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Coupling energy homeostasis with a mechanism to support plasticity in brain trauma

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ABSTRACT

Metabolic dysfunction occurring after traumatic brain injury (TBI) is an important risk factor for the development of psychiatric illness. In the present study, we utilized an omega-3 diet during early life as a metabolic preconditioning to alter the course of TBI during adulthood. TBI animals under omega-3 deficiency were more prone to alterations in energy homeostasis (adenosine monophosphate-activated protein kinase; AMPK phosphorylation and cytochrome C oxidase II; COII levels) and mitochondrial biogenesis (peroxisome proliferator-activated receptor gamma coactivator 1-alpha; PGC-1α and mitochondrial transcription factor A; TFAM). A similar response was found for brain-derived neurotrophic factor (BDNF) and its signaling through tropomyosin receptor kinase B (TrkB). The results from in vitro studies showed that 7,8-dihydroxyflavone (7,8-DHF), a TrkB receptor agonist, upregulates the levels of biogenesis activator PGC-1α, and CREB phosphorylation in neuroblastoma cells suggesting that BDNF–TrkB signaling is pivotal for engaging signals related to synaptic plasticity and energy metabolism. The treatment with 7,8-DHF elevated the mitochondrial respiratory capacity, which emphasizes the role of BDNF–TrkB signaling as mitochondrial bioenergetics stimulator. Omega-3 deficiency worsened the effects of TBI on anxiety-like behavior and potentiated a reduction of anxiolytic neuropeptide Y1 receptor (NPY1R). These results highlight the action of metabolic preconditioning for building long-term neuronal resilience against TBI incurred during adulthood. Overall, the results emphasize the interactive action of metabolic and plasticity signals for supporting neurological health.

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1. Introduction

The brain is a highly metabolic organ, such that disruptions of metabolic homeostasis can have dramatic consequences for information processing and cognitive function. Traumatic brain injury (TBI) is followed by a state of brain energy crisis [1], which compromises neuronal survival and long-term brain function. However, there is a paucity of studies investigating how metabolic adaptations contribute to build a neuronal reserve for supporting long-term plasticity. Nutritional imbalance during early life appears as a determinant factor for the risk imposed by metabolic diseases later in life [2], and this concept conforms to the basis for the postulate of “Metabolic Programming”. Accordingly, we embarked in studies to investigate how nutrition during early life could influence bioenergetics in the brain, which can alter neuronal resilience in TBI during adulthood. The omega-3 polyunsaturated fatty acid docosahexaenoic acid (DHA) is considered as an essential nutrient for proper brain development and function; [3] however, its action on metabolism is poorly understood. Here we report that omega-3 fatty acids support metabolic pathways involved in energy homeostasis, which are fundamental for proper brain function under homeostatic and injury conditions.

Patients suffering even mild concussive cerebral injury often exhibit long-term cognitive and emotional disturbances, in spite of minimal neurological damage [4,5]. In particular, depression and anxiety disorders are the most prevalent sequel in the survivors of TBI [6]. Recent clinical and experimental studies suggest that neuronal metabolic abnormalities occurring after TBI are important instigators of neurological and psychological aberrations [7,8]. An increasing line of research emphasizes the potential of DHA protecting against psychiatric disorders [9]. Accordingly, we have assessed the action of DHA on molecular

Abbreviations: AMPK, adenosine monophosphate-activated protein kinase; BDNF, brain-derived neurotrophic factor; FCCP, carbonyl cyanide-*p*-trifluoromethoxyphenylhydrazone; COII, cytochrome C oxidase II; 7,8-DHF, 7,8-dihydroxyflavone; DHA, docosahexaenoic acid; EPM, elevated plus maze; FPI, fluid percussion injury; LPOs, lipid peroxides; mtDNA, mitochondrial DNA; TFAM, mitochondrial transcription factor A; NPY1R, neuropeptide Y1 receptor; NRFs, nuclear respiratory factors; OXPHOS, oxidative phosphorylation; OCR, oxygen consumption rate; PGC-1α, peroxisome proliferator-activated receptor gamma coactivator 1-alpha; PUFAs, polyunsaturated fatty acids; ROS, reactive oxygen species; SOD2, superoxide dismutase; TBI, traumatic brain injury; TrkB, tropomyosin receptor kinase B

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systems positioned at the interface of cell metabolism and neuronal plasticity, and may determine the course of TBI.

Mitochondria are particularly susceptible to damage as they are the major bioenergetics machinery and source of oxidative stress in cells. Therefore, effective control of mitochondrial bioenergetics and turnover becomes critical for the maintenance of energy production, prevention of oxidative stress and neuronal function. Multiple endogenous and exogenous factors regulate mitochondrial biogenesis through peroxisome proliferator-activated receptor gamma coactivator-1alpha (PGC-1α), which is a transcriptional regulator of various transcription factors, including nuclear respiratory factors (NRFs). The NRFs activate the mitochondrial transcription factor A (TFAM) that regulates mitochondrial DNA (mtDNA) transcription and replication [10]. Brain-derived neurotrophic factor (BDNF), a neurotrophin classically recognized for its role on plasticity and behavior, likely plays a central role in the events that link metabolism and synaptic plasticity. New studies indicate that BDNF participates in a range of metabolic events, including glucose utilization and energy management [11,12]. Accordingly, we conducted studies using 7,8-dihydroxyflavone (7,8-DHF), a TrkB agonist, to explore the role of BDNF signaling on the regulation of mitochondrial function. This study provides crucial information to understand the potential of diet to modulate the interaction between mitochondrial homeostasis and synaptic plasticity as a mechanism that regulates the capacity of the brain to resist TBI.

2. Materials and methods

2.1. Animals & experimental design

Female Sprague–Dawley rats (250–280 g) were obtained from Charles River Laboratories (Wilmington, MA, USA) on the 3rd day of pregnancy. The animals were kept in polyacrylic cages under standard housing condition (22–24 °C) with 12 h light/dark cycle in two dietary groups. Pregnant female rats were fed an either omega-3 fatty acid adequate (n − 3 diet) or omega-3 fatty acid deficient (n − 3 def) diet through gestation and lactation. The two custom diets used were based on the composition of the American Institute of Nutrition diet (AIN-93G) and prepared commercially (Dyets Inc., PA, USA) as previously described [13]. Both diets had the same basal macronutrients, vitamins, minerals, and basal fats (hydrogenated coconut and safflower oils) (Table 1). The only difference between two diets was the amount of n − 3 fatty acids, which was achieved by adding 0.5% of flaxseed oil (source of 18:3n − 3; α-linolenic acid, ALA) and 2.7% of ProDHA oil (Nordic Naturals, Inc., CA, USA) to the n − 3 diet. The final concentration of DHA, EPA and other n − 3 fatty acids in n − 3 diet was 1.2%, 0.24% and 0.1% of total n − 3 diet respectively. The diets were provided in powder in a bowl and animals had free access to food and water.

Table 1
Composition of experimental diets.

Ingredient	Amount (g/100 g diet)	
	n − 3 adq	n − 3 def
Alacid 710, acid casein	20	20
Cornstarch	15	15
Sucrose	10	10
Dextrose	19	19.9
Maltose-dextrin	15	15
Cellulose	5	5
Salt-mineral mix	3.5	3.5
Vitamin mix	1	1
L-cystine	0.3	0.3
Choline bitartrate	0.25	0.25
TBHQ	0.002	0.002
Fat sources:		
Hydrogenated coconut oil	7.45	8.1
Safflower oil	1.77	1.9
Flaxseed oil	0.48	–
DHA[a]	1.2	–
EPA[a]	0.24	–
Other n3 s[a]	0.1	–

Note: Dashes indicate that component was not added.
[a] Procured from Nordic Naturals Inc., CA, USA ProDHA capsule that contains 45% (w/w) DHA, 9% (w/w) EPA and 4% (w/w) other n − 3 s.

The study consists of a total of 16 litters (8/diet group), and only male offspring were included in the experimental groups. At weaning on postnatal day (PND) 21, the offspring were fed on the same diet as their dams and randomly divided into four experimental groups in a way that each offspring within an experimental group (n = 8) was chosen from a different litter. The male offspring from dams fed an omega-3 adequate diet were split into (1) sham (n − 3 diet/Sham) and (2) fluid percussion injury (n − 3 diet/FPI) groups. The offspring from dams fed an omega-3 deficient diet were also split into (3) sham (n − 3 def/Sham) and (4) fluid percussion injury (n − 3 def/FPI) groups. At 17 weeks of age, the offspring from each dietary group were subjected to either sham or fluid percussion injury (FPI). To assess the anxiety-like behavior, the offspring were subjected to the elevated plus maze (EPM) test after 1 week of injury (i.e. at 18 weeks of age) and were killed immediately by decapitation after the behavioral test. Fresh brain tissues were dissected out, frozen in dry ice and stored at −70 °C until use. Brain tissues were shared from the same animals for fatty acid and immunoblotting analysis. All 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.

2.2. Fluid percussion injury

The injury was performed as previously described [14]. In brief, the animals were anesthetized by using a Laboratory Animal Anesthesia System (VetEquip Inc., CA, USA) that provides a mixture of isoflurane and oxygen. The animals were maintained in a deep anesthetic state during surgery with 2–5% isoflurane mixed with 100% O_2 at a flow rate of 0.4 l/min via nose cone. With the aid of a microscope (Wild, Heerbrugg, Switzerland) a 3.0-mm-diameter craniotomy was made over the left parietal cortex, 3.0 mm posterior to bregma and 6.0 mm lateral (left) to the midline with a high-speed drill (Dremel, WI, USA). A plastic injury cap was placed over the craniotomy with silicone adhesive and dental cement. When the dental cement hardened, the cap was filled with 0.9% saline solution. Anesthesia was discontinued and the injury cap was attached to the fluid percussion device. At the first sign of hind-limb withdrawal to a paw pinch, a mild fluid percussion pulse (1.5 atm) was administered to the epidural space. Immediately upon responding to a paw pinch, anesthesia was restored and the skull was sutured. Neomycin was applied on the suture and the rats were placed in a heated recovery chamber before being returned to their cages. Sham animals underwent an identical preparation with the exception of the lesion.

2.3. Elevated plus maze

After 1 week of FPI, the rats were subjected to elevated plus maze (EPM) test [15]. Briefly, the EPM apparatus made of laminated wood consisted of 2 opposing open arms (10 × 50 cm) and 2 opposing closed arms (10 × 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 over a period of 5 min. Each rat was placed in the middle of the maze facing the open arm that faced away from the experimenter. The time spent and the number of entries in each arm were measured using AnyMaze video tracking software (San Diego Instruments, CA, USA). The total number of times the rats entered in the arms during the EPM test were calculated to account for differences in general motor activity in the maze.

2.4. Fatty acid analysis

Fatty acids were measured by gas chromatography in cortical tissues and total lipids were extracted according to published methods [16]. Briefly, frozen brains were homogenized in chloroform/methanol (2:1 vol/vol), containing 50 μg/ml of butylated hydroxytoluene to prevent lipid oxidation during the procedure. Tricosanoic acid methylester (C23:0) was used as an internal standard. Tissues were grounded to powder under liquid nitrogen and subjected to extraction of total lipids. Fatty acid methylation was done by heating at 90 °C for 1 h under 14% (w/v) boron trifluoride–methanol reagent.

Extracted lipids were analyzed on Clarus 500 gas chromatograph (GC; PerkinElmer, MA, USA) equipped with a built-in Autosampler and flame ionization detector (FID). An Elite-WAX column (60 m, 0.32-mm internal diameter) was used, with hydrogen as the carrier gas. GC oven temperature was initially held at 140 °C for 2 min and raised with a gradient of 5 °C min^{-1} until 250 °C and held for 10 min. The total run time is 34 min. The injector and detector were maintained at 250 °C and 300 °C, respectively. A 1 μl 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).

2.5. Immunoblotting

The frontal cortex tissues were homogenized in a lysis buffer containing 137 mM NaCl, 20 mM Tris–HCl pH 8.0, 1% NP40, 10% glycerol, 1 mM phenylmethylsulfonylfluoride (PMSF), 10 μg/ml aprotinin, 0.1 mM benzethonium chloride, 0.5 mM sodium vanadate. The homogenates were then centrifuged, the supernatants were collected and total protein concentration was determined according to MicroBCA procedure (Pierce, IL, USA), using bovine serum albumin (BSA) as standard.

Briefly, protein samples were separated by electrophoresis on a 10% polyacrylamide gel and electrotransferred to a PVDF membrane (Millipore, MA, USA). Non-specific binding sites were blocked in Tris–buffered saline (TBS), pH 7.6, containing 5% non-fat dry milk. Membranes were rinsed in buffer (0.05% Tween-20 in TBS) and then incubated with anti-actin or anti-BDNF, anti-pTrkB, anti-TrkB, anti-AMPK, anti-cytochrome c oxidase II, anti-mtTFA (TFAM), anti-SOD-2 4HNE(1:500; Santa Cruz Biotechnology, CA, USA), anti-PGC-1α, anti-pCREB, anti-CREB (1:1000, Millipore, MA, USA), anti-pAMPK (1:1000; Cell signaling technology, MA, USA), anti-NPY1R (1:1000; Alpha Diagnostic Intl. Inc., TX, USA) followed by anti-rabbit or anti-goat or anti-mouse IgG horseradish peroxidase-conjugate (1:10,000; Santa Cruz Biotechnology, CA, USA). After rinsing with buffer, the immunocomplexes were visualized by chemiluminescence using the ECL kit (Amersham Pharmacia Biotech Inc., NJ, USA) according to the manufacturer's instructions. The film signals were digitally scanned and then quantified using ImageJ software. Actin was used as an internal control for Western blot such that data were standardized according to actin values.

2.6. In vitro experiments

2.6.1. Cell culture

Neuro-2a cells (mouse neuroblastoma cell line from American Type Culture Collection; ATCC, VA, USA) were maintained as monolayer cultures in Dulbecco's modified Eagle medium (DMEM) (Gibco by Life Technologies Inc., NY, USA) supplemented with 10% (v/v) fetal bovine serum (Gibco by Life Technologies Inc., NY, USA), penicillin (100 units/ml) and streptomycin (100 μg/ml) at 37 °C, in a humidified atmosphere containing 95% air and 5% CO_2. Cells were treated with dimethyl sulfoxide (DMSO; with a final concentration of 0.01%) or 100, 200 and 400 nM of 7,8-dihydroxyflavone (7,8-DHF) for 2 h. The doses and time of 7,8-DHF treatment were chosen on the basis of optimal activation of TrkB receptor as demonstrated in previous in vitro studies [17]. After 2 h, cell lysates were prepared in 1× RIPA lysis buffer containing protease inhibitors for immunoblotting. Total protein concentration was determined according to MicroBCA procedure (Pierce, IL, USA), using bovine serum albumin (BSA) as standard and immunoblotting was performed as described above.

2.6.2. Immunocytochemistry

N2a cells were plated onto 4-chamber slides for immunocytochemical analysis. Cells were cultured as described above and 7,8-DHF was added to the wells at a concentration of 200 nM. After 2 h incubation with 7,8-DHF, cells were fixed with 4% paraformaldehyde for 10 min, washed with PBS and incubated for 1 h at room temperature with 2% BSA in Tris-buffered saline containing 0.2% Triton-X-100 (TBST) to inhibit non-specific binding. Primary antibodies (CREB and PGC-1α from Millipore, MA, USA), diluted in 1% bovine serum albumin (BSA)/TBST, were added to the cells and incubated overnight at 4 °C in a humidified chamber. Cells were washed several times with PBS and were incubated with appropriate dilutions of fluorescent mouse or rabbit secondary antibodies (FITC; 1:1000, Cy3; 1:4000; Jackson ImmunoResearch Laboratories Inc., PA, USA) for 1 h at room temperature. Slides were washed in PBS and mounted using aquamount. Negative controls were performed by omission of the primary antibody. The staining was visualized under Zeiss microscope (Zeiss Imager.Z1) using the Axiovision 4.6 software.

2.6.3. Mitochondrial bioenergetics analysis

Mitochondrial bioenergetics functions were measured using an XF24 Extracellular Flux Analyzer (Seahorse Bioscience, MA, USA). One day before analysis, N2a cells were seeded in V7 plates (Seahorse Bioscience) at 4×10^4 cells/well and cultured as described above. Cells were incubated with 200 nM of 7,8-DHF and cellular respiration was measured after 4, 8 and 12 h treatment. Prior to the measurements, the culture medium was changed to unbuffered complete DMEM as described by Wu et al. [18]. Mixing, waiting and measure times were 3, 2 and 3 min, respectively. To assess mitochondrial functions, oxygen consumption rate (OCR) was measured in basal condition and after sequential injection of 0.75 μM oligomycin, 0.25 μM carbonyl cyanide-*p*-trifluoromethoxyphenylhydrazone (FCCP), and a mixture of 0.75 μM rotenone-myxothiazol. The ATP synthase inhibitor oligomycin allows the detection of ATP-linked respiration by decreasing OCR, thus indicating the extent of respiration to which cells are using mitochondria to generate ATP. The uncoupler FCCP reveals maximal mitochondrial respiratory capacity, while rotenone–myxothiazol blocks mitochondrial respiration at complexes I and III.

2.7. Statistical analysis

The results are represented as mean ± standard error of the mean (SEM). Protein results are expressed as percentage of n − 3 diet group for in vivo experiments and statistical analysis was performed by two-way analysis of variance (ANOVA; diet vs. injury). Post-hoc analyses were conducted using Bonferroni's multiple comparison tests to determine the significance of difference among various groups. For in vitro experiments, protein results are expressed as percentage of control group and data was analyzed by one-way ANOVA followed by Bonferroni's multiple comparison tests. The p-value < 0.05 was considered as statistically significant. Pearson correlation analysis was performed on individual samples to evaluate the association between variables.

3. Results

3.1. Brain trauma affected metabolic proteins primarily in n −3 fatty acid deficient animals

We have evaluated the vulnerability imposed by early nutrition on the effects of TBI in cortical tissues by focusing on the role of proteins

that control the balance and transduction of cellular energy. A two-way ANOVA analysis (diet vs. injury) showed a significant effect of diet ($F_{1,28} = 4.611$, $p < 0.05$), injury ($F_{1,28} = 6.904$, $p < 0.05$) and an interaction between diet vs. injury ($F_{1,28} = 4.715$, $p < 0.05$) for AMPK phosphorylation. The Bonferroni post-hoc test for multiple comparisons showed that the n−3 deficiency was a factor for the action of TBI, as FPI decreased AMPK phosphorylation only under n−3 deficiency (n−3 def/FPI vs. n−3 def/Sham group; $p < 0.01$). In turn, the n−3 diet appeared to protect the brain against the effects of the injury as FPI did not alter AMPK phosphorylation levels under the n−3 diet condition (n−3 diet/FPI vs. n−3 diet/Sham, $p > 0.05$; Fig. 1A).

We assessed the levels of COII, a mitochondrial protein, which provides an index of mitochondrial function/mass and plays an important role in mitochondrial oxidative phosphorylation (OXPHOS). Two-way ANOVA analysis revealed a significant effect of diet ($F_{1,28} = 9.069$, $p < 0.01$) and its interaction with injury ($F_{1,28} = 4.244$, $p < 0.05$) for COII level. The post-hoc analysis showed that COII levels were reduced under the n−3 deficiency (n−3 def/FPI vs. n−3 def/Sham group, $p < 0.05$), while the adequate levels of dietary n−3 fatty acid maintained the levels of COII after TBI (n−3 diet/FPI vs. n−3 diet/Sham, $p > 0.05$; Fig. 1B). The positive correlation between percentage time spent in the open arms and level of COII ($r = 0.538$, $p < 0.01$) suggests that anxiety-like behavior tested in the elevated plus maze may rely on the levels of mitochondrial proteins (Fig. 1C).

We assessed a possible association between AMPK action and regulation of the mitochondrial PGC-1α, and found that the AMPK phosphorylation varied in proportion to the level of PGC-1α ($r = 0.514$, $p < 0.01$; Fig. 1D).

3.2. *Brain trauma affected molecules associated with mitochondrial function primarily in n−3 fatty acid deficient animals*

We examined the levels of PGC-1α, which is a member of a family of transcription co-activators that plays a central role in the regulation of cellular energy metabolism. The significant effect of diet ($F_{1,28} = 72.082$, $p < 0.01$) and injury ($F_{1,28} = 4.723$, $p < 0.05$) as well as a significant interaction between diet vs. injury ($F_{1,28} = 6.733$, $p < 0.05$) were observed on PGC-1α levels by two-way ANOVA. The Bonferroni post-hoc test showed the effect of TBI on PGC-1α only in the state of n−3 deficiency (n−3 def/FPI vs. n−3 def/Sham, $p < 0.01$). The prior exposure of n−3 diet was able to counteract the effect of brain injury on levels of PGC-1α, as the PGC-1α levels remained similar in both of the n−3 diet groups (n−3 diet/FPI vs. n−3 diet/Sham, $p > 0.05$). We observed that n−3 deficiency resulted in a decreased expression of PGC-1α in sham animals (n−3 diet/Sham vs. n−3 def/Sham, $p < 0.01$; Fig. 2A).

The level of PGC-1α varied in proportion to the phosphorylation of cyclic AMP response element binding protein (CREB) ($r = 0.621$, $p < 0.01$), suggesting that association of CREB with co-activator PGC-1α (Fig. 2B). We also observed that the levels of PGC-1α were positively correlated with the percentage of time spent in the open arms of the elevated plus maze test ($r = 0.662$, $p < 0.01$) suggesting that an increased PGC-1α may contribute to decrease in anxiety-like behavior (Fig. 2C).

Several studies have been suggested that the PGC-1α works in close association with the reactive oxygen species (ROS)-detoxifying capacity of cells [19]. Reactive oxygen species (ROS), a byproduct of mitochondrial

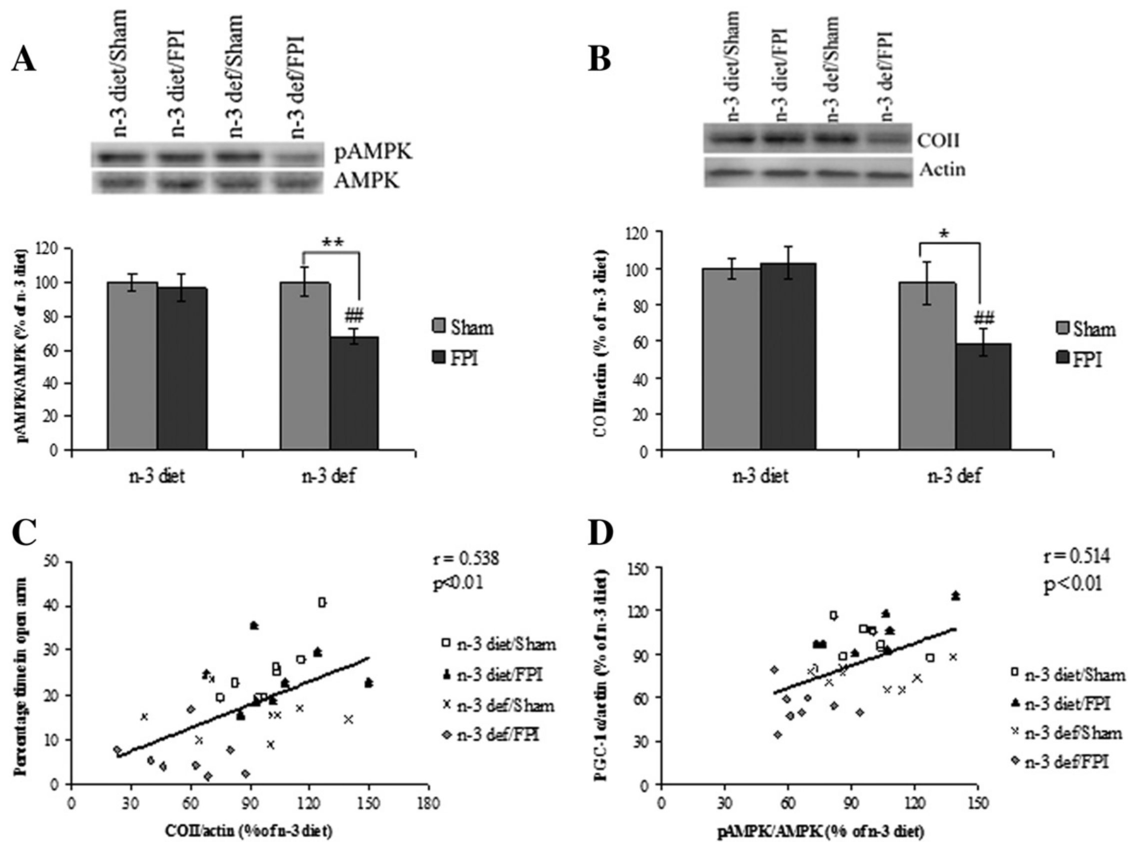

Fig. 1. (A) Phosphorylation of AMPK, (B) levels of COII protein, (C) correlation analysis of COII with percentage of time spent in open arms of EPM and (D) correlation analysis of AMPK phosphorylation with levels of PGC-1α in groups fed with either n−3 fatty acid (n−3 diet) or n−3 fatty acid deficient (n−3 def) diet and subjected to either sham or fluid percussion injury (FPI). Data are expressed as percentage of n−3 diet group (mean ± SEM). #$p < 0.05$ vs. n−3 diet/Sham, *$p < 0.05$, **$p < 0.01$ vs. n−3 def/Sham group; ANOVA (two-way) followed by post-hoc test with Bonferroni's comparisons.

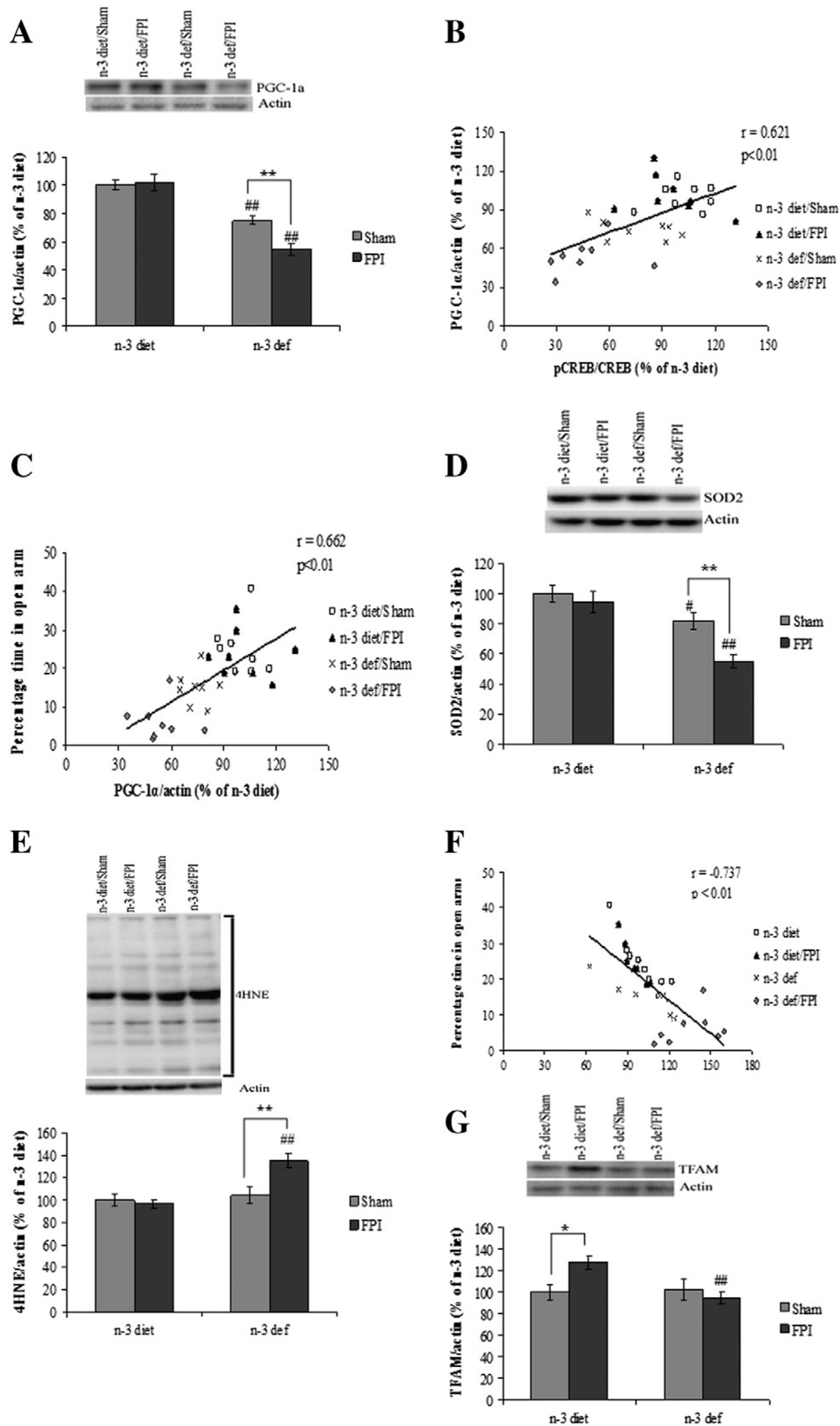

Fig. 2. (A) Protein level of PGC-1α, (B) correlation analysis of CREB phosphorylation with levels of PGC-1α, (C) correlation analysis of PGC-1α with percentage of time spent in open arms of EPM, (D) levels of SOD2, (E) levels of 4HNE, (F) correlation analysis of 4HNE with percentage of time spent in open arms of EPM and (G) levels of TFAM in groups fed with either n − 3 fatty acid (n − 3 diet) or n − 3 fatty acid deficient (n − 3 def) diet and subjected to either sham or fluid percussion injury (FPI). Data are expressed as percentage of n − 3 diet group (mean ± SEM). $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ vs. n − 3 diet/Sham, $^{*}p < 0.05$ vs. their respective n − 3 diet/Sham or n − 3 def/Sham group; ANOVA (two-way) followed by post-hoc test with Bonferroni's comparisons.

electron transport chain, leads to oxidative damage by increasing lipid peroxidation of unsaturated fatty acids. Therefore, we assessed the levels of mitochondrial protein superoxide dismutase 2 (SOD2), an antioxidant and levels of 4-hydroxynonenal (4HNE), a marker of lipid peroxidation, to have a comprehensive view of the effects of diet and TBI on oxidative stress. For the levels of SOD2, two-way ANOVA analysis

showed a significant effect of diet ($F_{1,28} = 26.473$ $p < 0.01$) and injury ($F_{1,28} = 8.260$, $p < 0.01$). TBI was able to reduce the level of SOD2, only in the absence of n − 3 in diet (n − 3 def/FPI vs. n − 3 def/Sham, $p < 0.01$). Pre-exposure to n − 3 fatty acid prevented the effects of TBI on SOD2 levels as there was no effect observed after FPI under n − 3 diet condition (n − 3 diet/FPI vs. n − 3 diet/Sham, $p > 0.05$). Exposure to dietary n − 3 fatty acid deficiency resulted in a significant decrease in the levels of SOD2 (n − 3 def/Sham vs. n − 3 diet/Sham, $p < 0.05$; Fig. 2D). With regard to the levels of 4HNE, a significant effect of diet ($F_{1,28} = 12.432$ $p < 0.01$) and injury ($F_{1,28} = 5.393$ $p < 0.05$) as well as their interaction ($F_{1,28} = 8.189$ $p < 0.01$) was observed by ANOVA (two-way) analysis. TBI elevated the levels of 4HNE under n − 3 deficiency (n − 3 def/FPI vs. n − 3 def/Sham, $p < 0.01$); however, the prior exposure of n − 3 diet was able to counteract the effect of TBI on 4HNE levels (n − 3 diet/FPI vs. n − 3 diet/Sham, $p > 0.05$). Omega-3 fatty acid deficiency had no effect on 4HNE levels in sham animals (n − 3 def/Sham vs. n − 3 diet/Sham, $p > 0.05$; Fig. 2E). The levels of 4HNE were negatively correlated with percentage of time spent in the open arms of elevated plus maze test ($r = -0.737$, $p < 0.01$) suggesting that an increased lipid peroxidation may contribute to increase anxiety-like behavior (Fig. 2F).

Further, we assessed the mitochondrial transcription factor TFAM, which is a key activator of mitochondrial transcription and mitochondrial genome replication. A significant effect of diet ($F_{1,28} = 4.584$, $p < 0.05$) and an interaction between diet vs. injury ($F_{1,28} = 5.856$ $p < 0.05$) were observed for the levels of TFAM by two-way ANOVA. The post-hoc test performed for multiple comparisons showed that TBI increased levels of TFAM in animals fed an n − 3 diet (n − 3 diet/FPI vs. n − 3 diet/Sham, $p < 0.05$). TBI had no effects on TFAM levels in animals exposed to the n − 3 deficient diet (n − 3 def/FPI vs. n − 3 def/Sham, $p > 0.05$; Fig. 2G).

3.3. BDNF signaling systems

BDNF signaling has emerged as a key player in several psychiatric conditions such as depression and anxiety disorders [20,21]. To assess the changes in BDNF signaling, we measured the phosphorylation level of tropomyosin receptor kinase B (TrkB) and its downstream effector CREB in groups subjected to TBI with or without n − 3 fatty acid in diet. Two-way ANOVA analysis showed a significant effect of diet ($F_{1,28} = 12.569$ $p < 0.05$) and injury ($F_{1,28} = 10.549$ $p < 0.01$), as well as, a significant interaction between diet and injury ($F_{1,28} = 4.958$ $p < 0.05$) was observed for BDNF levels. Bonferroni post-hoc test showed that FPI reduced the levels of BDNF in animals exposed to n − 3 deficiency (n − 3 def/FPI vs. n − 3 def/Sham, $p < 0.01$), while n − 3 fatty acid prior to the FPI prevented this reduction (n − 3 diet/FPI vs. n − 3 diet/Sham, $p > 0.05$; Fig. 3A). With regard to phosphorylation of TrkB, the significant effects of diet ($F_{1,28} = 58.763$ $p < 0.01$) and its interaction with injury ($F_{1,28} = 6.641$ $p < 0.05$) was observed by ANOVA (two-way) analysis. TBI reduced TrkB phosphorylation under n − 3 deficiency (n − 3 def/FPI vs. n − 3 def/Sham, $p < 0.01$); however, TBI had no effects on TrkB receptor in the presence of n − 3 fatty acid

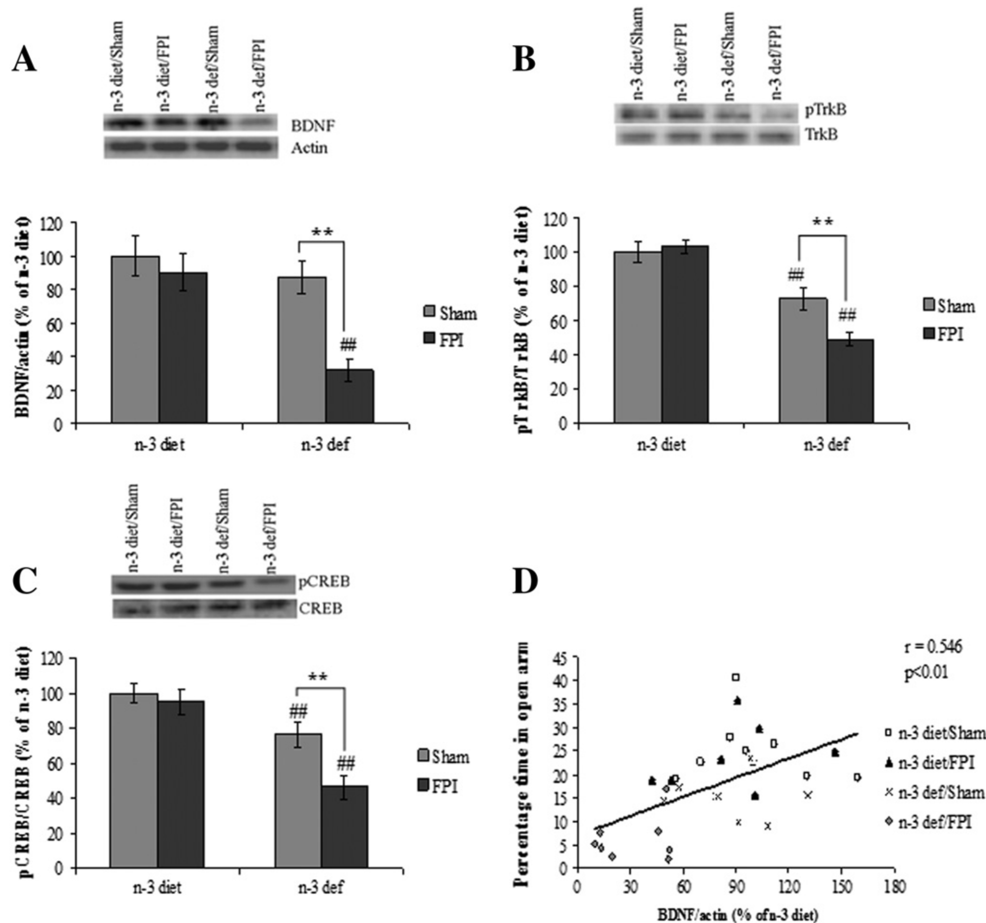

Fig. 3. (A) Protein level of BDNF, (B) TrkB phosphorylation, (C) phosphorylation of CREB and (D) correlation analysis of BDNF with percentage of time spent in open arms, in groups fed with either n − 3 fatty acid (n − 3 diet) or n − 3 fatty acid deficient (n − 3 def) diet and subjected to either sham or fluid percussion injury (FPI). Data are expressed as percentage of n − 3 diet group (mean ± SEM). #$p < 0.05$, ##$p < 0.01$ vs. n − 3 diet/Sham, *$p < 0.05$, **$p < 0.01$ vs. n − 3 def/Sham group; ANOVA (two-way) followed by post-hoc test with Bonferroni's comparisons.

in diet (n − 3 diet/FPI vs. n − 3 diet/Sham, $p > 0.05$). The dietary deficiency of n − 3 reduced levels of TrkB phosphorylation in sham animals (n − 3 def/Sham vs. n − 3 diet/Sham, $p < 0.01$; Fig. 3B). BDNF affects neuronal plasticity through the activation of CREB. The phosphorylation of CREB changed significantly with diet ($F_{1,28} = 31.821$ $p < 0.01$) and injury ($F_{1,28} = 7.867$ $p < 0.01$) by ANOVA (two-way) analysis. We found that FPI reduced CREB phosphorylation in the n − 3 deficient group (n − 3 def/FPI vs. n − 3 def/Sham, $p < 0.01$); however, FPI did not affect the phosphorylation of CREB in the presence of n − 3 diet (n − 3 diet/FPI vs. n − 3 diet/Sham, $p > 0.05$). Exposure to dietary n − 3 fatty acid deficiency resulted in a significant decrease in phosphorylation of CREB (n − 3 def/Sham vs. n − 3 diet/Sham, $p < 0.01$; Fig. 3C). There was a positive correlation between BDNF and percentage time spent in the open arms ($r = 0.546$, $p < 0.01$), suggesting an association between BDNF signaling and performance in the EPM (Fig. 3D).

3.4. Preponderant effects of brain trauma on anxiety-like behavior in n − 3 fatty acid deficiency

We corroborated the effects of TBI and dietary n − 3 fatty acid interventions on anxiety-like behavior and related molecular markers. Two-way ANOVA analysis showed a significant effect of diet ($F_{1,28} = 44.253$ $p < 0.01$) and injury ($F_{1,28} = 6.077$ $p < 0.05$) for percentage of time spent in the open arms in elevated plus maze. The post-hoc analysis showed a marked reduction in the percentage of time spent in the open arms after TBI in dietary n − 3 fatty acid deficient condition (n − 3 def/FPI vs. n − 3 def/Sham, $p < 0.01$). However, FPI had no effect on these variables after n − 3 feeding (n − 3 diet/FPI vs. n − 3 diet/Sham, $p > 0.05$). The dietary n − 3 fatty acid deficiency also decreased the percentage of time spent in the open arms in sham animals (n − 3 def/Sham vs. n − 3 diet/Sham, $p < 0.01$; Fig. 4A). The general motor activity, as measured by the total number of entries during the EPM test was similar among all the groups (data not shown) suggesting that the changes in anxiety-like behavior were not due to changes in motor activity.

We assessed levels of neuropeptide Y1 receptor (NPY-1R) based on its involvement in anxiety and depression-like behavior [22]. There was a positive correlation between NPY1R levels and the percentage time spent in the open arms ($r = 0.616$, $p < 0.01$) in EPM test (Fig. 4B). With regard to the levels of NPY1R, the significant effects of diet ($F_{1,28} = 41.670$ $p < 0.01$) and injury ($F_{1,28} = 8.298$ $p < 0.01$) as well as a significant interaction between diet vs. injury ($F_{1,28} = 4.468$ $p < 0.05$) were observed by two-way ANOVA. TBI reduced NPY-1R levels under dietary n − 3 deficiency (n − 3 def/FPI vs. n − 3 def/Sham, $p < 0.01$). TBI had no effects in these variable under n − 3 dietary condition (n − 3 diet/FPI vs. n − 3 diet/Sham, $p > 0.05$), which indicates that pre-exposure to dietary n − 3 fatty acid provides resistance to cope with the effects of TBI. The dietary deficiency of n − 3 fatty acid resulted in lower levels of NPY1R in sham animals (n − 3 def/Sham vs. n − 3 diet/Sham, $p < 0.01$; Fig. 4C).

3.5. Brain DHA contents

To confirm accretion of n − 3 fatty acid in the brain following the diet, we measured the levels of DHA by using gas chromatography as shown in Fig. 5. Significant diet ($F_{1,28} = 1169.98$ $p < 0.01$) and injury ($F_{1,28} = 5.462$ $p < 0.05$) effects were found for the levels of DHA by two-way ANOVA analysis. The n − 3 deficiency resulted in significant lower levels of DHA as analyzed by Bonferroni post-hoc test (n − 3 def/Sham vs. n − 3 diet/Sham, $p < 0.01$). TBI did not cause significant changes in DHA among n − 3 diet or deficient groups (n − 3 diet/FPI vs. n − 3 diet/Sham $p > 0.05$; n − 3 def/FPI vs. n − 3 def/Sham, $p > 0.05$).

3.6. Activation of BDNF–TrkB signaling recruits metabolic proteins

Several lines of evidence point to the idea that BDNF is involved in energy metabolism. We utilized the TrkB agonist 7,8-dihydroxyflavone (7,8-DHF) to stimulate TrkB signaling pathway in mouse Neuro-2a (N2a)-neuroblastoma cells. The exposure of 7,8-DHF for 2 h, stimulated the phosphorylation of TrkB and its downstream effector CREB in a dose dependent manner (100–400 nM; $p < 0.05$; Fig. 6A & B). The effect of

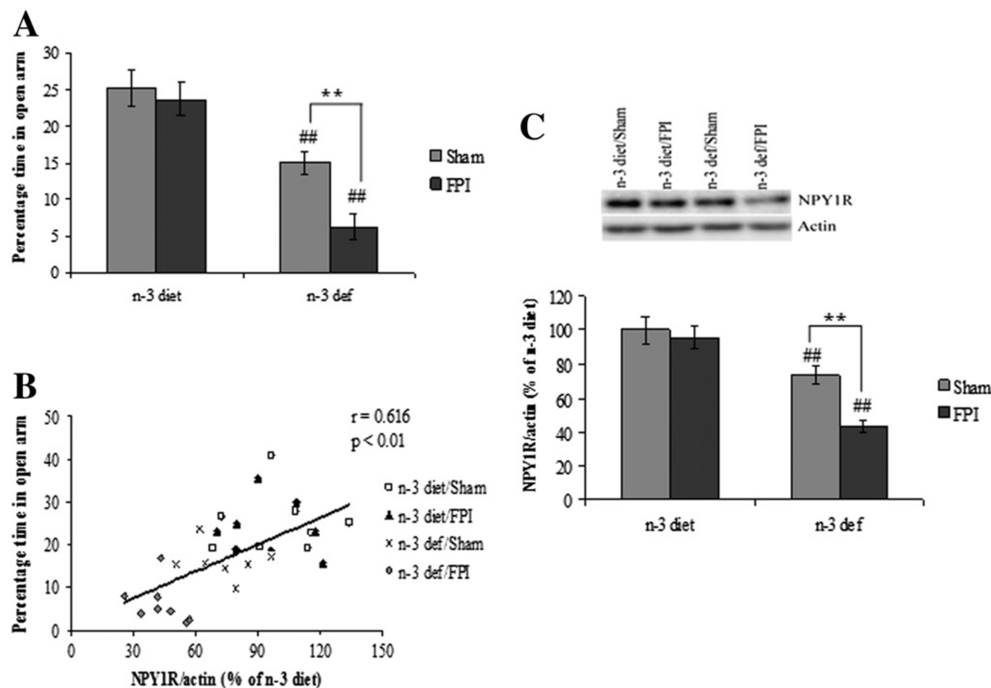

Fig. 4. (A) Percentage of time spent in open arms, (B) correlation analysis of NPY1R with percentage of time spent in open arms and (C) levels of NPY1R in groups fed with either n − 3 fatty acid (n − 3 diet) or n − 3 fatty acid deficient (n − 3 def) diet and subjected to either sham or fluid percussion injury (FPI). Data are expressed as percentage of n − 3 diet group (mean ± SEM). $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ vs. n − 3 diet/Sham, $^{*}p < 0.05$ vs. n-3 def/Sham group; ANOVA (two-way) followed by post-hoc test with Bonferroni's comparisons.

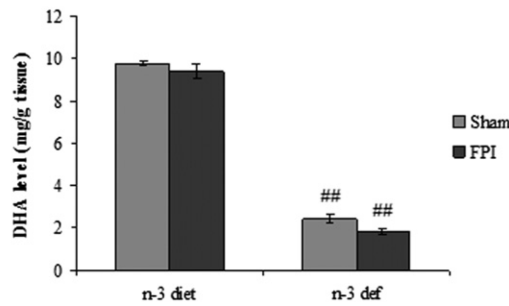

Fig. 5. Levels of docosahexaenoic acid (DHA; C22:6n−3) in groups fed with either n−3 fatty acid diet (n−3 diet) or n−3 fatty acid deficient diet (n−3 def) and subjected to either sham or fluid percussion injury (FPI). Data are expressed as mg/g tissue (mean ± SEM). ##$p < 0.01$ vs. n−3 diet/Sham group; ANOVA (two-way) followed by post-hoc test with Bonferroni's comparisons.

7,8-DHF (200 and 400 nM) was also observed on the levels of PGC-1α, which indicates that the activation of BDNF–TrkB signaling recruits metabolic signals ($p < 0.05$; Fig. 6C). Furthermore, we assessed the phenotypic expression of CREB and PGC-1α by immunocytochemical analysis, and found that they co-localized in the same cells of the N2a cell line. We observed a qualitative increase in the staining intensity of CREB/PGC-1α co-localization after 2 h of 7,8-DHF treatment (200 nM), which suggests that BDNF receptor activation recruits plasticity and metabolic molecules in the same cell (Fig. 6D).

3.7. BDNF–TrkB signaling improves mitochondrial functions

To assess mitochondrial functions, we measured cellular respiration in N2a cells with a XF24 Extracellular Flux Analyzer. Oxygen consumption rate (OCR) was measured in real time under basal condition and in

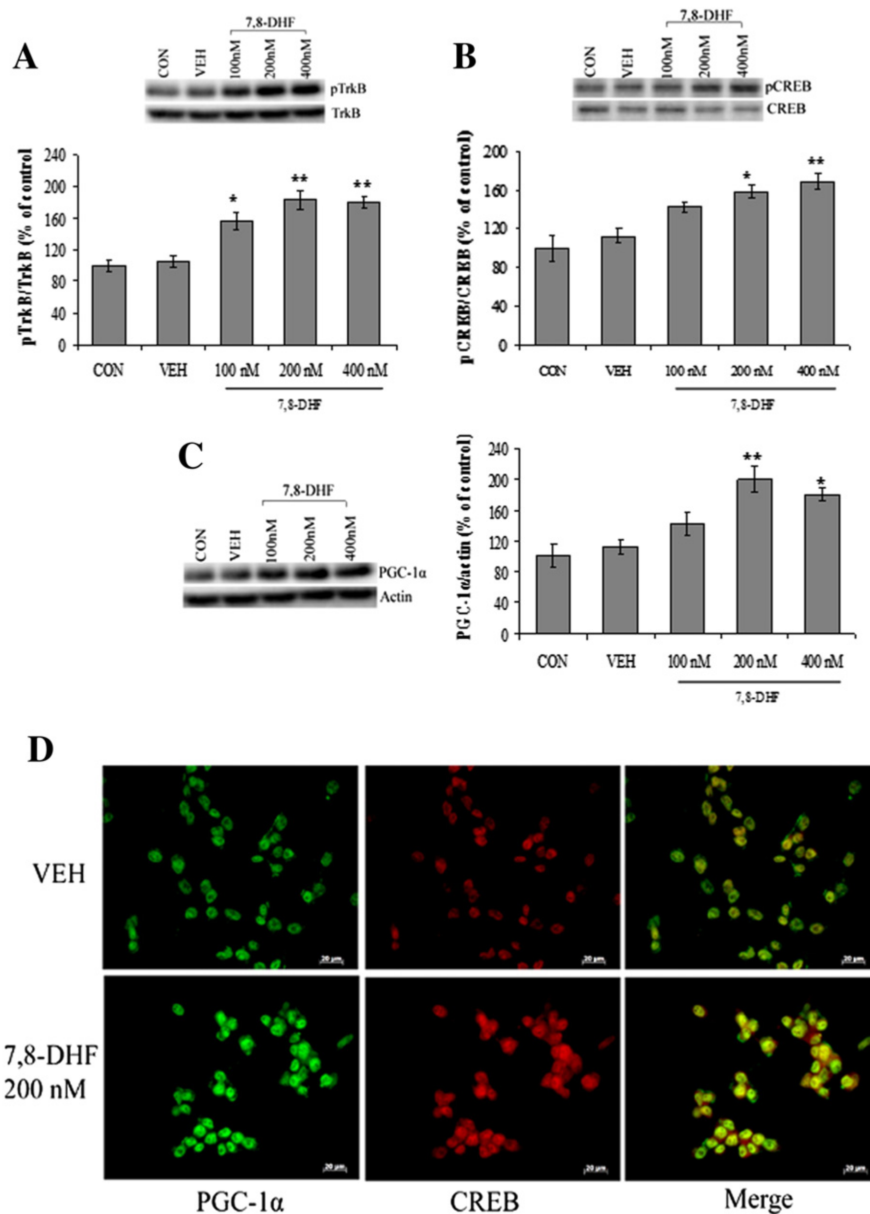

Fig. 6. Phosphorylation of (A) TrkB (B) CREB and (C) levels of PGC-1α in Neuro-2a (N2a)-neuroblastoma cells treated with 100, 200 and 400 nM of 7,8-dihydroxyflavone (7,8-DHF) for 2 h. Data are expressed as percentage of control group (mean ± SEM) of four independent experiments. *$p < 0.05$, **$p < 0.01$ vs. VEH group; ANOVA (one-way) followed by post-hoc test with Bonferroni's comparisons. Immunofluorescence staining of (D) PGC-1α (FITC) and CREB (Cy3) in N2a cells treated with 200 nM of 7,8-DHF for 2 h. Fluorescent immunocytochemical images reveals the co-localization of PGC-1α and CREB in N2a cells (green for PGC-1α, red for CREB and yellow for co-stained cells, scale bar: 20 μm).

response to sequential treatment with respiratory chain inhibitors (Fig. 7A). The ATP synthase inhibitor oligomycin was used to measure ATP production-associated respiration. According to our results, the treatment with 7,8-DHF (200 nM, 4–12 h) was not able to induce changes in ATP-linked respiration ($p > 0.05$; Fig. 7B). Next, the uncoupler FCCP was used to determine the maximal OCR of the cells. The FCCP-induced OCR represents the mitochondrial reserve or spare respiratory capacity of the cells, which is a strong indicator of mitochondrial functions that may not be apparent under basal conditions. In response to FCCP, the treatment of 7,8-DHF (200 nM) for 4 h was not effective to induce any changes in OCR levels (data not shown). However, OCR was higher after 8 and 12 h of 7,8-DHF (200 nM) treatment ($p < 0.05$; Fig. 7B), indicating that the activation of BDNF–TrkB signaling enhances the mitochondrial reserve capacity of cells, thereby improving mitochondrial function.

4. Discussion

We have found that consumption of an n − 3 diet during early life influences the capacity of the adult brain to resist the effects of TBI. These effects were deeply associated with alterations in molecules related to energy homeostasis such as nuclear (PGC-1α, TFAM) and mitochondrial proteins (COII, SOD2) that play a key role on mitochondrial biogenesis, and control of cell oxidative damage. A highlight of the results was the powerful action played by the BDNF receptor TrkB agonist, 7,8-DHF on the regulation of mitochondrial bioenergetics, biogenesis activator PGC-1α and CREB phosphorylation, which emphasize an association between plasticity and control of energy homeostasis. These events can be crucial to maintain neuronal function following TBI, when energy homeostasis is compromised. Our results also indicate that anxiety-like behavior alterations engage elements related to mitochondrial biogenesis. Given the crucial role of metabolism on brain plasticity and function, adequate n − 3 ingestion particularly during early life seems critical for fostering the plasticity necessary to abate the effects of brain insults during adulthood.

4.1. Effects of TBI and diet on molecular systems related to energy management

Clinical and experimental studies have documented the sequel of mild concussion on brain metabolic markers after 1 week of mild TBI [7,23]. We found that TBI, under dietary n − 3 fatty acid deficiency, compromised molecular systems important for the maintenance of energy homeostasis such as AMPK phosphorylation. AMPK is a serine–threonine kinase, which has the ability to sense the AMP/ATP ratio and activate or inhibit the appropriate molecules to re-establish the proper energy balance of the cell. Once activated, AMPK switches on catabolic pathways to produce ATP while simultaneously shutting down energy-consuming anabolic processes. This is achieved, in part, via activation of PGC-1α whose activity is regulated both by its expression level and by post-translational modifications involving AMPK-directed phosphorylation [24] and sirtuin-regulated deacetylation [25]. In our results, the presence of n − 3 appeared to normalize energy homeostasis probably by promoting conservation of ATP as evidenced by no changes in AMPK phosphorylation in n − 3 diet group with or without TBI. The positive correlation between AMPK phosphorylation and PGC-1α levels appears to support the above discussed association between PGC-1α and AMPK functions. The occurrence of TBI under dietary n − 3 deficiency compromised molecular system involved in energy balance as evidenced by a decrease in the activational state of AMPK and the levels of PGC-1α.

We also observed a significant decline in COII levels after TBI with dietary n − 3 fatty acid deficiency, which is likely associated with metabolic dysfunction. COII is a protein subunit of the terminal and highly regulated enzyme complex IV of the electron transport chain in mitochondria [26]. COII plays a key role in controlling ATP production as it helps to establish a transmembrane difference of proton electrochemical potential in the electron transport chain of mitochondria. The presence of n − 3 in diet prevented the changes in the COII level. The results showing that COII levels changed according to the times spent in the open arms of the EPM provides an indication on how mitochondrial activity can influence the anxiety-like behavior, and other neuropsychiatric disorders, as discussed below.

4.2. Mitochondrial homeostasis vs. neuronal plasticity

Mitochondria are particularly susceptible to damage as they are the main bioenergetic machinery and the source of oxidative stress in cells. Therefore, effective control of mitochondrial biogenesis and turnover becomes critical for the maintenance of energy homeostasis and prevention of oxidative stress. Our results show that diet and TBI influence mitochondrial proteins important for maintaining neuronal plasticity via activation of transcription regulators. In particular, PGC-1α is a co-transcriptional regulator that activates various transcription factors, including nuclear respiratory factors (NRFs). In turn, the NRFs activate the mitochondrial transcription factor TFAM, which stimulates mtDNA transcription. PGC-1α is a potent coactivator of a plethora of transcription factors that influence whole-body energy expenditure, and is an important mediator of several factors involved in mitochondrial homeostasis such as AMPK. Therefore, the fact that TBI decreases PGC-1α under n − 3 fatty acid deficiency implies that the lack of n − 3 predisposes the brain to the effects of TBI. The results showing that TBI increased the level of TFAM only under dietary n − 3 condition, emphasizes the role of n − 3 fatty acids to maintain a homeostatic balance

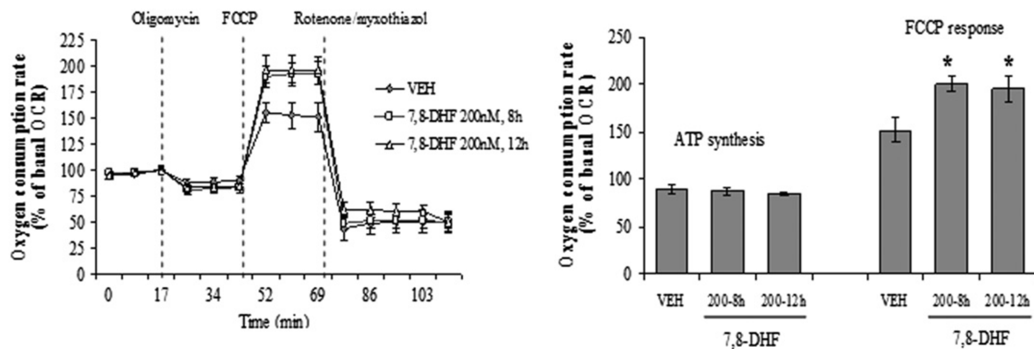

Fig. 7. Mitochondrial bioenergetics analysis in Neuro-2a (N2a)-neuroblastoma cells treated with 200 nM of 7,8-dihydroxyflavone (7,8-DHF) for 8 and 12 h. (A) Oxygen consumption rate (OCR) was measured with an XF24 Extracellular Flux Analyzer before and after the sequential injection of oligomycin, FCCP and rotenone/myxothiazol. (B) Respiration due to ATP production was calculated as response to the oligomycin, while maximal mitochondrial respiratory capacity was deduced from the response to treatment with FCCP. Data are expressed as percentage of basal OCR (mean ± SEM) of six independent experiments. *$p < 0.05$ vs. VEH group; ANOVA (one-way) followed by post-hoc test with Bonferroni's comparisons.

during energy crisis. Several studies reveal that the actions of PGC-1α are also related to control of oxidative stress [27].

Overproduction of reactive oxygen species (ROS), a byproduct of mitochondrial electron transport chain, has been implicated in acute brain injuries such as ischemia [19]. PGC-1α is a master regulator of scavenging enzymes including SOD2 and may contribute to cell survival. Our results showing that pre-exposure of n − 3 fatty acid helped to counteract the decreasing effect of TBI on SOD2 emphasizes the homeostatic role of n − 3 fatty acids in supporting antioxidant systems. An increase in 4HNE levels after TBI with n − 3 deficient diet, suggests that the deficiency of n − 3 may predispose the brain towards lipid peroxidation under challenging conditions. It is noteworthy that polyunsaturated fatty acids (PUFAs) are highly susceptible to oxidative damage such that lipid peroxidation could contribute to lower levels of n − 3. Our results showing that the n − 3 deficiency was not sufficient to alter 4HNE levels suggests that the n − 3 deficient diet was the main factor for the reduction in n − 3 levels. The negative correlation of 4HNE with anxiety-like behavior suggests that the lipid peroxides (LPOs) may influence the outcome of anxiety-like behavior.

4.3. The intermediate action of BDNF signaling on energy metabolism and plasticity

There are several indications that BDNF works at the interface of metabolism and synaptic plasticity such as by interacting with metabolic molecules [12], influencing mitochondrial energy management efficiency [28], and altering glucose utilization in neurons [29]. To explore a possible association between energy homeostasis and synaptic plasticity and behavior, we assessed the effects of our interventions on BDNF signaling. We found that TBI reduced the levels of BDNF related systems, such as its receptor TrkB, and its downstream effector CREB, only in the n − 3 deficient rats. CREB works under BDNF regulation to modulate synaptic plasticity and anxiety [30]. Our results showing that changes in CREB phosphorylation are in proportion to PGC-1α levels, together with reports that CREB is a potent activator of PGC-1α levels [31], emphasize the double function of CREB as a synaptic activator and mitochondrial biogenesis modulator. In addition, CREB has also been reported to regulate NPY [32] and BDNF [33] suggesting that the circuit BDNF–CREB–NPY may participate in the regulation of anxiety-like behavior. The overall results imply that the n − 3 deficiency may exacerbate the energy crisis occurring after TBI, thereby impacting synaptic plasticity and mental health.

We used the well-established ability of the BDNF receptor agonist 7,8-DHF to stimulate TrkB signaling in cultured cells. According to our results, 7,8-DHF stimulated the actions of key elements in the BDNF signaling cascade such as TrkB and CREB. In turn, the alteration in BDNF signaling had the ability to upregulate PGC-1α, as reflected by the elevated cellular expression of PGC-1α and CREB after 7,8-DHF treatment. CREB is an important molecule in BDNF–TrkB signaling, which converges pathways regulating synaptic activity and anxiety [30]. This implies that the effects of 7,8-DHF on increasing CREB activation may be significant for modulation of synaptic plasticity. BDNF–TrkB signaling acts through CREB to regulate PGC-1α [31], and this interaction is also supported by the results from in vivo experiments showing that levels of PGC-1α change in proportion to levels of CREB activation. The association between CREB and PGC-1α is also reflected at the cellular level as to CREB and PGC-1α immunostaining was co-localized in our in vitro studies. The ability of BDNF to modulate PGC-1α can be essential for the brain operation under the domain of metabolism, as PGC-1α is a master regulator of several important transcription factors in the mitochondria with the potential to affect a wide range of cellular functions. According to our results, the increased levels of PGC-1α after 7,8-DHF treatment indicates that the activation of BDNF–TrkB signaling recruits metabolic modulators. A potential action of BDNF–TrkB signaling on mitochondrial bioenergetics is supported by the results showing that 7,8-DHF increased mitochondrial respiration capacity.

4.4. Metabolic homeostasis and neuropsychiatric disorders

It is suspected that mitochondrial dysfunction predisposes the brain to neuropsychiatric abnormalities such as anxiety and depression [34,35]; however, how metabolic signals affect behavior has remained elusive. Reduced levels of NPY have been reported to be associated with anxiety and depression-like behavior [22]. In turn, NPY has also been related to metabolism as evidenced by its effects on insulin [36], glucose homeostasis [37], and energy balance [38]. Our findings showing that time spent in the open arms varied in proportion to the levels of NPY1R, BDNF, PGC-1α, and in turn PGC-1α varied in proportion to levels of CREB activation, appear to reflect an association between energy homeostasis and behavior. Given the discussed roles of BDNF on metabolism and plasticity, it is noteworthy that BDNF signaling has important implications for the pathophysiology of several neuropsychological disorders such as anxiety [21] and depression [39]. Therefore, our results showing that TBI reduced BDNF and its signaling molecules as well as elevated anxiety-like behavior, evoke the capacity of TBI to increase vulnerability to psychiatric disorders.

4.5. Metabolic programming as a modulator of adult brain plasticity

The concept of “Metabolic Programming” refers to an adaptive process whereby nutrition and other environmental factors during early life can impact adult health by altering elements related to control of cell metabolism. In compliance with this concept, our results provide an indication for the involvement of critical molecules that regulate energy metabolism on mechanisms that control brain plasticity. In addition, the results showing that the detrimental effects of TBI were observed only under n − 3 deficiency emphasize the power of n − 3 to protect the brain against challenges during adulthood. It is noteworthy that fatty acids such as n − 6 and n − 9 may influence the functions of n − 3 fatty acids [40,41]. In spite of the beneficial actions of n − 3 fatty acids, studies suggest that the n − 3 supplementation during pregnancy is not effective to lower the postpartum depression levels in mothers and cognitive improvement in their children [42,43]. Therefore, further studies are required to establish how diet provided on specific periods of brain development can affect adult brain plasticity.

In summary, our results show that TBI disturbs pathways associated with energy homeostasis and synaptic plasticity. Long-term dietary n − 3 influences the capacity of the brain to resist the effects of TBI incurred during adult life. This action of n − 3 was associated with key molecular events of mitochondrial bioenergetics, and synaptic plasticity in the brain, and had implications for anxiety-like behavior (Fig. 8). Further studies are required to determine the involvement of energy metabolites, which could provide a more direct index of energy metabolism. In addition, the results of the BDNF receptor activation indicate a potential mechanism by which synaptic plasticity is regulated in conjunction with mitochondrial function. The overall results provide insight to understand how metabolic and plasticity signals interact with each other, and how the product of this interaction influences long-term neuronal resilience against neurological and psychiatric disorders.

Disclosure/conflict of interest

There is no conflict of interest for any of the contributing authors.

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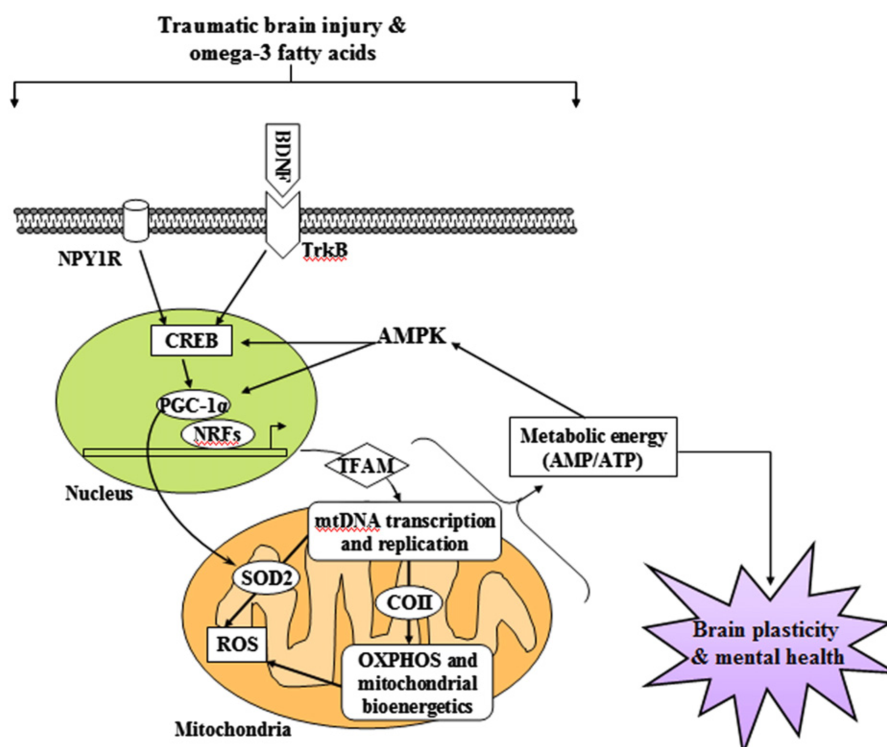

Fig. 8. Proposed mechanism by which traumatic brain injury (TBI) leads to disruption of brain energy metabolism with subsequent effects on cognitive performance. Dietary n − 3 fatty acid deficiency during early life may act as a vulnerability factor for the outcome of TBI. TBI and dietary n − 3 fatty acid alter NPY1 and TrkB receptor signaling, thereby affecting the co-transcriptional regulator PGC-1α via CREB. BDNF–TrkB signaling plays a pivotal action on mitochondrial bioenergetics through metabolic activators such as PGC-1α. PGC-1α activates important transcription factors, including nuclear respiratory factors (NRFs). In turn, NRFs activate the mitochondrial transcription factor A (TFAM), responsible for stimulating mitochondrial DNA (mtDNA) transcription, and mitochondrial proliferation. Trancription of mitochondrial genes leads to the synthesis of mitochondrial proteins including cytochrome c oxidase (COII) and superoxide dismutase (SOD2). COII regulates oxidative phosphorylation (OXPHOS) responsible for ATP production in mitochondria. Reactive oxygen species (ROS), a byproduct of mitochondrial electron transport chain, stimulates the activation of PGC-1α signaling pathway which in turn upregulates the mitochondrial antioxidant protein SOD2. Adenosine monophosphate-activated protein kinase (AMPK) helps to maintain the AMPK/ATP ratio, which is achieved, in part via activation of PGC-1α. These alterations in energy homeostasis are important for maintaining neuronal functions and synaptic plasticity via CREB. The interplay between energy management and plasticity systems appears crucial for the maintenance of neuronal function and neurological health.

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