Life Sciences 314 (2023) 121316

Contents lists available at ScienceDirect

Life Sciences

journal homepage: www.elsevier.com/locate/lifescie

High fat diet exacerbates long-term metabolic, neuropathological, and behavioral derangements in an experimental mouse model of traumatic brain injury

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ARTICLE INFO

Keywords:
Traumatic brain injury
High-fat diet
Metabolic syndrome
Neuroinflammation
Neurodegeneration
Oxidative stress

ABSTRACT

Aims: Traumatic brain injury (TBI) constitutes a serious public health concern. Although TBI targets the brain, it can exert several systemic effects which can worsen the complications observed in TBI subjects. Currently, there is no FDA-approved therapy available for its treatment. Thus, there has been an increasing need to understand other factors that could modulate TBI outcomes. Among the factors involved are diet and lifestyle. High-fat diets (HFD), rich in saturated fat, have been associated with adverse effects on brain health.

Main methods: To study this phenomenon, an experimental mouse model of open head injury, induced by the controlled cortical impact was used along with high-fat feeding to evaluate the impact of HFD on brain injury outcomes. Mice were fed HFD for a period of two months where several neurological, behavioral, and molecular outcomes were assessed to investigate the impact on chronic consequences of the injury 30 days post-TBI.

Key findings: Two months of HFD feeding, together with TBI, led to a notable metabolic, neurological, and behavioral impairment. HFD was associated with increased blood glucose and fat-to-lean ratio. Spatial learning and memory, as well as motor coordination, were all significantly impaired. Notably, HFD aggravated neuroinflammation, oxidative stress, and neurodegeneration. Also, cell proliferation post-TBI was repressed by HFD, which was accompanied by an increased lesion volume.

Significance: Our research indicated that chronic HFD feeding can worsen functional outcomes, predispose to neurodegeneration, and decrease brain recovery post-TBI. This sheds light on the clinical impact of HFD on TBI pathophysiology and rehabilitation as well.

1. Introduction

Traumatic brain injury (TBI) is a serious public health concern that remains one of the leading causes of death across all ages [1,2]. TBI is a disruption of the normal function of the brain that is caused by either penetrating or non-penetrating head impact [3]. The complications

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https://doi.org/10.1016/j.lfs.2022.121316
Received 20 October 2022; Received in revised form 13 December 2022; Accepted 19 December 2022
Available online 22 December 2022

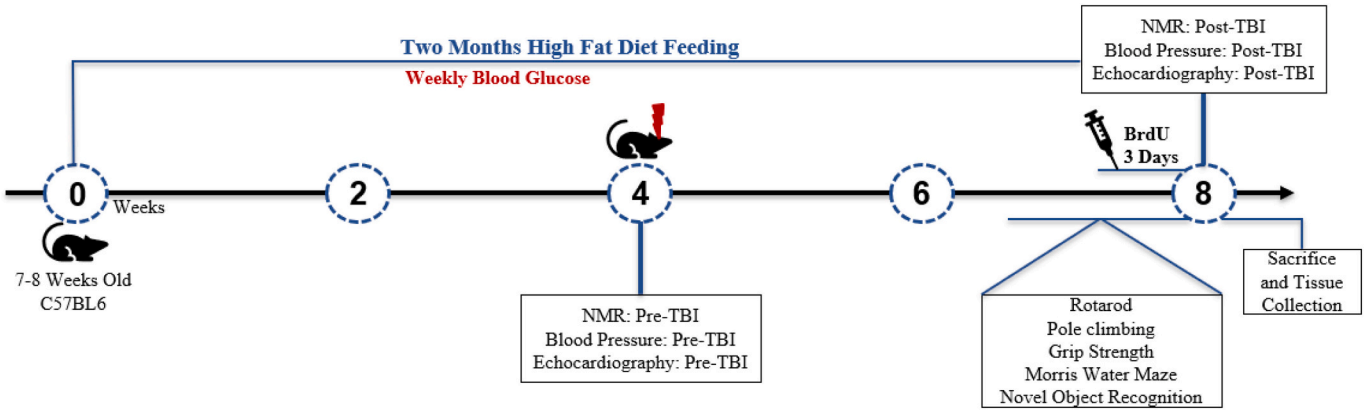

Fig. 1. Experimental timeline. Seven to eight-week-old C57BL6 mice were randomly allocated to 8 weeks of NC or HFD feeding. Calorie intake was calculated for the first four weeks. Mice were further subdivided into groups that underwent either a sham operation or a CCI to induce TBI ($n = 11$ mice/group). Bodyweight, fat composition, and cardiovascular parameters were assessed before the operation and 4 weeks after (week 8). Blood glucose levels were measured weekly from week 1 to week 8. Behavioral/neurological tests were conducted before sacrifice (week 8). Four days before sacrifice, the mice were injected with BrdU intraperitoneally for three consecutive days and sacrificed 24 h after the last BrdU injection.

arising from TBI are heterogeneous, as a single event of brain injury could alter diverse behavioral, cognitive, cellular, and molecular processes in the body [4]. Each year, over 50 million cases of TBI are reported worldwide, with most cases occurring in low- and middle-income countries [5]. In the USA alone, TBI accounts for over 2.5 million hospitalizations [6]. This is an under-representation of the actual number of TBI cases in the United States, as it does not account for several TBI victims who did not get adequate medical attention. Also, several complications from TBI do not become apparent immediately after the injury; this leads to TBI being under-reported; hence, making it a "silent epidemic" [6–8]. Of interest, the Middle East and North Africa (MENA) region suffers from political unrest precipitating conflict and war operations which contribute to the prevalence of TBI [9,10]. Indeed, despite the limited population-based research on TBI in the Middle East, a systematic review estimates a median incidence of about 45 in 10,000 individuals with around 10 % emergency-based mortality [10].

While the effect of the primary injury is often visible following the initial impact, the extent and severity of the secondary injury is an important indication of how critical a TBI is [11]. Also, because TBI has the potential to modulate the function of the autonomic nervous system, it can induce alterations in several organ functions [12]. Several studies have reported the development of cardiovascular complications post-TBI [13–16]. For instance, one study reported that 13 % of TBI patients developed cardiac dysfunction, with a predisposition to diabetes mellitus post-TBI [13]. While there are no currently FDA-approved drugs for brain injury treatment [17], diet and lifestyle have been postulated to be among the main key factors that can significantly impact the brain, and can potentially modulate TBI outcomes [18,19].

The relationship between the quality of diet and brain function has been poorly understood. In recent years, crosstalk between TBI and nutritional status has received heightened attention. This has revealed that a healthy diet leads to improved cognitive functions [20] while poor nutrition is a risk factor in several neurological disorders [21]. Importantly, studies have shown that diets rich in saturated fat, otherwise known as high-fat diets (HFD), are a significant risk factor for metabolic syndrome, and can cause adverse effects on the brain, leading to impaired cognitive function [22,23]. Besides being an important risk factor for cardiovascular diseases, metabolic syndrome (MeS) has been associated with the development of cerebrovascular deficits and neurodegenerative disorders [24,25]. Particularly, chronic consumption of HFD has been shown to induce cognitive and behavioral derangements [26]. Interestingly, the processes contributing to such impairment are similar to the ones culminating in secondary injury in TBI, namely oxidative stress, neuroinflammation, as well as disruptions of the blood-brain barrier (BBB) and autophagic processes [18,27]. This supports the postulation that HFD-induced MeS could exacerbate the secondary injury associated with TBI.

A better understanding of the mechanisms by which oxidative and neuroinflammatory processes impact cognitive and neuronal functions is still required and could eventually provide windows for intervention. Previous research has focused on studying the impact of dietary interventions on secondary injury and recovery post-TBI rendering "diet" as a potential target for neurotherapy [28–30].

However, there is a paucity of studies investigating the long-term consequences of the metabolic syndrome induced by HFD on TBI. It is unclear how the different metabolic insults affect cerebral and cardiovascular functions. For instance, obesity, a hallmark of metabolic syndrome, was found to be associated with more severe complications after a frontal collision [31]. In this study, we sought to determine the impact of a chronic HFD on the long-term consequences of TBI. C57BL6 mice were fed HFD for two months where the neurological and behavioral outcomes post-TBI were investigated. Our data linked chronic HFD consumption to exacerbated secondary consequences of TBI, aggravating neurological deficits and exacerbating neuropathological molecular pathways involved in neuroinflammation, neurodegeneration, and oxidative stress.

2. Materials and methods

2.1. Sample size calculation and experimental groups

All animal procedures were carried out in compliance with the guidelines of the Institutional Animal Care and Use Committee (IACUC Approval number: 21-09-589) at the American University of Beirut-Medical Center (AUB-MC). For this study, male C57BL6 mice, 6 to 8 weeks old, were obtained from the Animal Care Facility (ACF) at the AUB. Mice were housed in a temperature-controlled room, with a 12-hour light-dark cycle, with continuous access to food and water. Among the cognitive and motor tests we set out to conduct, the Morris water maze (MWM) behavioral test is the most prone to variability. Therefore, the optimal sample size for our studies was determined based on the usual data obtained from this test [32]. We estimated that at least 10 mice are required per study group in order to detect a difference with a 95 % confidence level and achieve a power of 90 %. A total number of forty-four mice were used for this study. The mice were randomly allocated into four groups based on the diet regimen [normal chow (NC) or high-fat diet (HFD)] and type of operation (sham *vs.* TBI): NC-sham, NC-TBI, HFD-sham, and HFD-TBI, with a total of $n = 11$ per group. As

Table 1
Primer sequences used for RT-qPCR.

Gene	Forward Primer (5′-3′)	Reverse Primer (3′-5′)
mCAT	TGAGAAGCCTAAGAACGCAATTC	CCCTTCGCAGCCATGTG
mSOD2	GGCCAAGGAGATGTTACAA	GAACCTTGGGACTCCCACA
mNrf2	CGAGATATACGCAGGAGAGGTAAGA	CGAGATATACGCAGGAGAGGTAAGA
mGAPDH	AGGTCATCCCAGAGCTGAA	CTGCTTCACCACCTTCTTGA

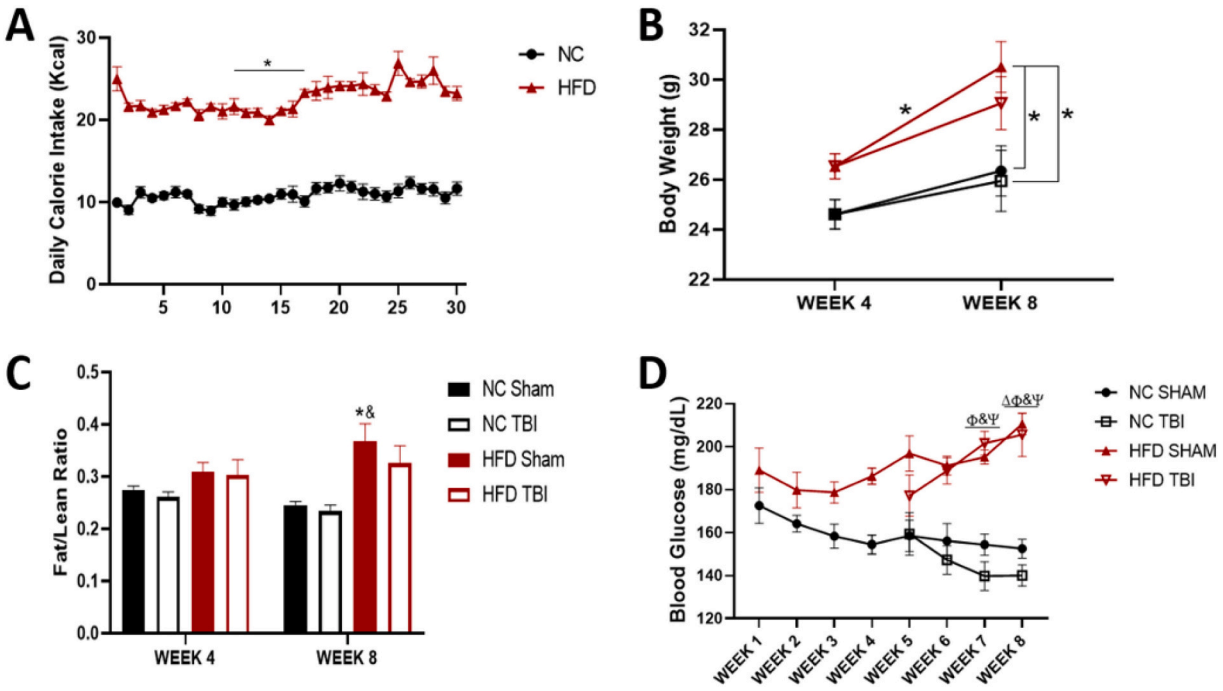

Fig. 2. Impact of HFD feeding and TBI on metabolic parameters. *A:* Daily caloric intake of NC and HFD-fed mice in the 28 days pre-operation, *B:* body weight change from pre- to post-operation (weeks 4 and 8, respectively), *C:* fat to lean ratio at pre- and post-operation (weeks 4 and 8, respectively), and *D:* changes in blood glucose levels throughout the experimental period (week 1 through 8) in NC Sham, NC TBI, HFD Sham, and HFD TBI mice ($n = 8$ male mice/group for all metabolic parameters). Data are presented as mean±SEM. Statistical significance was determined using two-way ANOVA for 2A and three-way ANOVA (with the following factors, 1: diet, 2: operation, & 3: time) for 2B, 2C, and 2D. * denotes $P < 0.05$ as indicated in 2A and 2B, while in figure 2C, * denotes $P < 0.05$ for HFD Sham vs NC fed groups. Δ denotes $P < 0.05$ for the effect of diet in sham-operated mice (NC Sham *vs.* HFD Sham), Ψ for the effect of diet on TBI mice (NC TBI *vs.* HFD TBI), Φ for NC Sham *vs.* HFD TBI, and & for HFD Sham *vs.* NC TBI.

shown in Fig. 1, Four weeks into the feeding, mice in the TBI groups were subjected to an open-head TBI, using the controlled cortical impact (CCI) device (described below). Meanwhile, the body weight changes of the mice were recorded before TBI (week 4) and after TBI (week 8). Following sacrifice, three animals per group ($n = 3$) were used to perform immunofluorescence, three animals (n = 3) per group for immunoblotting, and three animals per group (n = 3) for RT-qPCR. Molecular testing and immunohistochemistry were blinded to the researchers conducting the assessment.

2.2. Diet formulation

The HFD used in this study was prepared in-house to provide 4.8 kcal/g, of which 60 % is from fat [33,34]. The diet was prepared from the normal mice chow (70 % by weight) and fat (30 % by weight). NC diet was purchased from Teklad Rodent Diets (Madison, WI) and provides 3 kcal/g. The mice were fed for a total period of two months (NC or HFD), while their daily caloric intake was recorded for 4 weeks before TBI.

2.3. Surgical procedure and controlled cortical impact (CCI) model

Mice were first anesthetized by an intraperitoneal (IP) injection of a combination of ketamine and xylazine (50 mg/kg ketamine and 15 mg/kg xylazine). Following anesthesia, a midline skin incision was made using a blade, to expose the skull, and Xailin gel ointment (Xailin®, Nicox, France) was applied to the eyes to prevent them from damage during surgery. A craniectomy was first performed using a drill, after which an open-head TBI was induced using the Leica Impact One Angle with Leica Angle Two™ Stereotaxic Instrument (Biosystems, Buffalo Grove, IL, USA). The center of the impactor was placed above the somatosensory area of the parietal cortex of the brain using the following coordinates: +1.0 mm AP, +1.5 mm ML, and − 2 mm DV. The duration of impact was kept constant with a dwell time of 1 s, at a velocity of 4 m/s. The depth of the injury was set to 1.5 mm. The tip of the impactor was 1 mm in diameter. The size of the bone flap was 1.7 mm in diameter, which was carefully removed using a manual trephine to expose the dura matter without damaging it. After inducing the injury, the animal was removed from the frame, and placed on a heating pad. Mice in the sham group underwent the same procedure, except for the impact [35].

2.4. Nuclear magnetic resonance and blood glucose measurement

LF10 Minispec Nuclear Magnetic Resonance (NMR) machine (Bruker, MA, USA) was used to assess the mouse body composition, particularly the fat-to-lean ratio, at weeks 4 and 8, as previously

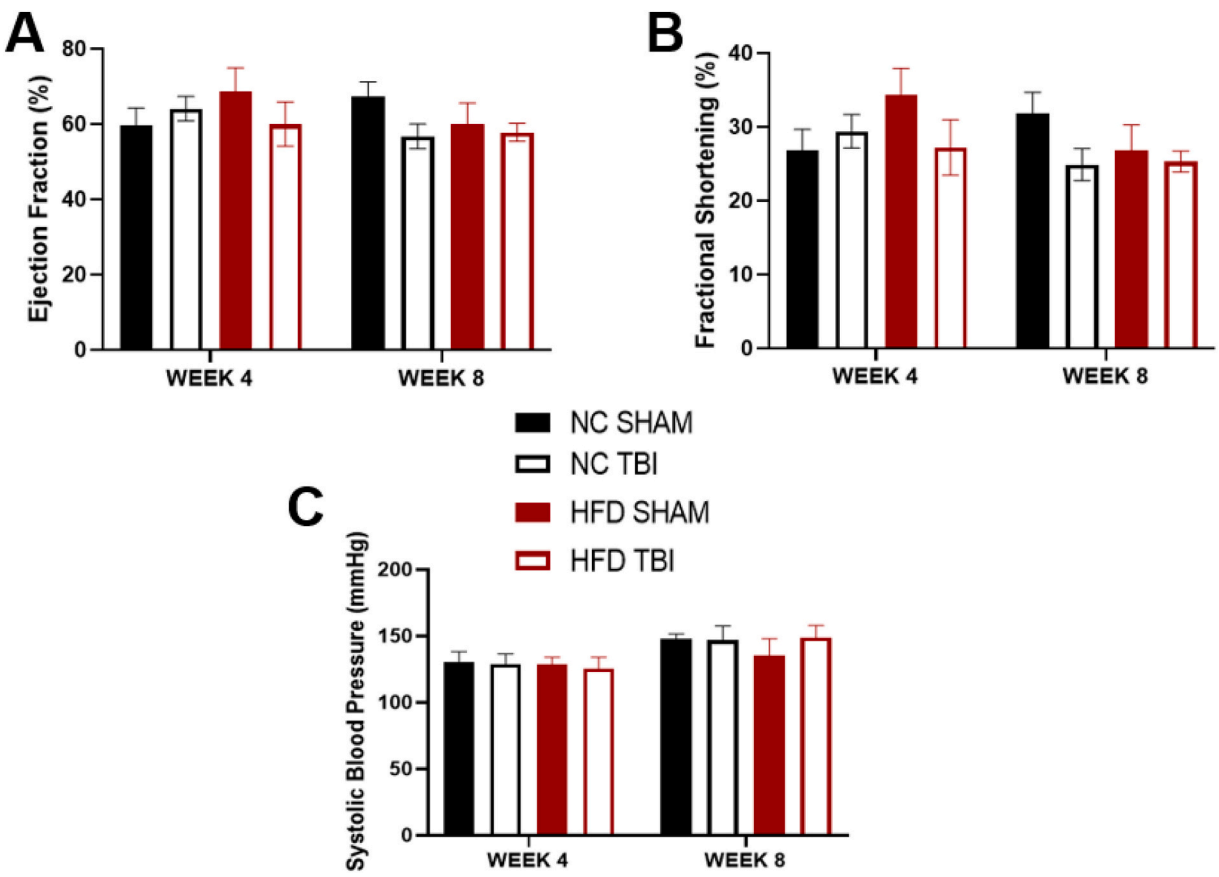

Fig. 3. Impact of HFD feeding and TBI on cardiovascular function. *A:* Ejection fraction of the control and experimental groups (NC Sham, NC TBI, HFD Sham, and HFD TBI), *B:* fractional shortening, and *C:* systolic blood pressure assessed pre-and post-operation (weeks 4 and 8, respectively) ($n = 7$ male mice/group for all cardiovascular parameters). Data are presented as mean±SEM. Statistical analysis was assessed using three-way ANOVA (factor 1: diet, 2: operation, & 3: time).

described [36–38]. NMR analysis returns masses of fat, lean, and free fluid. Accu-chek Performa glucometer (Roche Diagnostics, Basel, Switzerland) was used to measure random blood glucose levels by lateral tail vein puncture every week (weeks 1 to 8) [25].

2.5. Echocardiography and non-invasive blood pressure measurement

At two different time points (week 4, pre-TBI, and week 8, post-TBI), the blood pressure of the mice was measured noninvasively, using the CODA Tail-cuff High Throughput Monitor (Kent Scientific, Torrington, CT), as previously described [39]. Also, the cardiac structure and function were assessed by echocardiography along the parasternal long axis M and B modes performed on weeks 4 and 8, using SonixTouch Q+ ultrasound machine (BK ultrasound, Peabody, MA) [40].

2.6. Morris water maze (MWM)

The Morris water maze (MWM) test was conducted to examine spatial learning and memory as discussed previously [35,41]. In brief, mice were trained for five days in a row, beginning on day 24 after TBI and continuing for four days (learning trials), followed by a probe trial on day 5 (memory trial). The water maze pool was filled with water and maintained at room temperature (25–26°*C*). The water was rendered opaque by adding non-toxic white paint to it. The pool was divided into four quadrants, with an equal surface area each. An escape platform was placed in the northeast (NE) quadrant. Three cues were placed on the wall around the water maze pool and were kept consistent throughout the experiment. Three trials were performed per day during the learning phase. On the first trial, a flag was mounted on the escape platform to help guide the animals to the platform. This flag was removed in subsequent trials. During these trials, the time it took for the mice to reach the platform was recorded. All recordings were made using a video camera connected to the ANY-maze software (Stoelting Co., Wood Dale, Illinois, USA). On the fifth day, the platform was removed, and the mice were subjected to one trial for one minute. The time it took the mice to locate the target quadrant (NE), and the time they spent on this quadrant were both recorded.

2.7. Novel object recognition

Novel object recognition (NOR) was carried out according to the protocol described by Leger et al. [42]. The test consisted of 2 trials, 5 min each, with a 15-min inter-trial interval. Two identical objects were put equidistantly on the right and left sides of the box maze during the first trial (learning phase). The familiar object to the left was changed with a novel object during the second session (discrimination trial). Between trials, the maze and artifacts were cleaned up with 70 % ethanol to remove any lingering odors. The time the mice spent investigating the various objects was used to calculate the discrimination index (DI). DI was determined by calculating the difference in the exploration time of the novel and familiar objects, divided by the total exploration time of both objects [43].

2.8. Rotarod test

The rotarod machine (UgoBasile, 47750 – Rat Rota-Rod NG, Comerio, Italy) was used to assess motor coordination and balance [44], by comparing the time it takes the mouse to fall off the rotating rod and the average velocity of the rotarod across the groups. To perform this experiment, mice were placed on a horizontal rod that continually

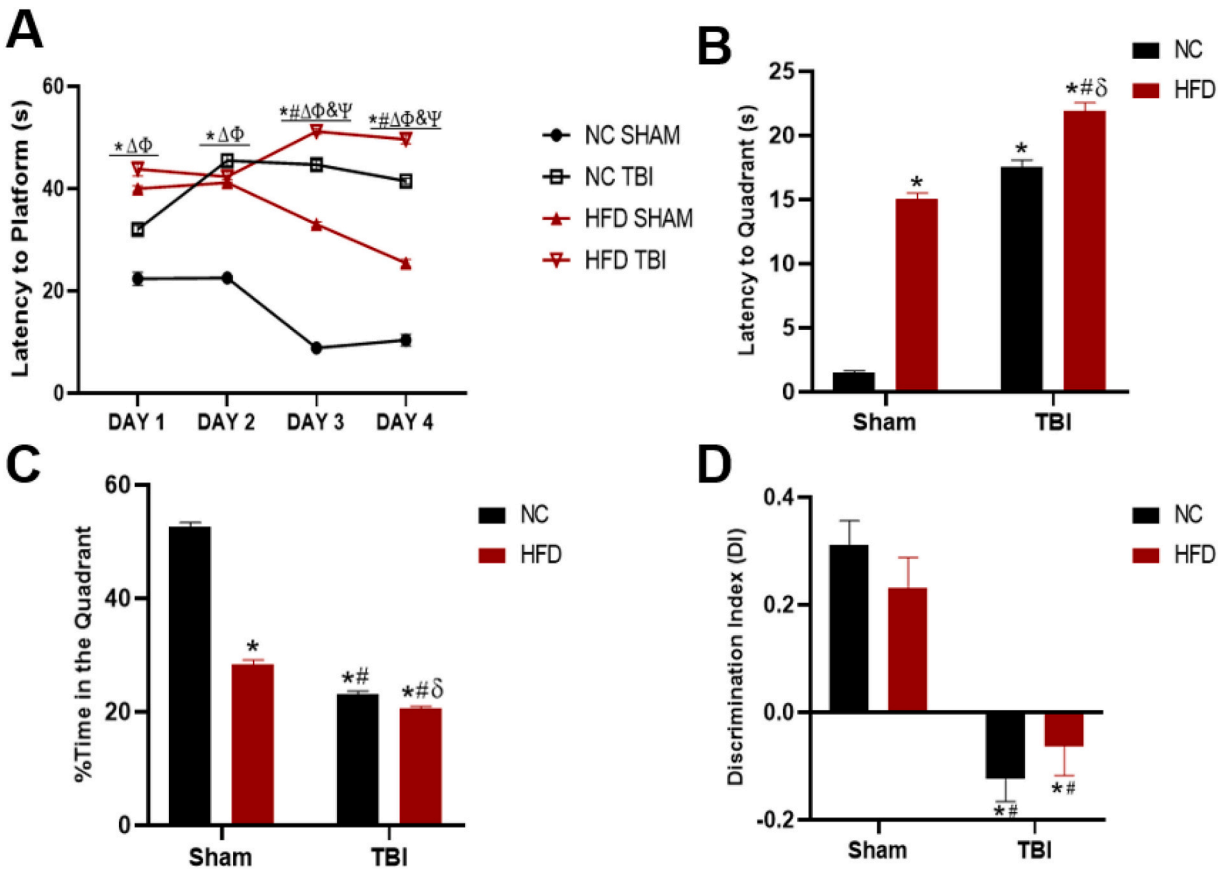

Fig. 4. Effect of diet and/or TBI on long-term neurocognitive function. *A-C:* Morris Water Maze test conducted at week 8 ($n = 8$ male mice/group for A, B, & C). *A:* Latency to escape platform recorded on four consecutive, learning days (spatial learning), *B:* latency to quadrant recorded on day five, when the platform had been removed, reflecting the ability of the mice to remember the quadrant that housed the platform (spatial memory), and *C:* percentage time spent in the quadrant, also evaluated on day five, representing the time each animal spent in the quadrant searching for the escape platform. *D:* Discrimination index derived from the novel object recognition test performed on week 8 (n = 8 male mice/group). Data are presented as mean±SEM. Statistical significance was determined using two-way ANOVA followed by Sidak's multiple comparisons or three-way ANOVA in 3A (with the following factors, 1: diet, 2: injury, 3: time). $P < 0.05$ was denoted by the following symbols in Fig. 3A: * for the effect of TBI in NC-fed mice (NC Sham *vs.* NC TBI), # for the effect of TBI in HFD-fed mice (HFD Sham *vs.* HFD TBI), Δ for the effect of diet in sham-operated mice (NC Sham *vs.* HFD Sham), Ψ for the effect of diet on TBI mice (NC TBI *vs.* HFD TBI), Φ for NC Sham *vs.* HFD TBI, and & for HFD Sham *vs.* NC TBI. In all other figures, * denotes $P < 0.05$ *vs.* NC Sham, # *vs.* HFD Sham, and δ *vs.* NC TBI.

rotates around its long axis (4–40 rpm over 5 min). Animals were placed on the rod one day before the testing day to habituate them to the apparatus.

2.9. Pole climbing

A pole climbing test was carried out to evaluate motor coordination [45,46]. The test apparatus consists of a pole that is 60 cm long, with a diameter of 1 cm. The pole was wrapped with tape to facilitate animal grip and was set perpendicular to an empty box. Animals were first habituated on the apparatus by conducting three trials before the actual experiment. The mice were then placed at the top of the pole, with their heads facing upwards, and were allowed to descend the pole freely. Three trials were performed for each animal. The time needed for each animal to get to the bottom of the pole was recorded.

2.10. Grip strength test

The grip strength test was used to assess neuromuscular function by recording the maximal muscle strength of the forelimbs using the 47,200-grip strength meter (UGO BASILE, Gemonio, Italy). To measure neuromuscular strength, the mouse was allowed to firmly grasp the grid component of the test apparatus and was then pulled back gently, until it releases the grid. For each mouse, three trials were recorded. All grip strength values obtained in "gf" were averaged and normalized against mouse body weight [47].

2.11. 5-Bromo-2′deoxyuridine (BrdU) Injection

Four days before sacrifice, mice were injected with 5-Bromo-2'deoxyuridine (BrdU, 100 mg/kg, Roche Diagnostics GmbH, Mannheim, Germany). Each mouse received a single dose of BrdU injection for three consecutive days as shown in the timeline (see Fig. 1), to investigate cell proliferation. The mice were sacrificed 24 h after the last BrdU injection.

2.12. Brain tissue preparation

All mice were sacrificed 30 days after the TBI. Mice from different groups were alternated among three preparatory tracks after sacrifice. For Western blot and RT-PCR, mice were sacrificed by cervical dislocation, and brains were isolated, dissected, and flash-frozen in liquid nitrogen, after which, they were stored at −80°C. For molecular staining, the mice were sacrificed by perfusion using phosphate-buffered saline (PBS) and fixed with 4 % paraformaldehyde (PFA), after which the tissues were placed in 30 % sucrose overnight, before being cut with a microtome (Leica Microsystems, USA), with each slice having a thickness of 40 μm. The third track was a subset of the second where

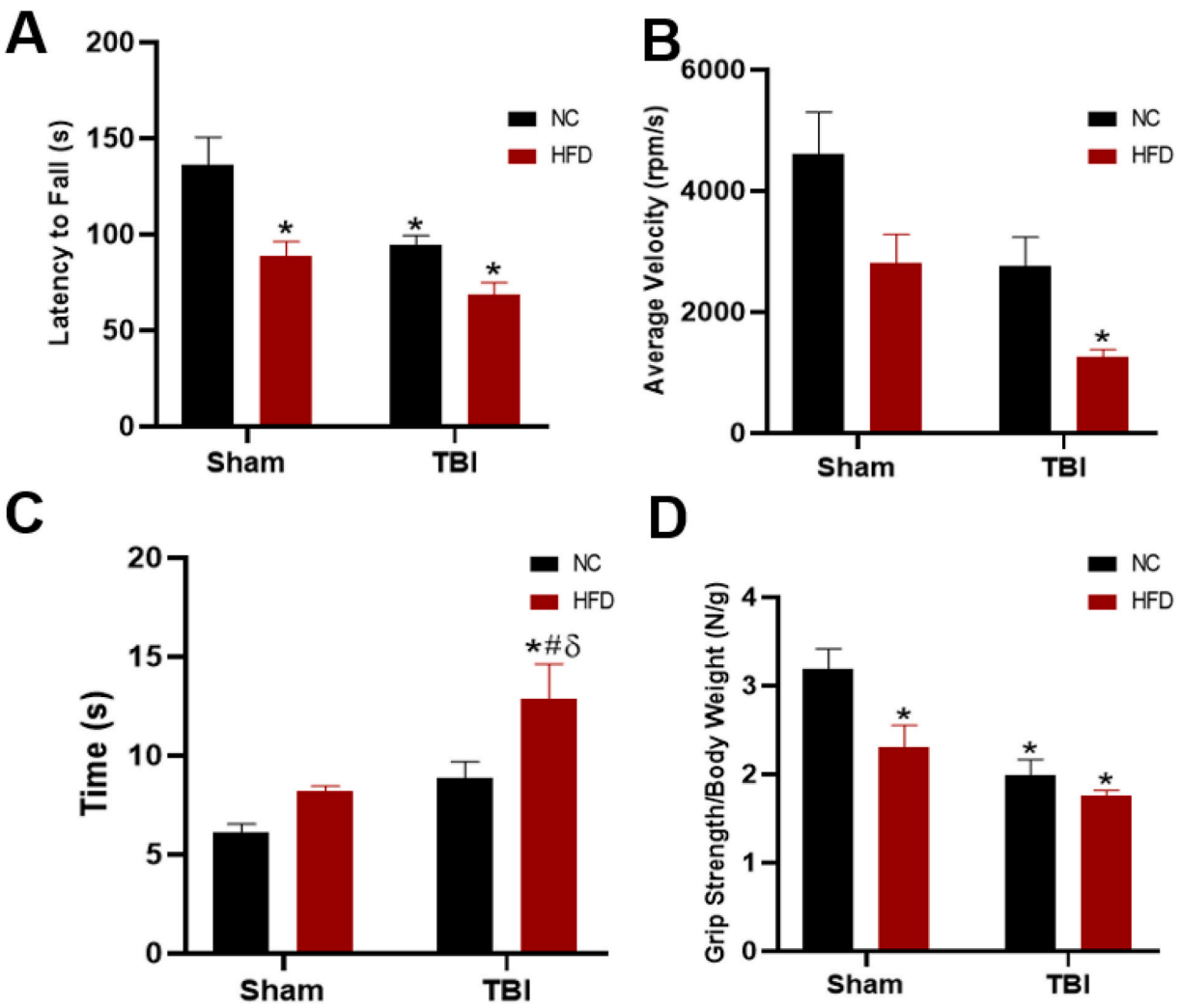

Fig. 5. Effect of diet and/or TBI on long-term motor coordination and neuromuscular strength. *A&B:* Rotarod test performed at week 8 (n = 8 male mice/group). *A:* Latency to fall from the rotarod and *B:* average velocity of the rotarod at the time of fall. *C:* The time it took for each mouse group to get to the bottom of a pole climbing apparatus (n = 8 male mice/group). *D:* The normalized neuromuscular strength of each group, was assessed using the grip strength test conducted at week 8 (n = 8 male mice/group). Data are presented as mean±SEM. Statistical significance was determined using two-way ANOVA (with the following factors, 1: diet, 2: injury), followed by Sidak's multiple comparisons. * denotes $P < 0.05$ *vs.* NC Sham, # *vs.* HFD Sham, and δ *vs.* NC TBI.

mice had been pre-treated with BrdU as described above.

2.13. Lesion volume

2.13.1. Hematoxylin and eosin (H&E) staining

Free-floating coronal brain sections (40 μm) were first mounted on microscope slides and then dipped in cold fixative (4 % PFA in PBS) for 10 min. Slides were then rinsed in a gentle stream of tap water for 1 min. To stain nuclear components, hematoxylin (Sigma-Aldrich, Hematoxylin Solution, Mayer's) was applied to the sections for 30 s. Another gentle stream of tap water was run over the sections for 1 min, after which the slides were dipped in PBS 1× for 20 s and then washed again with tap water for 1 min. Slides were submerged in 70 % ethanol (30 s) and then 95 % ethanol (30 s). To stain the cytoplasm, alcoholic eosin (Sigma-Aldrich, Eosin Y-solution 0.5 % alcoholic) was applied to the sections for 30 s. Dehydration was then achieved *via* 2 changes of 95 % ethanol (15 s each) and 3 changes of 100 % ethanol (15 s each). Ethanol was cleared through 3 changes of xylene (1 min each). Sections were then cover-slipped and imaged using the uSCOPE MXII Slide Scanner (Microscopes International).

2.13.2. Calculation of lesion volume

Using the NIH ImageJ software, the lesion volume was calculated as a percentage of the total area of the uninjured contralateral hemisphere [48] according to the following equation:

$$\text{Lesion volume} = \frac{\text{contralateral hemisphere} - \text{preserved portion of ipsilateral hemisphere}}{\text{contralateral hemisphere}} \times 100$$

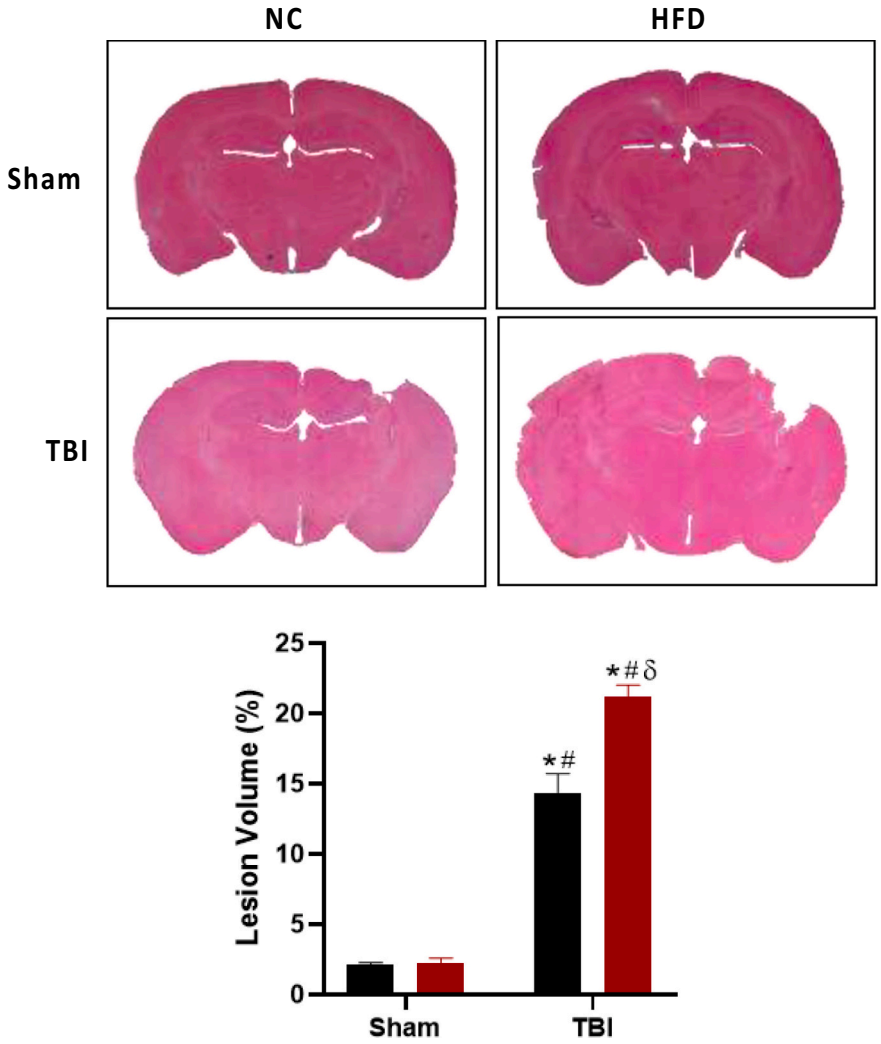

Fig. 6. Effect of chronic HFD feeding on TBI lesion volume. *A:* Representative micrographs of hematoxylin and eosin (H&E) staining on brain sections for the different groups (4×) and *B:* lesion volume calculated as the percentage change in ipsilateral hemisphere volume relative to that of the contralateral hemisphere ($n = 3$ male mice/group). Data are presented as mean±SEM. Statistical significance was determined using two-way ANOVA (with the following factors, 1: diet, 2: injury), followed by Sidak's multiple comparisons. * denotes $P < 0.05$ *vs.* NC Sham, # *vs.* HFD Sham, and δ *vs.* NC TBI.

2.14. Immunofluorescence staining

Immunofluorescence staining was used to assess neuroinflammation and brain injury markers. Free-floating mice brain sections were washed with PBS and PBS-Triton 0.1 % (PBS-T), after which they were incubated with 10 % heat-inactivated fetal bovine serum (FBS), prepared in PBS-T, for 1 h. Thereafter, the brain sections were incubated overnight at 4°*C* with primary antibodies, prepared in 1 % FBS. The following antibodies were used: glial fibrillary acidic protein (GFAP) (Abcam Ab7260; 1/1000) for astrocytes, Ionized calcium-binding adaptor molecule 1 (IBA-1) (Wako 091–19,741; 1/1000) for microglia, Phospho-Tau (Ser202, Thr 205, Invitrogen Mn1020; 1/500) for phosphorylated tau, and Aquaporin 4 (Rainbow, CSB-PA11137A0Rb; 1:200). The next day, the sections were washed with PBS-T, and then incubated with an appropriate fluorochrome-conjugated secondary antibody for 1 h, at room temperature. Samples were washed with PBS-T and PBS, counter-stained with DAPI, and then mounted on a slide.

For the BrdU staining, the sections were incubated with 2N HCl at 37 °C for 30 min, followed by 10 min of incubation in sodium borate (pH 8.5). After this, the sections were washed with PBS and incubated with anti-BrdU (Abcam Ab6326, 1:500) and Fox3/NeuN (1:1000, MCA-IB7, *Encor Biotechnology*, Gainesville, FL, USA; https://encorbio.com), for 24 h. The sections were further incubated with the appropriate fluorophore-conjugated secondary antibodies for 2 h before they were mounted on a slide using a fluoromount (F4680-25ML, Sigma Aldrich). Images were taken using the laser scanning confocal microscope (LSM 710, Carl Zeiss, Oberkochen, Germany).

2.15. Dihydroethidium (DHE) staining

DHE staining was done as previously described by de Montgolfier et al. [49]. Dihydroethidium (DHE) is an oxidative fluorescent dye that undergoes two-electron oxidation to create the DNA-binding fluorophore ethidium bromide. Cell membranes are permeable to DHE. It reacts with reactive oxygen species (ROS) and gets converted to ethidium, which binds to nucleic acid and gives the nucleus a red colour [50]. Slices of brain sections were prepared using the microtome. DHE (10 μ mol/L) was prepared in DMSO and applied to each of the tissue sections. The tissues were incubated with DHE in a light-protected chamber, for 40 mins, at a temperature of 37 °C. Fluorescent images were taken with a Zeiss Axio Observer microscope (Carl Zeiss, Oberkochen, Germany).

2.16. Western blot procedure

Protein expression of autophagy markers was assessed using western blotting. Hippocampal tissues that were stored at −80 °C were crushed under liquid nitrogen and then transferred into a RIPA lysis buffer. The samples were placed on a rocking platform for 90 min at 4 °C, after which the proteins were extracted and quantified using the DC™ Protein Assay Kit from Bio-Rad. Equal concentrations of extracted proteins (1 μg/μl) across the groups were used for SDS-PAGE followed by

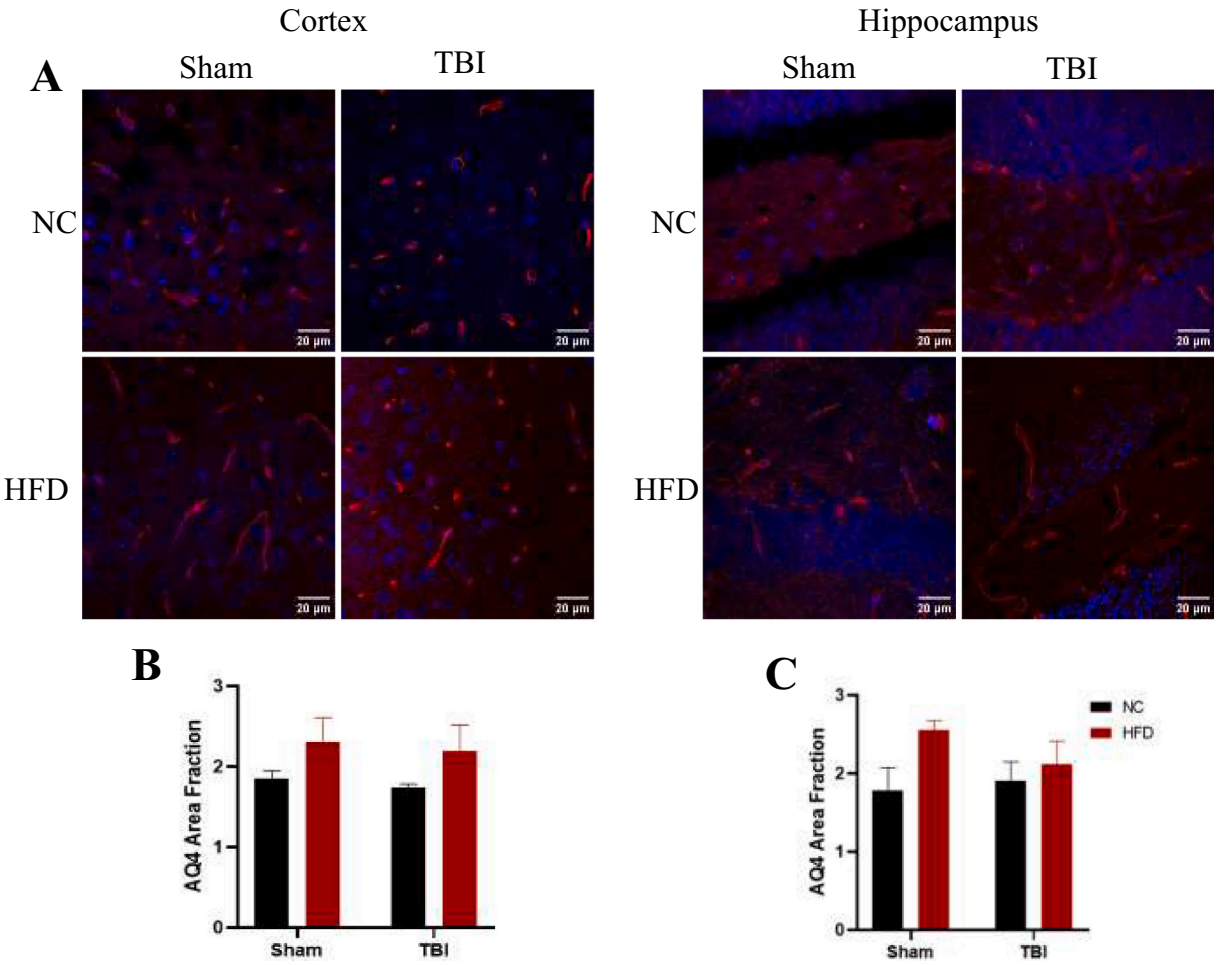

Fig. 7. Aquaporin 4 expressions in the cortex and hippocampus. *A:* Representative confocal images of aquaporin expression in both the cortex (left) and hippocampus (right) for NC Sham, NC TBI, HFD Sham, and HFD TBI. Tile scan images were taken with 40× Oil. Quantification values for AQ4 expression *B:* in the cortex and *C:* in the hippocampus ($n = 3$ mice/group for B & C). Data are presented as mean±SEM. Statistical significance was determined using two-way ANOVA (with the following factors, 1: diet, 2: injury), followed by Sidak's multiple comparisons.

immunoblotting where we loaded 25μg of proteins in each lane. Proteins were separated on 10 % gel and then transferred to nitrocellulose membranes. After transfer, the membranes were blocked for 1 h and then incubated overnight at 4°*C* with primary antibodies against LC3 (1:1000, Cell Signaling, #2775), Beclin (1:1000, Santa Cruz, D2908), mTOR (1:1000, Cell Signaling, #2972), ATG 14 (1:1000, Rainbow), β-actin (1:1000, Abcam, ab8227) and GAPDH (1:1000, Encor, RPCA-GAPDH). The membranes were then incubated with the appropriate HRP-conjugated secondary antibodies at room temperature for 1 h before being exposed to Clarity Western ECL substrate (Biorad, Hercules, CA). Subsequently, image detection was performed using the Chemidoc imaging system (Biorad, Hercules, CA). The protein bands were quantified using ImageJ software and normalized to β-actin or GAPDH. Three mice from each group were considered for statistical analysis.

2.17. RNA Extraction and RT-qPCR

Cortical and hippocampal RNA was isolated from the samples initially stored at −80°*C*, using Tri reagent (SIGMA-ALDRICH, USA), followed by DNA impurity removal using the TURBO DNA-free™ Kit (AM1907, Thermo Fisher Scientific, Waltham, Massachusetts, USA). iScript Transcriptase kit (Bio-Rad Laboratories, USA) was used to perform reverse transcription according to the manufacturer's instruction: 1.5 μg of the total extracted RNA was reverse transcribed into cDNA in a final volume of 20 μL. The reverse transcription was achieved in a BioRad T100 Thermo Cycler machine (Bio-Rad Laboratories, California, USA). Using the Qiagen Quantfast SYBR Green Supermix (Qiagen Strasse 1, Hilden Germany), polymerase chain reactions (PCR) were performed on the obtained samples in a CFX96 system (Bio-Rad Laboratories, California, USA). The primer oligonucleotide sequences mCAT, mSOD, mNrf2, and mGAPDH were synthesized and purchased from Macrogen Inc. Before usage, they were reconstituted, and a working solution of 10 μM was prepared. Results were calculated using the $2^{-\Delta\Delta CT}$ method and normalized to the housekeeping gene GAPDH. The forward and reverse murine primers used are presented in Table 1.

2.18. Quantification and statistical analysis

Image and band quantification were obtained using ImageJ (1.53e National Institute of Health, USA). Images for the different experimental interventions were acquired under the same laser and microscopic parameters for consistency. At least three sections per mouse were used for quantification with different levels to cover the hippocampus and cortex areas. The fields from all three sections were averaged for every animal. Statistical analysis was performed using GraphPad 8 (GraphPad Software, La Jolla, CA 92037, USA). Multiple comparisons among different groups were performed using either three-way (factor 1: operation, factor 2: diet, and factor 3: time) or two-way analysis of variance (ANOVA) followed by Sidak's multiple comparisons test. Student's *t*-test

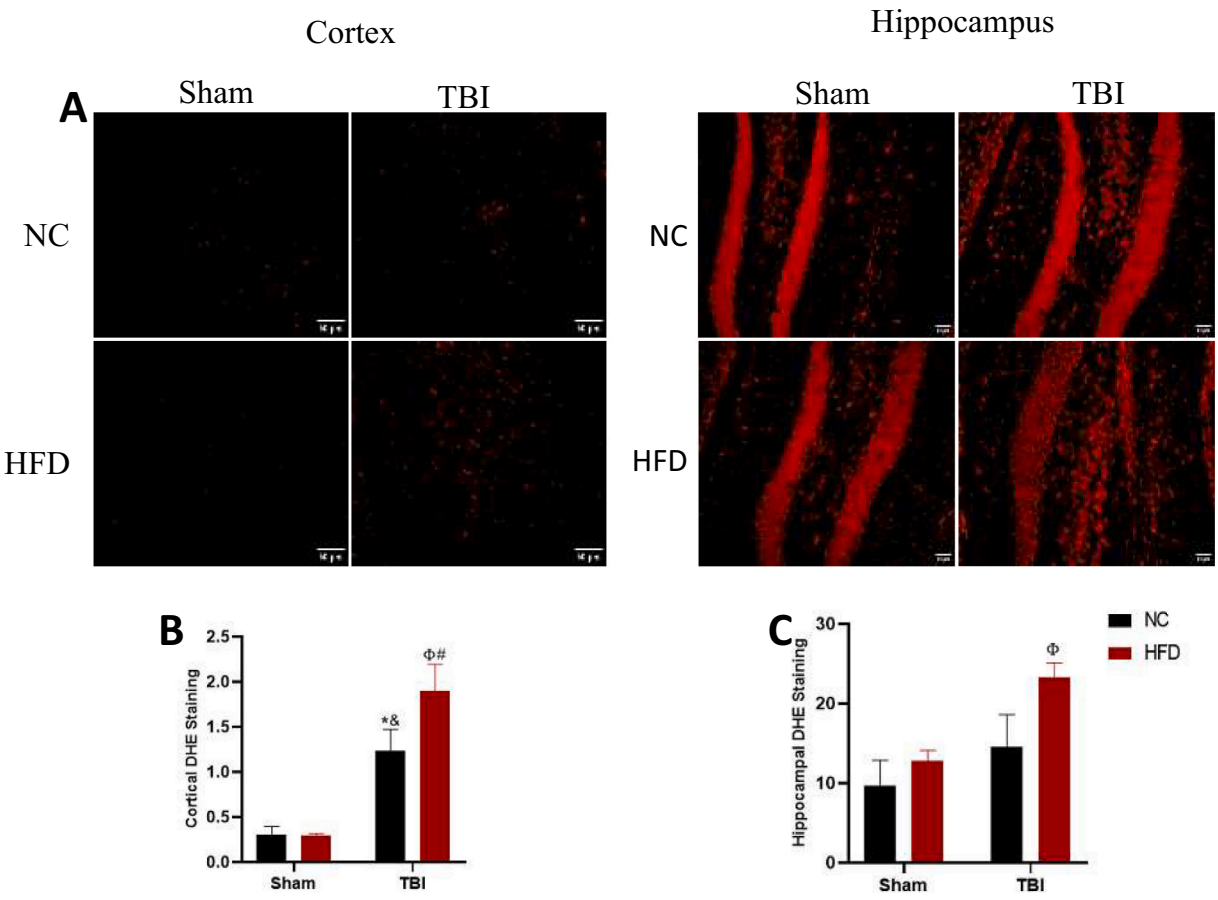

Fig. 8. Effect of HFD and/or TBI on hippocampal and cortical reactive oxygen species (ROS). *A:* Representative micrographs of DHE staining in both the cortex (left) and hippocampus (right). The red stain indicates the presence of ROS. Quantification values for the intensity of DHE staining *B:* in cortical and *C:* hippocampal sections (an average of 3 sections/mouse, $n = 3$ mice/group). Data are presented as mean±SEM. Statistical significance was determined using two-way ANOVA (with the following factors, 1: diet, 2: injury), followed by Sidak's multiple comparisons. * denotes $P < 0.05$ *vs.* NC Sham and # *vs.* HFD Sham. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

was used to compare lesion volume in NC- and HFD-fed mice exposed to TBI. Statistical significance was considered at $p < 0.05$.

3. Results

3.1. Effect of diet and/or TBI on metabolic parameters

Mice fed HFD consumed significantly more calories than those fed NC on all days before surgery (Fig. 2A) which was translated to a significantly higher body weight in HFD-fed mice compared to NC-fed ones ($p < 0.0001$, Fig. 2B). Indeed, HFD Sham mice had significantly higher body weights at week 8 compared to both NC-fed groups ($P < 0.05$). Additionally, only HFD Sham mice exhibited significant weight gain post-operation, *i.e.*, from week 4 to week 8. The latter was not observed in HFD-fed mice which had undergone TBI, indicating a lag in body weight following TBI and altered whole-body metabolism. On the other hand, a significant increase in the fat-to-lean ratio of the HFD-fed sham group was observed (Fig. 2C). Significantly, a strong interaction was found between diet and time of assessment with respect to blood glucose levels, in such a way that HFD-fed mice exhibited a gradual increase in blood glucose levels with time, while NC-fed mice did not (Fig. 2D). Indeed, starting week 7, HFD TBI mice possessed significantly higher blood glucose levels than NC-fed mice (TBI and sham-operated) and HFD Sham higher than NC TBI. At week 8, HFD sham mice also had higher blood glucose levels compared to their NC-fed counterparts. In summary, chronic HFD feeding triggered long-term glucose dysmetabolism post-TBI.

3.2. Effect of diet and/or TBI on cardiovascular function

Neither HFD-feeding nor induction of TBI produced any significant change in cardiovascular function. Indeed, cardiac function (assessed by ejection fraction and fractional shortening) appeared to be preserved across all groups (Figs. 3A and B). In addition, systolic blood pressure was not different among the various groups (Fig. 3C).

3.3. Effect of diet and/or TBI on cognitive and behavioral function

To investigate the impact of HFD and TBI on cognitive and behavioral profiles, a battery of behavioral and neurological tests was conducted to assess spatial learning and memory, motor coordination, and neuromuscular strength (Figs. 4 and 5). The MWM was used to assess spatial learning and memory to investigate how HFD-feeding affects hippocampal plasticity post-TBI (Fig. 4, A-C). Strong interactions were found between diet (NC *vs.* HFD) and operation (Sham *vs.* TBI), at different time points, in terms of latency to the platform, whereby HFD-fed and TBI mice spent more time to reach the platform with respect to NC-fed and sham-operated mice, respectively ($p < 0.0001$ for both interactions) (Fig. 4A). TBI significantly impaired learning abilities in the experimental mice, which was aggravated by the HFD (Fig. 4A). Mice in the sham groups were able to better learn the location of the escape platform over the training period and they took significantly less time to locate the platform starting on day 2. However, the TBI groups spent more time learning the location of the platform, with the HFD TBI spending a significantly longer time to reach the platform (latency time

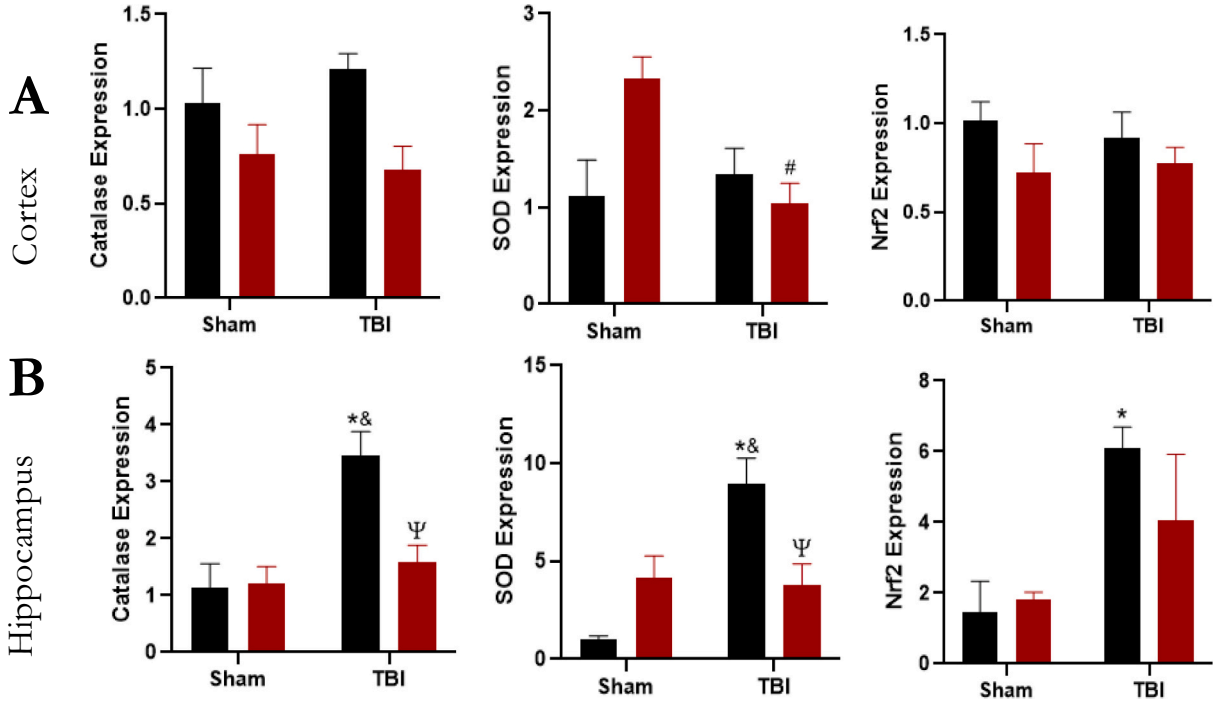

Fig. 9. Effect of HFD and/or TBI on antioxidant genes expression. The expression level was assessed using rt-PCR. *(A)* Cortical and *(B)* hippocampal mRNA expression levels of Catalase (left), SOD (middle), and Nrf2 (right) ($n = 3$ mice/group). Data are presented as mean±SEM. Statistical significance was determined using two-way ANOVA (with the following factors, 1: diet, 2: injury), followed by Sidak's multiple comparisons. * denotes $P < 0.05$ *vs.* NC Sham and # *vs.* HFD Sham, and δ *vs.* NC TBI.

in HFD TBI significantly greater than NC TBI and HFD sham on days 3 and 4). In the probe trial, spatial memory was found to be impaired by TBI and aggravated by HFD. The mice in the HFD TBI group spent the longest time locating the quadrant that initially had the platform ($p < 0.05$ for HFD TBI *vs.* NC Sham, HFD TBI *vs.* HFD Sham, and HFD TBI *vs.* NC TBI, Fig. 4B), and they also spent the least time in the quadrant searching for the platform (Fig. 4C). Furthermore, NOR test showed a significantly diminished recognition memory in both the NC TBI and HFD TBI group, as both groups showed a significantly reduced discrimination index, compared to the sham groups (Fig. 4D).

To assess the effect of HFD and TBI on motor coordination, rotarod and pole climbing tests were conducted (Fig. 5, A-C). In the rotarod test, as both TBI and HFD reduced the time spent on the rotarod before falling compared to the control group (NC sham), HFD TBI mice exhibited significantly lower latency time with respect to NC Sham mice (Fig. 5A). Similarly, mice in the HFD TBI group recorded significantly lower rotation velocities along the rotarod compared to NC Sham mice ($p < 0.05$, Fig. 5B). In the pole climbing experiment, HFD TBI mice took the longest time to get to the bottom of the pole ($p < 0.05$ *vs* NC sham, HFD sham, and NC TBI, Fig. 5C), reflecting an accentuated motor coordination deficit. Grip strength was employed to measure the effect of HFD and TBI on neuromuscular strength (Fig. 5D). TBI and HFD feeding significantly altered neuromuscular strength ($p < 0.05$ for NC TBI *vs.* NC sham and HFD sham *vs.* NC Sham), producing a significantly lower grip strength in HFD TBI mice compared to the control group (NC Sham).

3.4. Effect of chronic HFD feeding on TBI lesion volume

Hematoxylin and Eosin (H&E) staining of the brain sections of the injured animals revealed that the TBI groups exhibited cortical lesion 4 weeks after TBI induction (Fig. 6). Our CCI model produced cortical tissue damage, similar to what has been previously reported in the literature [51,52]. Evidently, TBI mice on HFD presented with significantly higher lesion volume compared to their NC-fed counterparts.

3.5. Long-term effect of diet and/or TBI on cortical and hippocampal aquaporin-4 expression

Aquaporin-4 (AQP-4) is the most abundant water pore channel in the brain and is involved in the BBB integrity as well as brain edema in the acute post-TBI stage [53]. Immunofluorescence staining for AQP-4 revealed no significant difference in aquaporin 4 expression in the cortices or the hippocampi across all experimental groups 4 weeks after surgery (Fig. 7).

3.6. HFD feeding is associated with accentuated oxidative stress in TBI

We assessed the relative levels of ROS in the ipsilateral hippocampus and cortex using DHE staining. HFD in combination with TBI significantly increased the intensity of DHE-positive cells in both regions of the brain to levels higher than NC Sham (Fig. 8). Importantly, cortical sections from HFD TBI mice had significantly higher oxidative stress than HFD Sham mice (Fig. 8, A&B). Moreover, the combined effect of chronic HFD-feeding and TBI exclusively triggered oxidative stress in the hippocampus, reflecting the deteriorative effect of HFD on TBI secondary injury. Concomitantly, in line with the increased oxidative load, the expression of the hippocampal and cortical antioxidant genes was repressed in HFD TBI mice (Fig. 9). Specifically, the cortical expression level of SOD was significantly lower in HFD TBI mice compared to their sham-operated counterparts (HFD sham) (Fig. 9A). Additionally, while TBI alone significantly increased the hippocampal gene expression levels of catalase, SOD, and Nrf2, chronic HFD-feeding significantly diminished catalase and SOD expression in TBI mice to levels lower than their NC-fed counterparts (NC TBI) (Fig. 9B). This is suggestive of the deleterious effect of chronic HFD feeding on TBI secondary injury.

3.7. HFD aggravates neuroinflammation 30 days post-TBI

To assess the effect of HFD on TBI-induced neuroinflammation, the expression of GFAP and IBA-1 as molecular markers of activated

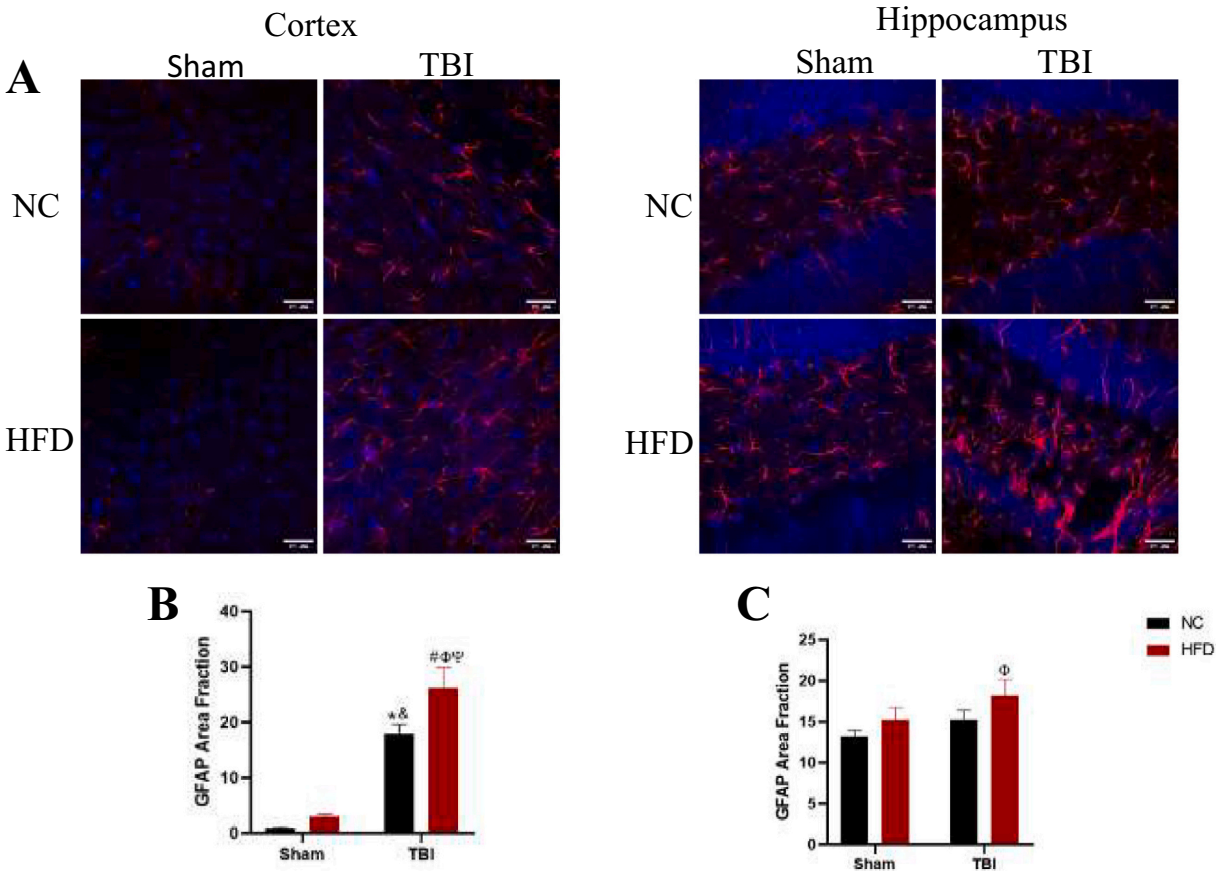

Fig. 10. Effect of HFD and/or TBI on GFAP expression in the ipsilateral cortex and hippocampus. *A:* Representative micrographs of GFAP in the cortex and hippocampus. Images were taken at 40× Oil and tile scan. Quantification values for GFAP expression *B:* in the cortex and *C:* in the hippocampus (average of 3 sections/mouse, $n = 3$ mice/group). Data are presented as mean±SEM. Statistical significance was determined using two-way ANOVA (with the following factors, 1: diet, 2: injury), followed by Sidak's multiple comparisons. * denotes $P < 0.05$ *vs.* NC Sham, # *vs.* HFD Sham, and δ *vs.* NC TBI.

astrocytes and microglia, respectively, was investigated in cortical and ipsilateral hippocampal samples using immunofluorescence. Staining for GFAP revealed that HFD aggravated neuroinflammation post-TBI in both the cortex and the hippocampus (Fig. 10). Cortical expression of GFAP was significantly increased in TBI groups, with HFD TBI mice showing the greatest intensity of GFAP-positive cells (Fig. 10, A&B). As for the ipsilateral hippocampus, only HFD TBI mice exhibited increased GFAP expression compared to NC Sham ($p < 0.05$, Fig. 10, A & C). Similarly, TBI induced an increase in IBA1 expression in the hippocampus and cortex of NC- and HFD-fed mice (Fig. 11). Of note, HFD TBI mice exhibited significantly higher expression of IBA-1 in both hippocampal and cortical sections compared to HFD Sham, further supporting the effect of HFD and TBI on neuroinflammation.

Effect of diet and/or TBI on autophagy markers expression.

Since secondary injury alters several homeostatic cellular and molecular pathways, we investigated whether autophagy was affected by HFD and TBI. The results showed that LC3B, ATG14, Beclin2, and mTOR expression levels were not significantly altered by chronic HFD feeding nor by TBI (Fig. 12).

3.8. Changes in markers of neurodegeneration and neuronal regeneration

To investigate the effect of diet on the long-term sequelae of TBI, the status of neurodegeneration was assessed in cortical and hippocampal sections *via* evaluating phosphorylated tau (p-Tau). p-Tau, as well as BrdU and NeuN, were used as markers of neurodegeneration and cellular proliferation, respectively.

Of interest, the p-Tau staining performed 30 days post-operation, showed similar results for the cortex and the hippocampus (Fig. 13). TBI groups expressed higher levels of p-Tau compared to their sham-operated counterparts. However, no significant difference in P-Tau expression was observed between NC TBI and HFD TBI groups.

BrdU incorporates into replicating DNA at the S-phase and is thus indicative of replicating cells. When compared to HFD or TBI alone, the combined effect of HFD and TBI resulted in a decrease in cellular proliferation in the hippocampus, as indicated by lower BrdU-positive cells (Fig. 14, A&B). Indeed, while the HFD sham and NC TBI mice expressed higher BrdU compared to the NC sham, reflecting activation of cellular proliferation, the number of BrdU-positive cells in HFD TBI mice was significantly lower than that in the NC sham. Additionally, HFD TBI mice had lower BrdU-positive cells than their sham-operated counterparts. No significant difference in NeuN expression was observed between the different groups.

4. Discussion

Diet and lifestyle are important factors that could influence the outcome of TBI, by either ameliorating or worsening the long-term consequences of the injury as recently reviewed by [21]. Clinically, it is established that TBI patients with metabolic comorbidities involving heart disease and diabetes are more vulnerable to secondary brain injury post-primary injury exacerbating the pathophysiological outcomes [54]. These outcomes are highly modulated by the nutritional supplementation that can shape the pathophysiology of TBI. In fact, several studies of TBI evaluating altered nutritional diets have focused on the neuropathological and behavioral outcomes "independent" of brain injury

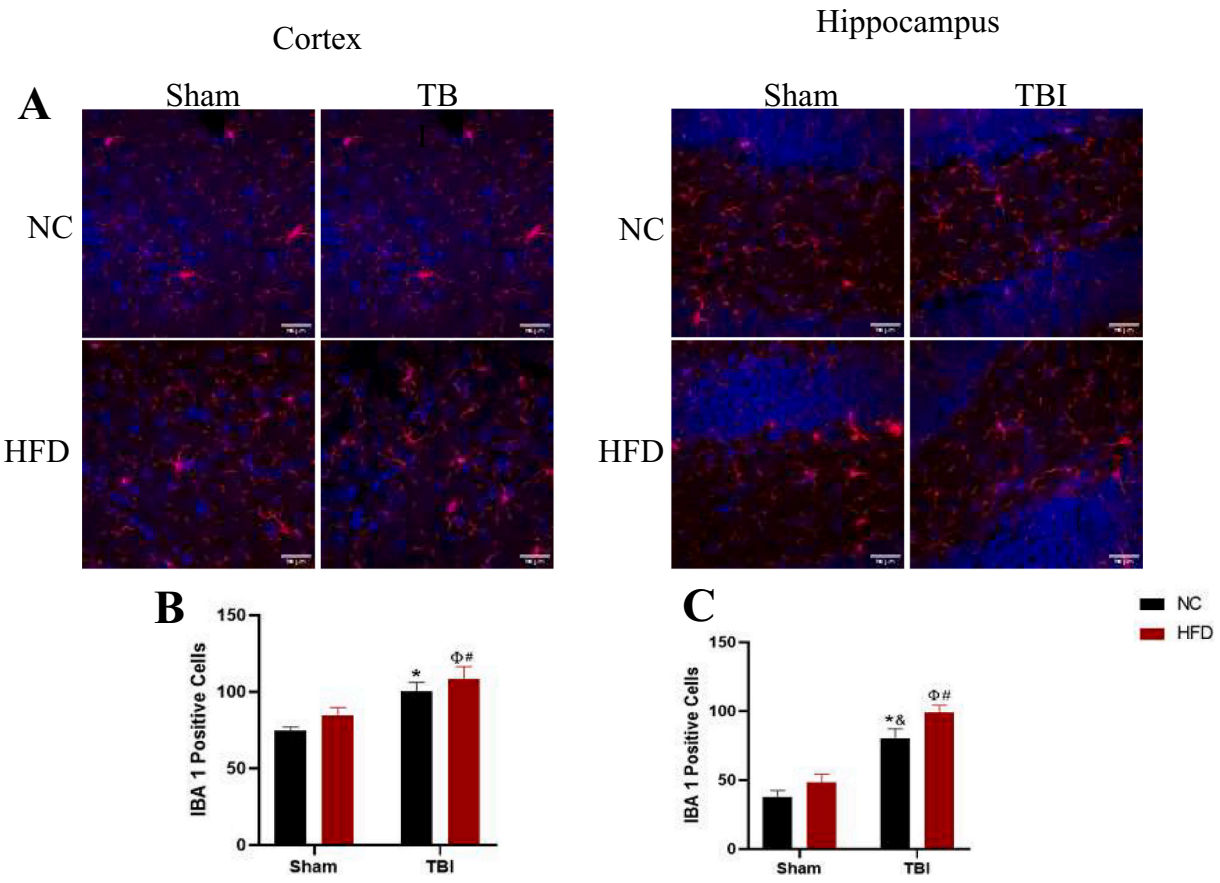

Fig. 11. Effect of HFD and TBI on IBA-1 expression in the ipsilateral cortex and hippocampus. *A:* Representative images of IBA-1 in the cortex and hippocampus. Images were taken at 40× Oil and tile scan. Quantification values for IBA 1 expression *B:* in the cortex and *C:* in the hippocampus (average of 3 sections/mouse, $n = 3$ mice/group). Data are presented as mean±SEM. Statistical significance was determined using two-way ANOVA (with the following factors, 1: diet, 2: injury), followed by Sidak's multiple comparisons. * denotes $P < 0.05$ *vs.* NC Sham and # *vs.* HFD Sham.

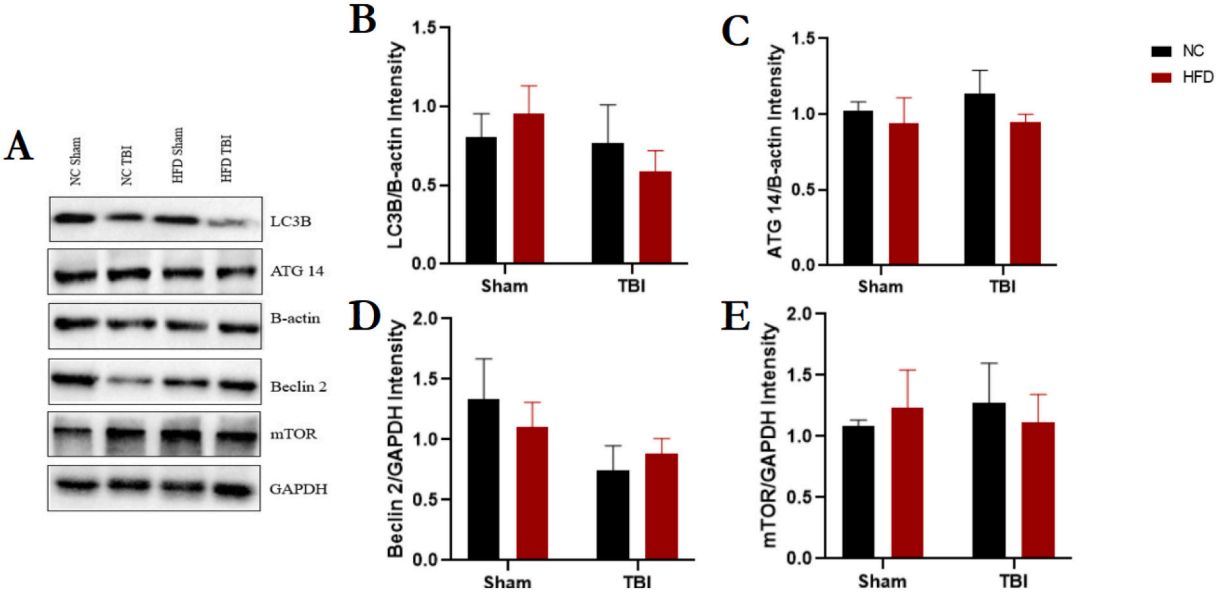

Fig. 12. Effect of HFD and TBI on autophagic machinery marker protein expression in hippocampus. *A:* Representative immunoblots for the expression level of various autophagy proteins (ATG 14, LC3B, Beclin 2, and mTOR). *B-E:* Quantification values of the protein band intensity of LC3B and ATG14, normalized to *β*-actin, as well as Beclin 2 and mTOR, normalized to GAPDH, respectively (n = 3 mice/group). Data were presented as mean ± SEM. Statistical significance was determined using two-way ANOVA (with the following factors, 1: diet, 2: injury), followed by Sidak's multiple comparisons.

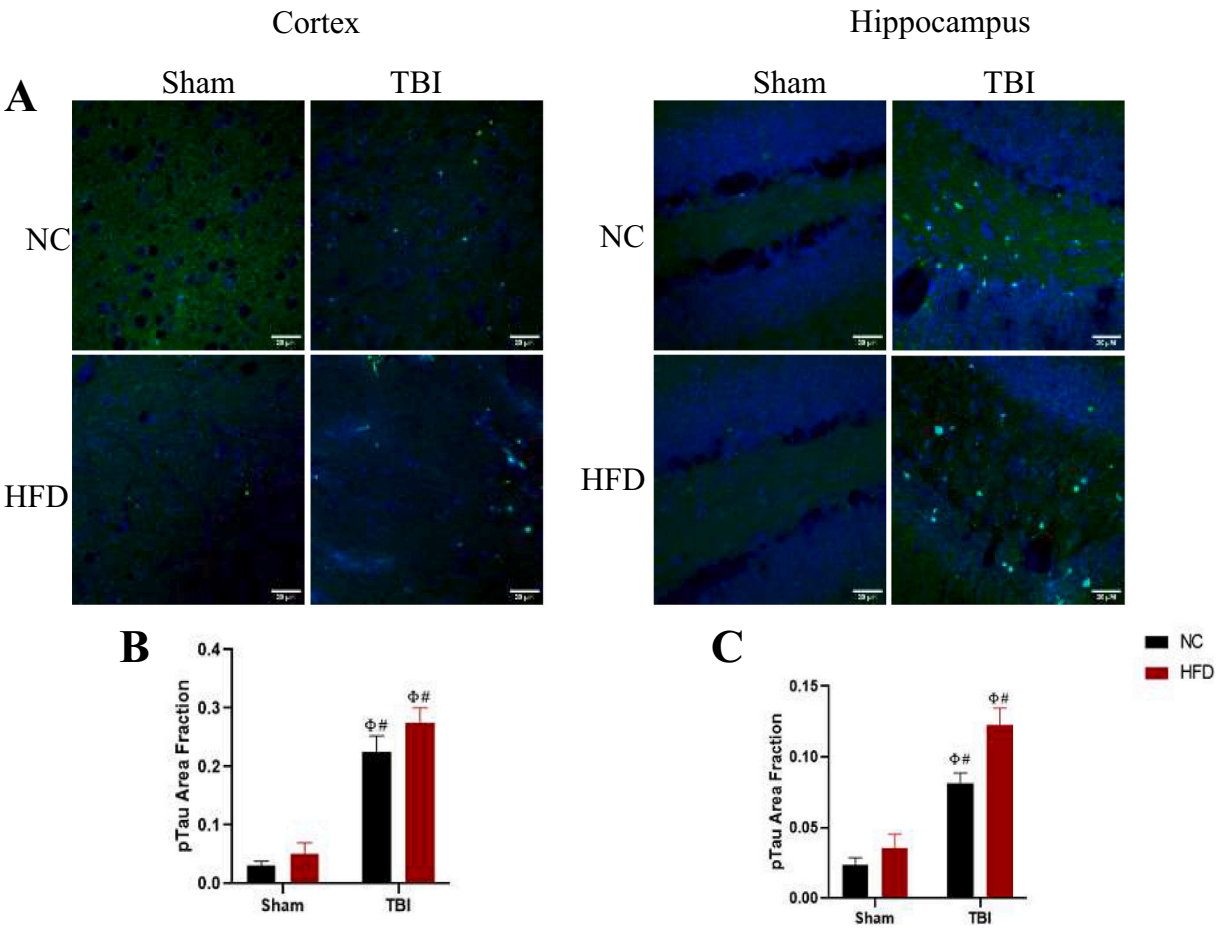

Fig. 13. Effect of HFD and TBI on p-Tau expression in the cortex and hippocampus. *A:* Representative immunofluorescent images of p-Tau in the cortex and hippocampus. Quantification values for pTau expression *B:* in the cortex and *C:* the hippocampus. Data are presented as mean±SEM (n = 3 mice/group). Statistical significance was determined using two-way ANOVA (with the following factors, 1: diet, 2: injury), followed by Sidak's multiple comparisons. * denotes $P < 0.05$ *vs.* NC Sham and # *vs.* HFD Sham.

modes or severity contexts whether it is closed head injury (mTBI) or more pronounced penetrating injury (moderate or severe), or even at the level timing of intervention (acute *vs* chronic long term assessments) which represents a key element in defining a specific TBI model [55–57]. The lack of these correlational studies depicts a major caveat in the field of nutritional supplementation/needs, specifically to the military personnel where optimal nutritional support would contribute to the acute, subacute, and chronic health outcomes of mTBI events [58].

As distinct cellular and molecular mechanisms characterize the acute and chronic post-TBI periods [59], we investigated the involvement of the different pathological pathways in HFD-induced exacerbation of secondary injury. Particularly, we assessed the status of oxidative stress, neuroinflammation, neurodegeneration, and neuroregeneration in the different mouse groups. To the best of our knowledge, our study is among the first to investigate the impact of chronic HFD feeding on the long-term consequences of TBI [60]. Our results indicate that HFD feeding exacerbates TBI secondary injury by increasing cortical and hippocampal oxidative and inflammatory load and by decreasing hippocampal compensatory cellular proliferation leading to a diminished healing capacity. Consistently, HFD-fed injured mice exhibited deranged spatial learning and memory as well as worsened motor coordination relative to their NC-fed counterparts.

In response to increased calorie intake, HFD-fed sham-operated mice exhibited significant weight gain. Conversely, a similar increase in body weight was not observed in injured mice, indicating a distinct metabolic state post-injury. The latter is consistent with results from a different study showing reduced weight gain in obese brain-injured mice [61]. Such reduction was attributed to increased hypothalamic microglial activation, like that seen in our model, and an alteration along the hypothalamus-pituitary-adrenal axis resulting in reduced corticosterone levels [61]. Indeed, as corticosteroids are anabolic fat-accumulating hormones, it comes as no surprise that HFD-fed TBI mice did not accumulate fat similar to their sham-operated counterparts. Alternatively, both HFD-fed groups developed hyperglycemia after 6 weeks.

Obesity and type 2 diabetes have both been reported to result in a state of increased systemic pro-inflammatory cytokines originating from changes in adipose tissue microenvironment and gut microbiota populations and resulting in diminished integrity of the BBB [62]. The latter contributes to cerebral alterations precipitating an elevated risk of neurological disorders and neurodegenerative diseases [63,64]. In fact, TBI and metabolic syndrome have been shown to trigger common pathological pathways of neuronal injury [18]. However, the impact of prolonged metabolic insult on the long-term consequences of TBI remains poorly addressed.

On the behavioral level, eight weeks of HFD feeding aggravated the impaired learning and memory abilities observed in TBI. Similar observations have been reported previously in the literature, where it was demonstrated that HFD has the potential to aggravate TBI-induced neurological deficits [65–67]. Learning and memory are very critical functions of the brain which are carried out by several molecular signaling pathways. For instance, brain-derived neurotrophic factor (BDNF) and CREB, which are important mediators of synaptic plasticity, play a role in the induction of long-term potentiation leading to improved learning capabilities [68]. HFD has been reported to alter

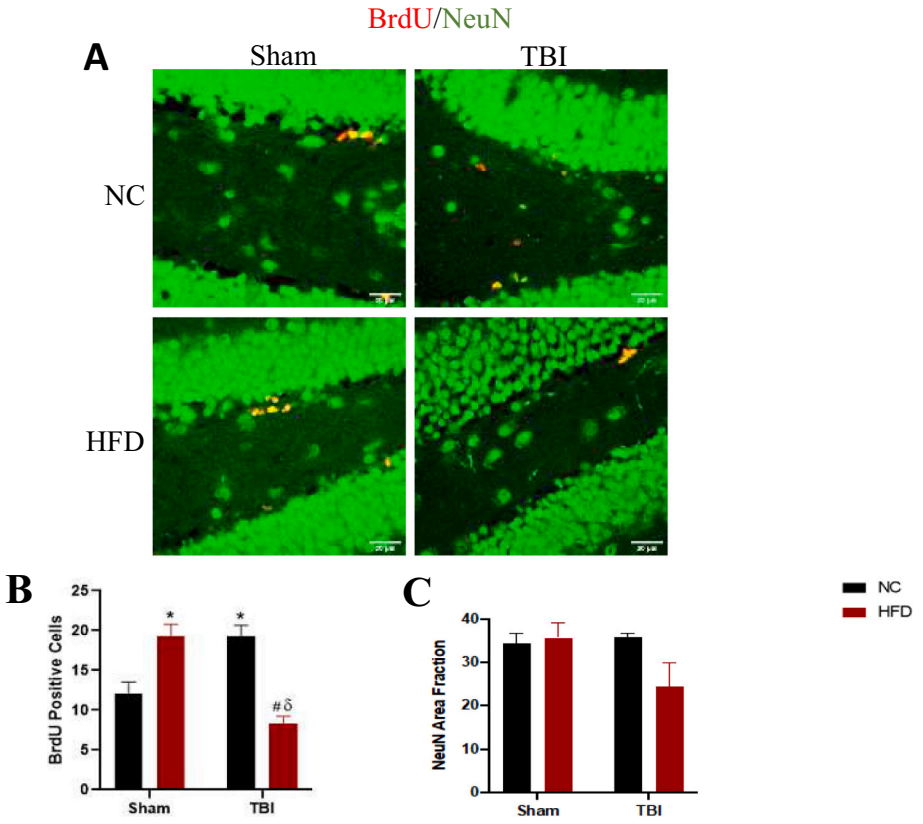

Fig. 14. Effect of HFD and TBI on BrdU and NeuN levels in the hippocampus. *A:* Representative immunofluorescence micrographs of BrdU (red) and NeuN (green) staining in the hippocampus. *B:* Quantification values for BrdU-positive cells in the hippocampus. *C:* Quantification of NeuN expression in the hippocampus. Data are presented as mean±SEM (n = 3 mice/group). Statistical significance was determined using two-way ANOVA (with the following factors, 1: diet, 2: injury), followed by Sidak's multiple comparisons. * denotes $P < 0.05$ *vs.* NC Sham, # *vs.* HFD Sham, and δ *vs.* NC TBI. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

BDNF levels, which in turn results in impaired cognitive abilities [22]. Moreover, altered BDNF signaling is associated with worse cognitive outcomes in high-fat-fed mice one-week post-TBI [57]. Concurrently, motor coordination and neuromuscular strength were impaired by HFD and TBI, thereby implicating HFD as a significant risk factor for aggravated assaults pots-TBI [69,70].

To evaluate the mechanism through which HFD elicited these prolonged aggravated effects, different molecular pathways were explored. TBI induces an alteration of the BBB, a condition that has also been reported to be produced by HFD through the downregulation of gap junction proteins [62,71,72]. One important indicator of BBB dysfunction after TBI is edema, which is mediated by an increased expression of the water channel protein aquaporin [72]. As aquaporin is involved in acute post-TBI brain edema, it was not surprising to find that its expression levels were normalized 4 weeks post-TBI [73]. Indeed, Xiong et al. showed that while AQ4 levels peaked at 12- and 72 h post-TBI, normal levels were restored 15 days after injury [73].

Oxidative stress, which results from an imbalance between the pro-oxidative processes and antioxidative defense mechanisms, is a major contributor to secondary brain injury [74,75]. Research over the years has shown that HFD induces oxidative stress [76]. Expectedly, our findings reflect accentuated ROS production in HFD-TBI mice. Along the same lines, the antioxidant defense system of TBI mice fed with HFD was repressed explaining the oxidative stress aggravation. For instance, HFD and TBI downregulated the cortical and hippocampal expression of SOD and Catalase. Nrf2 is a transcription factor, that gets activated in response to oxidative stress. When activated, it induces downward signaling that modulates the activity of SOD and Catalase [77]. SOD, on the other hand, catalyzes the dismutation of superoxide radicals to hydrogen peroxide, which is further converted to water and oxygen by catalase, thereby leading to a reduced level of ROS [78]. Interestingly, the reduced expression of SOD and catalase correlated with an increased level of DHE. Independent of TBI, HFD has been reported to alter antioxidant defense mechanisms by repressing the expression of SOD and catalase [79].

The role that autophagy plays in the pathologies of secondary TBI has generated a lot of conflicting data. While autophagy has been shown to exert a neuroprotective effect and aid brain recovery after TBI [80–82], some studies have reported that autophagy exerts deleterious effects post-TBI and that its inhibition is associated with improved functional outcomes [83,84]. Nevertheless, HFD has been reported to impair the autophagic flux, which was associated with inflammation and apoptosis [85]. At this stage, neither HFD nor TBI had a significant impact on the levels of autophagy markers. Although we have not seen changes in the autophagic machinery, we cannot preclude the possibility of altered apoptosis, especially in light of elevated neuroinflammation [86].

The implication of neuroinflammation in the pathology of TBI has been greatly studied. Of interest, the role of brain resident microglia and astrocytes, which are the primary immune cells of the brain, has been well reported in the literature [87]. Previous studies from our laboratory have reported chronic astrocytic and microglial activation post-TBI [35,52,88]. This is consistent with another study showing the persistence of astrocyte gliosis of the ipsilateral hippocampus six months after TBI and its association with brain aging and hippocampal neurodegenerative changes [89]. In the acute post-injury stage, serum levels of triglycerides significantly correlated with neuroinflammation, implicating a disrupted BBB and central delivery of triglycerides in exaggerated neuroinflammation in response to HFD [90]. Chronically, we found that HFD aggravated microglia and astrocyte activation. In fact, deteriorating neurocognitive functions in HFD-fed TBI mice can be attributed to heightened neuroinflammation as selective inhibition of microglia-derived NLRP3 inflammasome was reported to ameliorate the long-term neurological effects of TBI [91,92]. Interestingly, the ability of HFD to elicit chronic activation of microglia and astrocytes could hinder brain recovery post-TBI and predispose the brain to neurodegeneration [93,94].

Previous studies have reported that TBI induces cellular

proliferation, as part of the brain's attempt to repair and recover from the injury [95,96]. Interestingly, cell proliferation, which was assessed by BrdU staining, was shown to be significantly reduced by the HFD in the TBI group, with a concomitant increase in the lesion volume of HFD-fed TBI mice. The inhibition of injury-induced cell proliferation has been linked to reduced brain recovery and worsened functional outcomes after TBI [97].

Furthermore, neurodegeneration is one of the chronic consequences of TBI, which usually leads to the formation of neurofibrillary tangles (NFT) [98]. NFTs result from the misfolding and aggregation of Tau protein, otherwise known as tauopathies, and are a significant hallmark of neurodegeneration [99]. Previous studies have linked HFD consumption to worsened outcomes in several neurodegenerative and neurological disorders [100–102]. In our study, HFD in combination with TBI accentuated p-Tau levels suggesting increased neurodegeneration.

Our study unfolds a network of pathways through which HFD exacerbates TBI long-term outcomes. From elevating oxidative stress to aggravating chronic neuroinflammation, HFD slows down brain repair by repressing cell proliferation, thereby leading to more chronic complications with implications in neurodegeneration. Future studies should attempt to study the impact of Western-like diets, which are comprised of combinations of high fat and high sugar, on the above-described processes. Additionally, it is worth investigating the impact of the dietary switch to normal chow post-TBI on these deleterious mechanisms seen with high-fat feeding.

CRediT authorship contribution statement

AFE, FK, SN, YM, AE and HH conceived and designed the study. SI, CB, JN, NB, SM, MAR, and FA collected the data. FK, AFE, SI, FA, NB, MAR, and JN contributed to data analysis. SI, FA, JN, MAR, and NB performed the experiments. SI, FK, AFE drafted the manuscript. All authors reviewed and edited the manuscript. All authors read and approved the final manuscript.

Ethics approval

Declarations Ethics Approval and Consent to Participate: All the animal experiments in our study were approved by the Institutional Animal Care and Use Committee (IACUC: 21–09-689) at the American University of Beirut, Lebanon.

Consent for publication

All authors read and approved the final manuscript.

Declaration of competing interest

The authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Data availability

All data generated or analyzed during this study are included in this published article.

Acknowledgment

This work was funded by grants from the Science, Technology and Innovation Funding Authority to AFE (45912) and American University of Beirut Faculty of Medicine, Medical Practice Plan (AUB-FM MPP) to FK (320112). We thank the members of Dr. Ahmed El-Yazbi lab. (Nahed Mogharbil & Dr. Rana Alaaeddine) for their sincere help in conducting the cardiovascular experiments. We thank Leila Nasrallah and Yara Yehya for their help in the neurological experiments. We thank Dr. Gerry Shaw, the CEO of EnCor Biotechnology Inc. Gainesville, Fl, USA for helping us with the antibodies.

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