effects of diet transition to HFD on the levels of fatty acids in brain

To assess the effect of diet transition to HFD, we measured the levels of various fatty acids in brain by using gas chromatography. Detailed composition of fatty acids in the hippocampus is shown in Table 2. Most importantly, we found significant decrease (13.48 ± 0.11, $n=9$, $p<0.001$, Figure 2A) in the levels of DHA in the animal group fed on high-fat diet as compared to the DHA fed diet (14.81 ± 0.05, $n=5$, p,0.05, Figure 2B) counterpart (Table 2).We observed a positive correlation of hippocampal DHA levels with distance travelled in open field ($r= 0.6567$; $p<0.05$, Figure2C). The ratio of n-6/n-3 PUFA also showed a strong negative correlation with distance travelled in open field ($r=-0.7746$; $p<0.01$, Figure 2D) suggesting that change in dietary n-3 levels in HFD animals is associated with increased anxiety-like behavior.

Effects of n-3 deficiency on molecules associated with anxiety-like behavior: NPY1

Neuropeptides in the brain not only regulates the stress-induced activation of the HPA axis, but also mediates the behavioral and autonomic changes associated with stress-related illnesses

206 including anxiety, depression, and cardiovascular disease. The anxiety-reducing effects of NPY
207 and the anxiety-enhancing effects of antagonists of NPY receptors are fairly well-documented,
208 providing strong evidence for NPY's role in modulating anxiety responses. In the present study
209 we checked the modulating effects of DHA or HFD diet on the levels of NPY1 receptor. We
210 found a significant decrease in frontal cortex (22%, $p<0.05$; Fig. 3A) and hippocampus (35.5%,
211 $p<0.05$; Fig. 3A), when rats were fed with HFD as compared to DHA diet rats.
212

213 ***Effects of n-3-deficiency on molecules associated with synaptic***
214 ***plasticity***

215 ***BDNF signaling in frontal cortex and hippocampus***

216 The results showed that the percentage levels of BDNF were decreased to 31.1 % in the frontal
217 cortex of rats fed on HFD diet ($P<0.0001$; Fig. 4A), and in hippocampus the decrease observed
218 was 35.5 % ($p<0.0001$; Fig. 4A), as compared to DHA diet. Reports suggest that mice lacking
219 functional full-length *TrkB* specifically in the newborn neuron population of four to six weeks of
220 age exhibited a markedly enhanced anxiety- like behavior as evidenced by their decreased
221 explorative activity in the open field and elevated plus maze tests. (34). In our present study, we
222 observed a significant decrease in frontal cortex (26.33 %, $p<0.05$; Fig.4B), in the levels of
223 phosphorylated *TrkB* in HFD compared to rats fed on DHA diet. Currently, MAP kinase and PI-
224 3 kinase pathways are two of the best-studied BDNF/*TrkB*-mediated signaling pathways. Both
225 MAPK and PI-3K signaling pathways lead to the regulation of transcription factor, cyclic AMP
226 response element binding protein (CREB), which has been reported to be a key mediator of cell

survival (35). We assessed level of pCREB to further elucidate the effects of HFD on BDNF signaling. Again we found that levels of phosphorylated form of this nuclear factor dramatically decreased in frontal cortex (29.22 %, $p<0.0001$; 4C), and in hippocampus (15.33 %, $p<0.05$; Fig. 4C) of HFD animals as compared to DHA fed animals.

We observed reduced activation of CaMKII levels as suggested by reduced levels of p-CaMKII in frontal cortex (19.5%; $p<0.05$) and in hippocampus (29.11%; $p<0.001$) after 3 weeks of HFD, whereas, levels of total CaMKII were not changed. The ratio of p-CaMKII/CaMKII protein levels is also reduced in hippocampus (23.44%; $p<0.001$) of animals fed HFD as compared to DHA fed animals. The levels of GAP-43 protein significantly decline in frontal cortex (28.56%; $p<0.001$) and in hippocampus (10.39%; $p<0.05$) of HFD animals, as compared to DHA fed animals.

HFD induced alterations in synaptic plasticity protein "p-synapsin"

After 3 weeks of HFD the levels of synaptic plasticity marker protein p-synapsin decline significantly in frontal cortex (22.06%; $p<0.001$) and in hippocampus (32.96%; $p<0.01$) as compared to DHA fed animals.

HFD induced alterations in BDNF signaling & plasticity related proteins are associated with behavioral deficits

We observed that the BDNF levels in frontal cortex ($r=0.6089$; $p<0.05$) and hippocampus ($r=$ 7787; $p<0.001$) are strongly correlated to the outcomes in open field such as distance travelled. The results are presented in figure 5. The number of open arm entries made in elevated plus

maze are positively correlated with the levels of hippocampal p-CREB (r= 0.6189; $p<0.05$). There is a positive correlation between ratio of p-CaMKII/CaMKII protein levels and distance travelled in open field (r=0.8566; $p<0.001$). The levels of p-CamKII in frontal cortex (r=0.5328; $p<0.05$) are positively correlated with the number of open arm entries. The time spent in open arms of elevated plus maze is positively correlated to the levels of hippocampal GAP-43 protein levels (r=0.6857; $p<0.01$). The levels of GAP-43 in frontal cortex (r=0.6382; $p<0.05$) are positively correlated with the distance travelled in open field. The distance travelled in open field is positively correlated to the levels of p-synapsin protein in frontal cortex (r=0.7453; $p<0.01$) and hippocampus (r=0.6110; $p<0.05$).

Discussion

The purpose of the present study is to understand how changes in dietary habits e.g. transition from a healthy diet rich in omega-3 fatty acids to a junk food diet deficient in omega-3 but rich in saturated fatty acids, leads to vulnerability for psychiatric disorders. Here we show that consumption of a junk HFD for 3 weeks is enough to induce maladaption in anxiety & depression like behavior, and molecular systems associated with these behaviors. We found that the HFD reduced brain DHA contents, and that reduced levels of DHA were associated with increase in anxiety and depression-like behaviors in these rats. These data emphasize the detrimental effects of the HFD on brain function and behavior that are particularly manifested from switching from a healthy diet. The results of this study have important implications for public health, in terms of the risk imposed by poor dietary practices on mood disorders.

HFD increases risk for anxiety and depression-like behaviors

The rationale for the present study stemmed from our recent findings that animals fed on a diet deficient in omega-3-fatty acids during gestation, prenatal and postnatal growth periods were more prone to anxiety-like behavior as compared to animals fed on DHA supplemented diet (36). Given the positive effects of the DHA diet, it was reasonable to assume that switching to an unhealthy diet could have detrimental results for brain function. We found that 3 weeks of HFD increased the vulnerability for anxiety and depression-like behaviors. A recent study reported the ability of high-fat diet to exacerbate the depressive-like behavior in a rat model of genetic depression (37). The pro-depressive effects of high-fat diet in the current study may be mediated by the pro-inflammatory signalling induced by the high-fat diet consumption. There is substantial evidence that rodent diet-induced obesity model involves an inflammatory reaction in key hypothalamic areas critical for regulating food intake (38). A recent study has reported that hypothalamic inflammation was evident just after 1 to 3 days after onset of high-fat diet consumption prior to any substantial weight gain (12). In order to evaluate the effects of the HFD on the body, we measured several metabolic markers in blood and found elevations in glucose, cholesterol, tryglicerades, and uric acid. This implies that the HFD influences several parameters associated with obesity, in conjunction with its effects on the brain. The effects of the HFD highly contrast with the know roles of essential omega-3 polyunsaturated fatty acids on body and brain. Omega-3 fatty acids are crucial for brain function during development and adulthood, and their deficiency are considered risk factors for anxiety-like behavior in various animal models (39, 40).

Dietary effects on frontal cortex and hippocampus

To further elucidate possible differential effects of the diet across brain regions associated with anxiety-like behavior, we centered our studies in the frontal cortex and hippocampus. The human orbitofrontal cortex receives reciprocal connections from the hippocampus, nucleus accumbens, and hypothalamus (41) and is thought to play a significant role in hedonic and emotional processes implicated in the psychiatric disorders. The frontal cortex, together with hippocampus, amygdala and hypothalamus, are limbic regions forming part of well-defined anxiety and fear-related circuits in the forebrain Owing to the fact that all these limbic regions play an important role in mood disorders, it is significant that dietary fatty acids manipulation showed to affect the hippocampus and frontal cortex. Accordingly, we assessed neuropeptide Y (NPY) based on its role both anxiety and depression like behavior, particularly in the frontal cortex and limbic regions (43). It has been suggested that NPY produces an anxiolytic effects via NPY 1-type receptors (NPY-1R) (44). The anxiety-reducing effects of NPY and the anxiety-enhancing effects of antagonists of NPY receptors are fairly well-documented, providing strong evidence for NPY's role in modulating anxiety responses. Our results showed that n-3 deficiency decreased the levels of NPY-1R in the frontal cortex, hypothalaus and hippocampus, in agreement with the anxiolytic involvement of NPY-1R. In addition, these findings suggest that a radical shift in dietary omega-3 fatty acids intake to HFD can hinder the animal's natural ability to face challenges further in their life and leads to more anxiety-like behavior.

Molecular mechanisms: a potential link between dietary omega-3 fatty acid & BDNF receptor signaling

Our present study shows that HFD significantly reduced the levels of BDNF in frontal cortex and hippocampus. BDNF has been associated with the action of treatments for anxiety (45).

Previously it has been reported that changes in BDNF signaling in different areas of the adult brain may be implicated in the pathophysiology of psychiatric disorders, such as depression (46), (47), (48). Not only this, manipulations of the early environment can affect the expression of neurotrophins both during development and adulthood (49), (50), (51). BDNF binds with high affinity to the tropomyosin-related kinase B transmembrane receptor, (*TrkB*) resulting in BDNF signaling. Deficiency in *TrkB* activation has been linked to psychiatric illness in humans (47), (52). Furthermore a very recent report showed that an 11 base pair (bp) deletion in the *TrkB* promoter could have effects on the anxiety related traits in human (53).

Our current results show a reduction in the activation of BDNF receptor *TrkB* in the rats fed on HFD diet in hippocampus. These results hold well with previous findings that mice lacking functional full-length *TrkB* signaling, specifically in the newborn neuron population, exhibit a markedly enhanced anxiety-like behavior (54). The fact that DHA is a structural component of the plasma membrane important for membrane fluidity and function of transmembrane receptors, suggests that DHA regulate the function of *TrkB* receptors.

Effects of junk HFD on downstream molecular systems to BDNF

The transcription factor, cyclic AMP-dependent response element binding protein (CREB), regulates the expression of many genes, including BDNF (55), (56) and NPY-1 ((57). It has been shown that decreases in CREB phosphorylation and NPY expression in the central amygdala might be associated with anxiety-like behaviors in models of ethanol withdrawal in rats (58). In our studies, we showed reduction in the activation of CREB with HFD in hippocampus and frontal cortex. CREB has been implicated in the pathophysiology of depression as well as of bipolar disorder. Accordingly, the ability of the HFD to reduce CREB phosphorylation, in

conjunction with BDNF receptor activation, could be related to elevated risk for anxiety-like behavior.

Adoption of a HFD is an increasingly common event observed in the modern society which is tighly related to today's obesogenic environment where high calorie food is readily available. According to our results, the switch from a healthy n-3 PUFA diet to the HFD may be responsible for increased vulnerability to mood disorders, in addition to metabolic dysfunction. The transition to junk HFD reduced markers of synaptic plasticity in the frontal cortex and hippocampus. We found that the brain DHA contents were associated with levels of the BDNF in these brain regions. These data emphasize the importance of maintaining a healthy diet in order to support substrates that determine the balance between brain health and disease.

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Conflict of interests

All authors report no financial interests or potential conflict of interests

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Figure Legends

Fig 1 **Effect of diet switch to junk HFD on body weight gain and anxiety & depression-like behaviors** (A) Diet switch to a junk HFDfood significantly increased body weight gain as early as second week ($p<0.0001$) which remains significantly higher at the end of three weeks of junk HFD ($p<0.0001$) as compared to the rats on a healthy omega-3 supplemented diet. (B) Open field: significant decrease in the distance travelled in the open field ($p<0.001$) in rats switched to a junk HFD was noticed after 3 weeks of diet switch. (C-D) EPM: a non-significant trend toward decrease in percentage open arm entries in the rats subjected to diet switch to a junk HFD and a significant decrease in percentage time spent in open arm ($p<0.05$) in rats subjected to diet switch to a junk HFD was observed. Values are expressed in mean ±SEM. *$p<0.05$, *** $p<0.001$ Vs DHA diet.

Fig 2 **Effect of diet switch to junk HFD on brain DHA levels and their association with pro-depressive-like behavior** (A) A significant decrease ($p<0.01$) in brain DHA levels in rats subjected to a diet switch to junk HFD for 3 weeks as compared to the counterpart rats which were fed omega-3 supplemented diet (B) An increase ($p<0.001$) in the ratio of omega-6 (arachidonic acid) to omega-3 (DHA) fatty acids in rats switched to junk HFD was observed. The distance travelled in the open field was directly proportional to brain DHA levels (C; $r = 0.5657$; $p<0.05$), while inversely proportional to the ratio of n6/n3 PUFA (D; $r = 0.7746$; $p<0.001$); indicating that reduced brain DHA levels may be compensated by a corresponding increase in the brain arachidonic acid. The Values are expressed in mean ±SEM. **$p<0.01$, *** $p<0.001$ Vs DHA diet.

Fig 3 **Effects of diet switch to junk HFD on plasticity markers. Levels of** (A) neuropeptide Y (NPY) 1 receptor significantly decreased in frontal cortex ($p<0.05$) as well as in hippocampus ($p<0.05$). (B) Phosphorylated *TrkB* (p*TrkB*) showed significant decrease in hippocampus ($p<0.001$). (c) Brain derived neurotrophic factor (BDNF) showed a significant decrease in hippocampus ($p<0.001$) and frontal cortex ($p<0.001$). (D) A significant decrease in activation of CREB as depicted by levels of pCREB was observed in the hippocampus ($p<0.001$) and frontal cortex) $p<0.001$). Representative western blot bands are shown for BDNF, p*TrkB*, pCREB and actin in hippocampus and frontal cortex. Values are expressed in mean ±SEM. *$p<0.05$, $^{***}p<0.001$ Vs DHA diet.

Fig 4 **Effects of diet switch to junk HFD on GAP-43 levels.** (A) A significant reduction in the levels of GAP-43 was observed in hippocampus ($p<0.05$) and frontal cortex ($p<0.001$). (B) The GAP-43 levels in frontal cortex increased proportional to the distance travelled in the open field ($r = 0.6382$; $p<0.05$). Values are expressed in mean ±SEM. *$p<0.05$, $^{***}p<0.001$ Vs DHA diet.

Fig 5 **Association of plasticity markers with anxiety and depression-like behavior** (A-B) The distance travelled in open field was found to be positively associated with the levels of BDNF in hippocampus ($r = 0.7787$; $p<0.001$) and frontal cortex ($r = 0.6089$; $p<0.05$). (C-D) The distance travelled in open field was positively correlated with the levels of p-CREB in frontal cortex ($r = 0.6908$); $p<0.001$). The number of open arm entries made in elevated plus maze was positively correlated with the levels of hippocampal p-CREB ($r= 0.6189$; $p<0.05$). Values are expressed in mean ±SEM. *$p<0.05$, $^{***}p<0.001$ Vs DHA diet.

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553 **Table 1: Effects of diet switching to a high fat diet on metabolic syndrome**

554 **related molecules in blood**

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Diet	Glucose (mg/dL)	Cholestrol (mg/dL)	Triglycerides (mg/dL)	Uric Acid (mg/dL)
DHA	93.83 ± 2.915	62.5 ± 3.51	193 ± 13.22	2.983 ± 0.0654
HFD	112.3 ± 4.729*	85.89 ± 6.981*	370 ± 60.42*	3.767 ± 0.176**

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564 **Table 2: Effects of diet transition to HFD on the levels of fatty acids**
565 **in brain**

Fatty acid	DHA	HFD
C14:0	0.22±0	0.25± 0.01
C16:0	18.4±0.13	17.93± 0.13
C16:1	0.44±0.02	0.45± 0.01
C18:0	19±0.14	18.38± 0.16
C18:1	15.6±0.18	15.19± 0.17
C18:2n6 (LA)	0.56±0.01	0.84± 0.03
C20:0	0.61±0.06	0.60± 0.03
C20:1	1.47±0.06	1.59± 0.03
C20:2	0.6±0.06	1.05± 0.09
C20:3n6	0.6±0.05	1.26± 0.06[a]
C20:4n6 (AA)	9.36±0.09	9.83± 0.12
C22:4n6	2.68±0.06	2.91± 0.06
C22:5n6 (DPA)	0.03±0.01	0.24±0.03
C24:0	1.61±0.09	1.16± 0.03
C22:6n3 (DHA)	14.8±0.05	13.48± 0.11[a]
Ratio n-6/n-3 PUFA	0.67±0.01	0.81±0.01[a]

566 Each parameter is presented as percentage mean relative to total fatty acids (±SEM) in frontal
567 cortex. Statistically significant changes are represented [a]$p<0.05$ compared with DHA diet. Data
568 are analyzed by using two tailed unpaired t-test.

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