Dietary Docosahexaenoic Acid Improves Cognitive Function, Tissue Sparing, and Magnetic Resonance Imaging Indices of Edema and White Matter Injury in the Immature Rat after Traumatic Brain Injury

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Abstract

Traumatic brain injury (TBI) is the leading cause of acquired neurologic disability in children. Specific therapies to treat acute TBI are lacking. Cognitive impairment from TBI may be blunted by decreasing inflammation and oxidative damage after injury. Docosahexaenoic acid (DHA) decreases cognitive impairment, oxidative stress, and white matter injury in adult rats after TBI. Effects of DHA on cognitive outcome, oxidative stress, and white matter injury in the developing rat after experimental TBI are unknown. We hypothesized that DHA would decrease early inflammatory markers and oxidative stress, and improve cognitive, imaging and histologic outcomes in rat pups after controlled cortical impact (CCI). CCI or sham surgery was delivered to 17 d old male rat pups exposed to DHA or standard diet for the duration of the experiments. DHA was introduced into the dam diet the day before CCI to allow timely DHA delivery to the pre-weanling pups. Inflammatory cytokines and nitrates/nitrites were measured in the injured brains at post-injury Day (PID) 1 and PID2. Morris water maze (MWM) testing was performed at PID41–PID47. T2-weighted and diffusion tensor imaging studies were obtained at PID12 and PID28. Tissue sparing was calculated histologically at PID3 and PID50. DHA did not adversely affect rat survival or weight gain. DHA acutely decreased oxidative stress and increased anti-inflammatory interleukin 10 in CCI brains. DHA improved MWM performance and lesion volume late after injury. At PID12, DHA decreased T2-imaging measures of cerebral edema and decreased radial diffusivity, an index of white matter injury. DHA improved short- and long-term neurologic outcomes after CCI in the rat pup. Given its favorable safety profile, DHA is a promising candidate therapy for pediatric TBI. Further studies are needed to explore neuroprotective mechanisms of DHA after developmental TBI.

Key words: controlled cortical impact; DTI; Morris water maze; pediatric

Introduction

PEDIATRIC TRAUMATIC BRAIN INJURY (TBI) is the leading cause of acquired disability in children and affects nearly half a million children in the United States annually.^{1–4} Despite the enormity of the problem, therapies to decrease disability after pediatric TBI are lacking.

Neurologic disability may be blunted by decreasing inflammation and oxidative damage, which are important mechanisms of secondary injury after TBI. The developing brain is more vulnerable to inflammatory and oxidative injury than is the mature brain. This developmental difference could help explain the correlation between greater cerebral immaturity and worse impairment after

TBI.^{5–14} Docosahexaenoic acid (DHA) is a candidate neuroprotectant that decreases neuroinflammation in the adult rat.¹⁵ DHA is an essential nutrient for normal brain function and development and is the most abundant omega-3 polyunsaturated fatty acid (22:6n-3) in the mammalian brain. DHA appears to have a favorable safety profile at any age.^{16–19} Treatment with DHA is associated with decreased white matter injury, oxidative stress, and cognitive impairment in the adult rat after TBI.^{20–26} While DHA is thus an appealing candidate therapy for pediatric TBI, little is known about DHA's effects on the developing brain after TBI.

To our knowledge, there is only one published study on DHA treatment of TBI in immature animals. This study reported short-term measures of motor, but not cognitive, outcome.²⁷ The

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objective of our study is to complement this previous work by testing the effects of DHA on long-term cognitive and histologic outcomes in the developing rat after TBI. In addition, we sought to assess DHA effects on oxidative stress, inflammation, and white matter injury in the developing brain after TBI.

To meet this objective, we used our established model of pediatric TBI, controlled cortical impact (CCI) in 17 d old (post-natal Day 17 [P17]) rats.^{28–33} We selected this age because rat brain maturation at P17 is comparable to that of the human infant/young toddler, the pediatric age group at highest risk for cognitive deficits after TBI.^{7,8,34–36} We tested DHA's effects on functional, histologic, and imaging outcomes in rat pups after CCI. Additionally, we examined DHA's effects on early oxidative stress and inflammation.

Methods

Animals

All experimental protocols were approved by the Animal Care and Use Committees at the University of Utah, in accordance with U.S. National Institutes of Health (NIH) guidelines and carried out at the University of Utah. All surgical procedures were performed using aseptic technique.

Briefly, male Sprague-Dawley rats were obtained from Charles Rivers Laboratories (Raleigh, NC) on P7-P10. We studied only males to eliminate any potential confounding effects of sex. Rats were housed in litters of 10 with the lactating dam until weaning on P21-P23. After weaning, rats were housed three to five per cage and allowed free access to food and water. All cages were kept in a temperature- and light-controlled (12 h on/12 h off) environment.

Rats were divided into two groups: those who received 0.1% DHA rodent diet (DHA) and those who received standard rat chow (REG). The DHA rodent diet (Harlan-Teklad, WI) substitutes 0.1% of the soybean oil in standard chow with purified DHA (U-84-A, Nu-Chek Prep, MN). This substitution results in the same macronutrient content and caloric density (3 kcal/g) as standard rat chow. This 0.1% DHA diet provides DHA as 1.8% of total fat. All dams and pups were kept on REG diet (Harlan Teklad 8640), with the exception of rats in the DHA CCI group. Seventeen-day-old rat pups depend exclusively on dam milk for their intake. Gavage feeding rat pups with DHA soon after surgery carries a significant risk of pulmonary aspiration. Instead, we fed dams the DHA diet 1 d before CCI, guided by data that breast milk DHA peaked at 10 h and lasted 24 h after ingestion of a DHA supplement.³⁷ Once rats were weaned (at P21) they consumed exclusively the DHA/REG chow for the duration of each experiment.

There were three experimental groups: rats who received DHA and CCI (DHACCI); rats who received REG and CCI (REGCCI); and rats who received REG and sham surgery (SHAM). To form the experimental groups, 400 total rats were split equally among the three groups. In order to minimize litter effects in the experimental groups, rats from different litters were culled by Charles River prior to shipment to our laboratory to generate litters of 10 male pups per dam. We further minimized litter effects by randomly selecting seven to eight rats from every 40 rats after CCI or sham surgery to form functional testing groups of 21–24 rats. Different animals were used for all experiments, with one exception: rats used for MWM were subsequently used for post-injury Day (PID) 50 tissue sparing analyses.

CCI procedure

CCI was carried out as previously described.³¹ At P17, rats undergoing CCI were anesthetized with 3% isoflurane for induction. Anesthesia was maintained with 2%–2.5% isoflurane for the duration of surgical preparation using a VetEquip Bench Top Isoflurane

Anesthesia System (Pleasanton, CA). The rats' core temperature was monitored via a rectal probe and continuously controlled at $37 \pm 0.5^\circ\text{C}$ using a servo-controlled heating pad. Each rat was placed into a stereotaxic frame (David Kopf, Tujunga, CA). After shaving, prepping with povidone-iodine and incising the scalp, a craniotomy (6 mm $\times$ 6 mm) was performed over the left parietal cortex (centered at the point 4 mm posterior and 4 mm lateral to bregma). Care was taken to not perforate the dura. Once the craniotomy was complete, anesthesia was reduced to 1% isoflurane for a 5 min equilibration period. CCI was then delivered (Pittsburgh Precision Instruments, Pittsburgh, PA) to the left parietal cortex using a 5 mm rounded tip to deliver a 2.0 mm deformation at 5 m/sec velocity and 100 msec duration. Immediately after CCI, isoflurane was increased to 2%–2.5% and the bone flap was replaced and secured with dental cement (Patterson Dental, Salt Lake City, UT). The scalp incision was sutured and triple antibiotic ointment and bupivacaine 0.5% were applied topically. Isoflurane was stopped and rats were allowed to recover in a temperature-controlled chamber. Once fully awake, rats were returned to their dams and littermates. SHAM rats underwent the same surgical craniotomy, equilibration, and closure procedures without CCI.

Outcomes

Outcomes were measured at various time-points spanning between PID1 and PID50. We assessed motor outcomes using rotarod testing at PID12 and PID35. Cognitive outcome was measured using Novel Object Recognition (NOR) and Morris water maze (MWM) testing at PID14 and PID41 after CCI, respectively. DHA's effects on oxidative stress and inflammation were assessed at PID1 and PID2 using brain levels of nitrates/nitrites, and of the inflammatory cytokines tumor necrosis factor α (TNF α), interleukin-1 β (IL-1 β), IL-6, IL-2, IL-10, and chemokine (CC-motif) ligand 2 (CCL2), also known as monocyte chemoattractant protein 1. Other outcomes included histology at PID3 and PID50 to assess tissue sparing, and magnetic resonance imaging (MRI) at PID12 and PID28 to assess edema and white matter injury.^{38–41}

Rotarod testing

We used the IITC Rotarod, Series 8 (IITC Life Science, CA), to test motor coordination and balance.⁴² One set of rats was tested at PID12 and a different set of rats at PID35. Each set was comprised of eight rats from each of the three groups. Each rat was placed on a cylinder that was initially rotating at a slow speed (4 rpm). The rotational speed gradually increased over time (up to 5 min) to a maximum speed of 45 rpm. In order to stay on the cylinder, the rat had to continuously walk forward. Measures included time to fall off the cylinder, the rpm at time of fall, and total meters traveled. Each rat underwent four daily rotarod trials that were repeated for three consecutive days per set of rats.

NOR

We used a black Plexiglas[®] NOR chamber (52 $\times$ 52 $\times$ 30 cm) as described for juvenile rats (P29–P40)⁴³ to test rat pups at PID14. Seven rats from each of the three groups were tested. The box was housed in a small room with soft lighting. Objects used for testing were small, plastic, and easily cleaned, and were secured to the box using Velcro[®]. The box and objects were cleaned between rat pups with 70% alcohol to remove odor traces. There were three phases to the NOR: habituation, familiarization, and testing. During the habituation phase, each rat pup was allowed to individually explore the empty box for 15 min per day over 2 d to ensure acclimation to the testing environment. During the 5 min familiarization phase, a single rat was placed in the arena containing two objects. The rat was returned to the arena 24 h later for testing. During the 5 min testing phase, the rat was exposed to one object from the familiarization phase and to one novel object. During testing, the rat's interaction with an object was measured using

automated video tracking and data analysis equipment from EthoVision[®] v 7.1 (Noldus Information Technology, Wageningen, the Netherlands) and confirmed by dual observer analysis of the videotaped session when needed. The rat's exploration was timed as the duration that the rat's nose was within 2 cm of the object as determined by a digitally drawn arena. Time spent rearing, if present, was not considered exploration. Performance on the testing phase was measured by the percent novel exploration time ($100 \times [N/(N+F)]$), where N is the time spent on the new object and F on the familiar.

MWM testing

Spatial memory and learning were assessed using the MWM. Fourteen injured (DHACCI, $n=7$; REGCCI, $n=7$) and six SHAM rats were tested starting at PID41. The pool was maintained at $26 \pm 2^\circ\text{C}$ and was located in a dimly lit room, surrounded by visual cues. Rats were placed in a pool of water that contains a submerged platform. The submerged platform was positioned in one of the four equal pool quadrants. The starting quadrant was varied over the four daily trials per day using the same pattern on a given day for all study groups. Serial testing (4 trials per day; maximum swim time, 120 sec per trial; 14 min rest between trials) was performed for 5 d with a submerged platform from PID41 to PID45. Raised platform testing was performed on PID46 and PID47 to assess ability to reach the visible platform. Latency to find the platform, swim speed, swim path, and thigmotaxis (time spent swimming close to the sides of the pool) were recorded for each trial. On PID48, a probe trial (no platform present) was performed in which the time spent in each quadrant over a 60 second trial was recorded. Each trial was recorded using automated video tracking and data analysis equipment from HVS Tracking System. Brains harvested from the 20 rats after completing MWM testing were used for tissue sparing analyses.

Molecular studies

For molecular studies, eight rats from each of the three groups were used for each assay and time-point (total of 96 rats). Rats were anesthetized with intraperitoneal xylazine (8 mg/kg) and ketamine (40 mg/kg) for tissue collection. For cytokine and nitrate/nitrite analyses, ipsilateral (left) hippocampi and cortices were dissected on ice, snap frozen in liquid nitrogen, and stored at -80°C .

Cytokine levels and nitrate/nitrite concentration in tissues ipsilateral to impact

Total protein samples were extracted by homogenizing ipsilateral hippocampi and cortices separately in ice-cold lysis buffer (150 mM NaCl, 50 mM Tris pH 7.4, 1 mM EDTA, 0.5% Na-deoxycholate; 1% Igepal CA-630) with protein inhibitors (Roche Applied Science, Indianapolis, IN). After centrifugation at 13,000 rpm at 4°C for 10 min, the supernatants were stored at -80°C until use. Protein concentration was determined by a colorimetric assay, the bicinchoninic acid method (Pierce Protein Research Products, Rockford, IL) and used to calculate volume for equal protein loading.

Cytokine levels were analyzed using the multiplexing immunoassay Rat Cytokine Magnetic Bead Panel (RECYTMAG-65K, Millipore, MA) for TNF α , IL-1 β , IL-6, IL-2, IL-10, and CCL-2 as recommended by the manufacturer. Mean fluorescence intensity values were converted into pg/mL and normalized by protein concentration for each sample.

Nitrate/nitrite concentrations were measured using chemiluminescence. Nitric oxide combined with oxidative species, such as peroxide and superoxide, produces nitrogen radicals. The nitric oxide and nitrogen radicals react with tissue and serum proteins, creating nitrates and nitrites in fluid (NO_x). Concentrations of NO_x provide a measure of acute/subacute nitrosative oxidative stress.

NO_x was measured using a chemiluminescence analyzer (Sievers NOA 280i; GE Analytical Instruments, Boulder, CO) according to manufacturer's protocol. Micromolar NO_x results were normalized by protein concentration for each sample.

Tissue sparing analyses

Whole fresh brains were removed and snap frozen at PID3 and PID50. At PID3, eight rats from each of the three groups were used. At PID50, brains were taken from all twenty rats used for MWM testing. Starting at the hippocampal commissure, 12 equally-spaced $16 \mu\text{m}$ cryosections through the damaged area were obtained from each animal and stained with hematoxylin and eosin. Slides were visualized under a light microscope, photographed at low power to cover the entire section and hemispheric area measurements were performed using Image J (v. 1.47; NIH, Bethesda, MD). Tissue sparing was calculated by dividing the hemispheric volume for the injured side of the brain by the mean volume for the opposite hemisphere, contralateral to impact.^{44,45}

Imaging

Imaging was done at PID12 and PID28. For imaging at PID12, 18 injured rats (DHACCI, $n=9$; REGCCI, $n=9$) and six SHAM rats were tested. A separate set of rats—10 injured (DHACCI, $n=5$; REGCCI=5) and five SHAM—were imaged at PID28. More rats were imaged at PID12 than at PID28 because we anticipated higher variability in T2-measures of edema earlier after injury.

MRI acquisition

Imaging experiments were conducted at PID12 and PID28 using a 7-Tesla horizontal-bore Bruker Biospec MRI scanner (Bruker Biospin, Ettlingen, Germany), interfaced with 12 cm actively shielded gradient insert capable of producing a magnetic field gradient up to 600 mT/m. Rats were anesthetized using 1%-3% isoflurane and 0.8 L/min O_2 and their vital signs (respiration, temperature, heart rate, and oxygen saturation) were continuously monitored using magnetic resonance-compatible physiological monitoring system (SA Instruments, Stony Brook, NY). Rats were placed in a 72-mm volume coil for signal transmission and a quadrature surface coil was placed on the head for signal reception (Bruker Biospin, Ettlingen, Germany).

T2-weighted scans were obtained using a rapid acquisition with relaxation enhancement pulse sequence with a repetition time (TR) of 5000 msec, an effective echo time (TE) of 50 msec, 8 echoes per image, two averages, 30 coronal 0.75 mm-thick slices, a field of view of $3.0 \times 2.5 \text{ cm}$, and an in-plane resolution of $117 \mu\text{m} \times 98 \mu\text{m}$. Diffusion tensor imaging (DTI) scans were acquired using spin echo diffusion-weighted sequence with single-shot echo planar readout, with a TR of 7500 msec, TE of 44 msec, 10 coronal 1 mm-thick slices, a field of view of $3.0 \text{ cm} \times 2.5 \text{ cm}$, and an in-plane resolution of $234 \mu\text{m} \times 195 \mu\text{m}$. Thirty diffusion-weighted gradient directions, uniformly spaced over unit sphere⁴⁶ and five non-weighted images were acquired with two signal averages, with diffusion gradient duration 7 msec, separation 20 msec, and diffusion encoding sensitivity 700 sec/mm^2 .

MRI analyses

Edema volume was quantified using T2-weighted images at PID12 but not at PID28, to avoid confounding effects of late cystic changes in the lesion cavity. Regions of high signal intensities in the T2 images, excluding ventricular zones, were delineated by blinded investigator (MS) using Image J software and expressed as percent of contralateral hemispheric volume.

We used two commonly measured directional indices of water diffusivity: axial diffusivity (AD), parallel to the axonal direction; and, radial diffusivity (RD), perpendicular to the axonal fibers in

homogeneous white matter tracts. Myelinated, undisrupted axons allow faster water diffusion parallel to the axon than perpendicular to it.⁴⁷ We also measured fractional anisotropy (FA), a scalar measure of the anisotropy of diffusion that ranges from zero to one. In the brain, FA is thought to reflect fiber density, axonal diameter, and myelination. Diffusion tensors were estimated on pixel-by-pixel basis using the manufacturer's software (Paravision version 5.1, Bruker Biospin, Ettlingen, Germany) and quantified using the FA index.⁴⁸ Post-processing for DTI was performed using manual region of interest (ROI)-based analysis in Image J and tract-based analysis in DSI Studio (National Taiwan University: <http://dsi-studio.labsolver.org>). We used age-matched SHAM rats to control for the normal developmental changes in myelination, and hence FA, in the immature rat. DTI parameters for SHAM rats were similar to those obtained in naïve rats at the same developmental age.⁴⁹

ROI-based analyses were performed on the DTI scalar maps using Image J software. The ROIs (proximal corpus callosum [CC] and external capsule [EC]) were manually drawn by blinded investigator (M.S.) on the FA maps for both hemispheres through five coronal slices per rat, as illustrated in Figure 5A and 5B, based on the Rat Atlas of Paxinos and Watson as previously described.⁵⁰ The CC and EC were chosen because they are known to be affected by CCI and because these two regions can be more reliably identified even in the presence of a large contusion injury.^{40,51} The CC was further subdivided into the genu, body, and splenium (slices 1, 2-4, and 5, respectively, as shown in Fig. 5C) to control for the known variability in axonal diameter and FA signal from anterior (genu) to posterior (splenium).⁵²⁻⁵⁴

Tract-based analysis for CC fibers connecting the two hemispheres was used to complement ROI-based analysis, a method dependent on visual inspection, given the anatomic disruption in the most lateral portions of the CC resulting from the CCI impact (Fig. 5C). Tractography was performed using imported diffusion-weighted raw magnetic resonance magnitude images to reconstruct pixel-wise diffusion tensors. On the FA images, rectangular ROI seeds were drawn by a separate investigator (O.A.) in the midline part of the CC in serial coronal imaging slices from genu to splenium. The following tracking parameters were used for all rats: FA threshold, 0.2; angular threshold, 20°; step size, 0.05; minimum tract length, 5; maximum tract length, 10; and number of tracts, 1000. The tracking parameters were empirically optimized such

that only CC/EC fibers connecting the two hemispheres were tracked. Any cingulum fibers that were inadvertently tracked were manually segmented out of the final tracking results. For a given tract, DSI studio return averaged measurements of FA, AD, RD, and tract volume for each rat.

Statistical analysis

MWM and rotarod data were analyzed using repeated measures analysis of variance (ANOVA) with Tukey's post hoc tests. NOR results were analyzed using one-way ANOVA. For tissue sparing analyses, significant differences between groups were determined by two-way (treatment x trial) ANOVA. Non-parametric ANOVA was used for all other analyses, using a Kruskal-Wallis test for group first to determine if a difference existed between the three groups and for those with group differences to determine if DHACCI and REGCCI groups differed from each other. Statistical analyses were performed using R version 2.15.1.⁵⁵

Results

Overall survival did not differ between the three study groups and ranged between 97.0% and 98.5%. Similarly, baseline weight (38-39 g) and weight gain after injury (averaging 4.5-4.7 g/d for the first 14 d and 5.8-6.3 g/d thereafter) were not different between groups.

CCI did not affect motor performance at PID12 or PID35 as measured by rotarod testing

For rotation time at PID12, neither the test for interaction between group and time nor the test for group was significant ($F_{4,66}=0.58$, $p=0.68$, and $F_{2,66}=2.22$, $p=0.1$). Average times on the Day 3 trial in seconds were 87.3 ± 37 for DHACCI, 78.4 ± 18 for REGCCI, and 90.1 ± 34 for SHAM rats. Similarly, there were no differences between groups for distance or rotations traveled. Average distances on the Day 3 trial in meters were 3.6 ± 1.9 for DHACCI, 2.9 ± 1 for REGCCI, and 3.7 ± 2 for SHAM rats. Similar results were obtained at PID35; neither the total distance traveled, time spent on the rod nor the number of rotations differed between groups.

FIG. 1. Docosahexaenoic acid (DHA) improved rat pup performance on Morris water maze testing after controlled cortical impact (CCI). The graphs depict the average latency (A) or distance (B) to find the hidden platform as a function of day of testing (post-injury Day [PID]41-PID47) for each group of rats. DHACCI rats are represented by dotted triangles, those who received standard rat chow (REGCCI) by black rectangles, and SHAM rats by white circles. Latency is expressed in seconds $\pm$ standard error of the mean (SEM) and distance in meters $\pm$ SEM; $n=6-7$ /group. * $p < 0.05$ relative to SHAM and DHACCI rats. REGCCI rats took significantly more time and traveled longer distances on PID43 and PID45 than did DHACCI or SHAM rats. Time or distance to reach the visible platform (PID46-PID47) did not differ between groups.

FIG. 2. Docosahexaenoic acid (DHA) increased interleukin (IL)-6, IL-10, and IL-2 protein levels in rat pup hippocampus on Day Two after controlled cortical impact (CCI). (A-F) depict protein levels in CCI hippocampus (HPC) and cortex (COR) ipsilateral to injury measured at post injury Day (PID)1 and PID2. Results are presented as pg/mg $\pm$ standard error of the mean ($n=8$); * $p<0.05$ relative to SHAM rats, & $p<0.05$ relative to those who received standard rat chow (REGCCI). DHACCI rats are shown in gray bars, REGCCI rats in black bars, and SHAM rats in white bars. (A) CCI increased tumor necrosis factor α (TNF α) protein levels at PID1 and PID2 in HPC, but only at PID1 in COR. (B) CCI also increased interleukin 1β (IL- 1β) protein levels at PID1 and PID2 in HPC, but only at PID1 in COR. (C) Chemokine (C-C motif) ligand 2 (CCL2) increased dramatically in both DHACCI and REGCCI tissues relative to SHAM rats during the first 2 d after injury, as shown by the asterisks. Differences from SHAM rats at Day 2 in the cortex did not reach significance because variability in CCI rats was high ($p=0.1$). (D) IL-6 protein increased in both DHACCI and REGCCI tissues relative to SHAM rats during the first 2 d after injury, as shown by the asterisks. At PID2, hippocampal IL-6 protein decreased in REGCCI rats relative to SHAM rats, and increased in DHACCI rats relative to REGCCI and SHAM rats. (E) Hippocampal IL-10 protein increased in DHACCI rats relative to REGCCI and SHAM rats at PID2. (F) Hippocampal IL-2 protein increased in DHACCI rats relative to REGCCI and SHAM rats at PID2.

DHA did not affect memory at PID14 after CCI as shown by novel object recognition testing

Higher novel exploration time represents better memory. Scores of 50%, representing 50% of time exploring the novel object, are no better than those achieved by chance alone. There were no statistically significant differences between groups ($F_{2,18} = 1.94$, $p = 0.17$), though estimated exploration by REGCCI rats relative to SHAM rats nearly decreased ($51\% \pm 4\%$ vs. $67\% \pm 5\%$ sham; $p = 0.07$). DHACCI rats explored similarly to SHAM rats ($63\% \pm 8\%$ vs. $67\% \pm 5\%$).

DHA improved learning at PID41-PID45 after CCI as shown by MWM testing

REGCCI rats performed poorly on MWM testing. DHACCI rats' performance was not different from that of SHAM rats. REGCCI rats' latency and path distance to find the platform were significantly increased relative to both SHAM and DHACCI rats (latency, $F_{2,80} = 4.36$, $p = 0.02$; distance, $F_{2,80} = 3.74$, $p = 0.03$) driven by differences at Day 3 and Day 4 of testing as shown in Figure 1A and 1B, respectively. Latency and path distance to the visible platform on Days 6 and 7 did not reveal any group differences in latency ($F_{2,32} = 0.75$, $p = 0.48$) or path distance ($F_{2,32} = 1.05$, $p = 0.36$). Similarly, neither thigmotaxis nor swimming speed differed between groups. During the probe trial, there were no group differences in percent of time spent in the correct quadrant ($F_{2,16} = 0.49$, $p = 0.62$).

DHA increased IL-6, IL-10, and IL-2 hippocampal cytokine levels at PID2

TNF α , IL-1 β , CCL2, and IL-6 protein levels increased in DHACCI and REGCCI rats relative to SHAM rats in the hippocampus and

cortex at PID1. Hippocampal TNF α , IL-1 β , and CCL2 protein levels increased in DHACCI and REGCCI rats relative to SHAM rats at PID2. Interestingly, mean TNF α and IL-1 β levels in injured rat brain regions ranged between one to four times the corresponding SHAM levels (Fig. 2A, 2B), while CCL2 levels ranged between 10-140 times SHAM levels (Fig. 2C). There were no statistically significant differences between DHACCI and REGCCI rats for TNF α , IL-1 β , or CCL2 at any of the studied time-points.

In contrast, several DHACCI and REGCCI protein levels differed in the PID2 hippocampus. IL-6 protein levels increased in DHACCI relative to REGCCI hippocampi at PID2 (149 ± 7 vs. 115 ± 11 pg/mg, respectively, $\chi^2_1 = 5.34$, $p = 0.02$), as shown in Figure 2D. At PID2, IL-10 and IL-2 protein levels increased in DHACCI relative to REGCCI (4.9 ± 0.3 vs. 2.9 ± 0.4 pg/mg, $\chi^2_1 = 8.65$, $p = 0.003$, and 28.7 ± 1 vs. 20.4 ± 2 pg/mg, $\chi^2_1 = 5.83$, $p = 0.02$, respectively) and to SHAM hippocampi as shown in Figure 2E and 2F. There were no IL-10 or IL-2 differences between groups at the other studied time-points.

DHA decreased nitrate/nitrite (NO $_x$) concentration in the injured cortex at PID1

Hippocampal NO $_x$ did not differ between DHACCI, REGCCI, SHAM groups at PID1 or PID2. In contrast, DHA blunted the CCI-induced increase in cortical NO $_x$ at PID1. Cortical NO $_x$ levels increased in REGCCI rats at PID1 relative to SHAM rats ($4.9 \times 10^{-7} \pm 4.2 \times 10^{-8}$ vs. $3 \times 10^{-7} \pm 3.7 \times 10^{-8}$ mean $\mu\text{mol NO}_x/\mu\text{g protein}$; $\chi^2_1 = 6.35$, $p = 0.01$) but decreased in DHACCI relative to REGCCI rats ($3.2 \times 10^{-7} \pm 2.8 \times 10^{-8}$ vs. $4.9 \times 10^{-7} \pm 4.2 \times 10^{-8}$ mean $\mu\text{mol NO}_x/\mu\text{g protein}$; $\chi^2_1 = 6.35$, $p = 0.01$). By PID2, cortical NO $_x$ levels no longer differed between groups.

FIG. 3. Docosahexaenoic acid (DHA) increased tissue sparing after controlled cortical impact (CCI). (A) The figure shows hematoxylin and eosin (H&E) stained coronal brain slices from rats who received standard rat chow (REGCCI), DHACCI, and SHAM rats with tissue sparing values close to the mean for each group at post-injury Day (PID)3. (B) The figure shows H&E stained coronal brain slices from REGCCI, DHACCI, and SHAM rats with tissue sparing values close to the mean for each group at PID50. Decreased volume loss in DHACCI relative to REGCCI was significant at PID50 (78 ± 3 vs. 71 ± 3 sparing, respectively) and nearly so at PID3 (83 ± 3 vs. $77 \pm 2\%$ sparing, respectively). At PID3 and PID50, both CCI groups had decreased tissue sparing relative to SHAM rats ($p < 0.0001$).

DHA improved tissue sparing at PID50

Tissue sparing in both CCI groups decreased from an average of $79.9\% \pm 2\%$ at PID3 to $74.5\% \pm 2\%$ of contralateral at PID50, as expected given the known time-dependent increase in volume loss after CCI.⁵⁶ As expected, SHAM rats had no hemispheric volume loss over time and thus complete tissue sparing ($103.2\% \pm 1.2\%$ and 98.9 ± 0.9 of contralateral hemispheric volume at PID3 and PID50, respectively). At PID3 and PID50, CCI rats had decreased tissue sparing relative to SHAM rats ($F_{2,17} = 51.3, p < 0.0001$). Between CCI groups, increased sparing in DHACCI rats at PID3 approached but did not reach statistical significance (83 ± 3 in DHACCI vs. $77\% \pm 2\%$ sparing in REGCCI; $F_{1,17} = 4.22, p = 0.056$). At PID50, tissue sparing differences between DHACCI and REGCCI rats ($78\% \pm 3\%$ vs. $71\% \pm 3\%$, respectively) reached statistical significance ($F_{1,17} = 4.82, p = 0.042$). Figure 3A and 3B show PID3 and PID50 slices with tissue sparing values closest to the mean values for the groups.

MRI

T2 results: DHA decreased edema volume as shown by T2 imaging at PID12. At PID12, edema volume in DHACCI rats decreased relative to REGCCI rats. Edema volumes averaged $3.2 \pm 0.6\%$ in DHACCI versus $6.1 \pm 1.3\%$ in REGCCI ($\chi^2_1 = 4.55, p = 0.03$). SHAM rats did not have any edema shown by T2 imaging ($\chi^2_2 = 16.6, p < 0.001$ relative to CCI rats). One representative T2 image per rat is shown for REGCCI, DHACCI and SHAM rats in Figure 4A, 4B, and 4C, respectively.

PID12 DTI results: DHA normalized radial diffusivity in the body of the CC. Diffusivity values are reported in Tables 1, 2, and 3. As expected, FA decreased in both CCI groups relative to SHAM rats in the ipsilateral body ($\chi^2_2 = 8.05, p = 0.02$) and splenium ($\chi^2_2 = 8.23, p = 0.02$) of the CC. FA in the contralateral hemisphere did not differ between groups for any ROI except for the splenium, where again FA decreased in both CCI groups relative to SHAM rats ($\chi^2_2 = 9.74, p = 0.01$). FA did not differ between CCI rats, though it appeared to be somewhat higher in DHACCI relative to REGCCI in the ipsilateral body and bilateral splenia ($\chi^2_1 = 2.39, p = 0.1$ for each ROI).

Both CCI groups showed evidence of white matter injury as shown by decreased AD and increased RD. AD decreased in both CCI groups relative to SHAM rats in the ipsilateral body ($\chi^2_2 = 8.06, p = 0.02$) and bilateral splenia ($\chi^2_2 = 6.82$ and $8.33, p = 0.03$ and 0.02) but did not differ between DHACCI and REGCCI rats. DHA normalized the increased RD induced by CCI in the contralateral body of the CC ($\chi^2_1 = 8.24, p = 0.004$ between DHACCI and REGCCI). Increased RD in the contralateral splenium and the ipsilateral genu in REGCCI rats relative to SHAM rats approached but did not reach statistical significance ($\chi^2_2 = 5.68, 5.75$ and $p = 0.06$ for each ROI). There were no FA, AD, or RD differences between groups in the ipsilateral or contralateral EC.

PID28 DTI results: DHA did not modulate CCI effects on DTI indices. Diffusivity values are reported in Tables 1, 2, and 3. As expected, FA decreased in both CCI groups relative to SHAM

FIG. 4. Docosahexaenoic acid (DHA) decreased hemispheric edema ipsilateral to impact at Day 12 after controlled cortical impact (CCI). These are representative T2 images of rat pups at post-injury Day (PID)12. Each image corresponds to one of the 12–13 slices analyzed per rat, encompassing cerebral cortex and midbrain as outlined in the Methods. Areas of T2-intense (white) regions, excluding those within the lateral ventricles or extra-axial locations such as the scalp, were normalized to contralateral hemispheric volume. (A) Representative T2 images for REGCCI (i.e., those who received standard rat chow) rat pup brains. (B) Representative T2 images for DHACCI rat pup brains. (C) Representative T2 images for SHAM rat pup brains.

FIG. 5. Region of interest (ROI)-based and tract-based diffusion tensor imaging (DTI) analyses. **(A)** This is a schematic illustrating the ROIs (ipsilateral and contralateral corpus callosum and external capsule), drawn at the level of the splenium. For comparison, fractional anisotropy (FA) image number 5 in **(B)** is taken at the level of the splenium. **(B)** FA images of a SHAM rat brain are shown to illustrate the corpus callosum (CC) sections used in ROI-based DTI analyses. Image 1 shows the genu, or anterior CC, and image 5 the splenium, or posterior CC. Images 2-4 show the slices between genu and splenium, or the slices averaged to obtain DTI values for the body of the CC. DTI values for the external capsule (EC) resulted from the average of all slices. **(C)** These FA images illustrate tract-based DTI analysis, using representative sham and controlled cortical impact (CCI) brains. Tract-based analyses of the CC were undertaken to complement ROI-based studies given that the anatomic disruption after CCI, as evident in this image, hindered visual analyses in more lateral regions of the CC and EC. On the FA images, rectangular ROI seeds were drawn in the midline part of the CC in serial coronal imaging slices from genu to splenium. The tracking parameters were empirically optimized to track only CC/EC fibers connecting the two hemispheres. Color image is available online at www.liebertpub.com/neu

rats in the EC bilaterally, in the ipsilateral body and in bilateral splenia ($\chi^2_2=9.14, 7.02, 7.28, 10.26$ and 9.38 ; $p<0.03$ for all). DHACCI rats showed a slight trend towards increased FA relative to REGCI rats in the ipsilateral splenium ($\chi^2_1=3.15, p=0.08$).

Consistent with white matter injury, AD decreased and RD increased in both CCI groups relative to SHAM rats in the ipsilateral splenium ($\chi^2_2=7.26$ and 9.62 ; $p<0.03$ for both). RD increased in both CCI groups relative to SHAM rats in the ipsilateral body, and the contralateral splenium and EC ($\chi^2_2=7.94, 6.32$, and 7.26 ; $p<0.05$).

Tract-based analyses. Tract-based analyses supported the direction of ROI-based results, though they were generally less sensitive to the regional effects of CCI. Specifically, RD results showed a similar direction but were less commonly significant. For example, RD of the tracked fibers appeared to increase in both CCI groups relative to SHAM rats ($\chi^2_2=5.61, p=0.06$) and in REGCCI relative to DHACCI ($\chi^2_1=3.70, p=0.05$) at PID12. At PID28, RD increased in both CCI groups relative to SHAM rats ($\chi^2_2=9.13, p=0.01$) but RD did not differ between DHACCI and REGCCI groups. Similar to ROI-based analyses, there were no differences in FA or AD between the DHACCI and REGCCI groups at PID12 or PID28.

Tract volumes decreased in both CCI groups relative to SHAM rats at PID12 and PID28 ($\chi^2_2=7.81$ and 10.8 ; $p=0.02$ and 0.004 , respectively) but did not differ between CCI groups at either time-point.

In contrast to ROI-based results showing that CCI generally decreased FA relative to sham injury, on tract-based analysis FA did not differ between groups at PID12 ($\chi^2_2=4.81, p=0.09$) or 28 ($\chi^2_2=5.08, p=0.08$). Similarly, CCI generally decreased AD relative to sham injury in ROI-based but not in tract-based analyses ($\chi^2_2=4.36$ and $2.04, p=0.1$ and 0.4 at PID12 and PID28 for tractography data).

Discussion

Dietary DHA provided before experimental TBI and continued thereafter decreased long-term cognitive dysfunction and tissue loss in rat pups, associated with increased brain IL-10 levels and decreased brain oxidative stress, edema, and DTI indices of white matter injury after CCI.

This study contributes important new information on the effects of DHA on cognitive function, tissue loss and imaging after experimental TBI in the immature brain. Russell and colleagues²⁷ reported that DHA improved motor function 7 d after CCI in the

TABLE 1. FRACTIONAL ANISOTROPY

ROI	PID12			PID28		
	DHACCI	REGCCI	SHAM	DHACCI	REGCCI	SHAM
Body Ipsi	0.465 ± 0.011*	0.441 ± 0.009*	0.523 ± 0.023	0.456 ± 0.024*	0.447 ± 0.030*	0.542 ± 0.016
Body Con	0.490 ± 0.010	0.457 ± 0.014	0.505 ± 0.025	0.502 ± 0.022	0.508 ± 0.012	0.558 ± 0.017
Genu Ipsi	0.594 ± 0.012	0.570 ± 0.014	0.552 ± 0.017	0.639 ± 0.043	0.558 ± 0.049	0.572 ± 0.022
Genu Con	0.565 ± 0.020	0.541 ± 0.019	0.545 ± 0.020	0.625 ± 0.035	0.558 ± 0.050	0.589 ± 0.024
Splen Ipsi	0.567 ± 0.029*	0.524 ± 0.022*	0.637 ± 0.024	0.553 ± 0.039*	0.453 ± 0.030*	0.733 ± 0.027
Splen Con	0.569 ± 0.020*	0.524 ± 0.025*	0.649 ± 0.024	0.545 ± 0.020*	0.555 ± 0.010*	0.725 ± 0.016
EC Ipsi	0.416 ± 0.014	0.418 ± 0.015	0.458 ± 0.035	0.424 ± 0.027*	0.424 ± 0.027*	0.458 ± 0.035
EC Con	0.432 ± 0.021	0.440 ± 0.026	0.461 ± 0.033	0.482 ± 0.018*	0.482 ± 0.018*	0.461 ± 0.033

*PID, post-injury Day; ROI, region of interest; DHACCI, Docosahexaenoic acid controlled cortical impact group; REGCCI, standard rat chow controlled cortical impact group; Ipsi, ipsilateral; Con, contralateral; Splen, splenium; EC, external capsule.

17 d old rat. Our study complements their findings by extending the observation period and by adding cognitive testing, histology, and imaging outcomes. Our model differs from that of Russell and colleagues in two ways. First, their CCI parameters should produce a more severe brain injury than ours. Russell and colleagues used a greater depth (3 mm rather than 2 mm) and smaller diameter (3 mm, rather than 5 mm) of impact for a longer duration (300 msec rather than 100 msec) than we employed.⁵⁷ Second, DHA administration was slightly different. They gavaged fed rat pups daily with DHA for 7 d, commencing 30 min before CCI. We changed the DHA content of the rat chow given to the dam the day before CCI until the end of each experiment. DHA dosing is not directly comparable between the two models. We estimate that our rat pups consumed 150–200 mg DHA /kg/d in the first week of life, while rat pups in their study received 134 mg/kg/d DHA. In both studies, DHA was safe, well tolerated, and improved outcomes when given before CCI and continued daily thereafter.²⁷

DHA improved rat pup cognitive dysfunction induced by CCI as shown by MWM testing. CCI in the 17 d old rat produces histologic hippocampal damage and impairs performance on the MWM, a classic test of hippocampus-based learning and memory.^{58–60} Effects of DHA on recognition memory could not be ascertained because CCI did not impair NOR performance in our model. We speculate that CCI at P17 impairs rat performance on NOR testing to a lesser degree than on MWM testing. In agreement with this, others have noted that CCI has inconsistent effects on NOR performance.⁶¹ Similarly, we were unable to test DHA effects on motor dysfunction after CCI in the immature brain because our model did not produce motor impairments detectable by rotarod nor by visible platform testing. Russell and colleagues showed that

DHA improved rat pup motor function 7 d after a CCI injury that was likely more severe than ours.²⁷ We speculate that DHA effects on motor dysfunction are most evident when greater motor deficits are present.

DHA improved long-term cognitive function in rat pups after CCI, associated with decreased tissue loss in the injured hemisphere. Mechanisms of DHA's neuroprotection after TBI are unknown in rats of any age. DHA improved cognitive outcomes in adult rats after TBI, associated with decreased axonal and oxidative injury.^{23,26} DHA improved function in adult rats after experimental spinal cord injury, associated with decreased spinal cord tissue loss, edema, and inflammation.⁶² Similarly, in adult rats after cerebral ischemia, DHA decreased brain tissue loss, edema, and inflammation.⁶³ We speculate that the improved long-term cognitive function and tissue sparing we observed in DHACCI rats may have resulted from early decrements in edema, oxidative stress, and inflammation.

DHA decreased cerebral edema, as measured by T2 imaging, in rat pups after CCI. Previous studies support the possibility that DHA decreases cerebral edema by decreasing inflammation and blood–brain barrier breakdown, important mediators of cerebral edema after TBI.^{64,65} In adult rats after ischemic stroke, DHA reduced cerebral edema and neuroinflammation.⁶⁶ Russell et al reported that DHA reduced blood–brain barrier breakdown and decreased TBI-induced upregulation of matrix metalloproteinase 9 (MMP9), a pro-inflammatory protease important in blood–brain barrier breakdown.^{27,67} Our findings add to the existing evidence that DHA decreases cerebral edema after TBI.

DHA blunted the increase in NO_x, a measure of oxidative stress, in rat pup brain after CCI. DHA could directly blunt oxidative stress

TABLE 2. AXONAL DIFFUSIVITY

ROI	PID12			PID28		
	DHACCI	REGCCI	SHAM	DHACCI	REGCCI	SHAM
Body Ipsi	1.191 ± 0.027*	1.236 ± 0.035*	1.327 ± 0.030	1.295 ± 0.020	1.299 ± 0.033	1.270 ± 0.036
Body Con	1.205 ± 0.018	1.280 ± 0.035	1.301 ± 0.032	1.290 ± 0.013	1.266 ± 0.048	1.295 ± 0.038
Genu Ipsi	1.405 ± 0.039	1.526 ± 0.056	1.469 ± 0.054	1.579 ± 0.073	1.592 ± 0.057	1.548 ± 0.105
Genu Con	1.401 ± 0.029	1.519 ± 0.049	1.433 ± 0.032	1.681 ± 0.059	1.582 ± 0.024	1.445 ± 0.077
Splen Ipsi	1.354 ± 0.052*	1.302 ± 0.074*	1.579 ± 0.057	1.414 ± 0.069*	1.423 ± 0.043*	1.715 ± 0.067
Splen Con	1.391 ± 0.050*	1.347 ± 0.067*	1.633 ± 0.062	1.448 ± 0.052	1.373 ± 0.147	1.490 ± 0.016
EC Ipsi	1.213 ± 0.075	1.283 ± 0.054	1.304 ± 0.057	1.249 ± 0.038	1.249 ± 0.038	1.304 ± 0.057
EC Con	1.157 ± 0.030	1.274 ± 0.043	1.263 ± 0.036	1.306 ± 0.034	1.306 ± 0.034	1.263 ± 0.036

*PID, post-injury Day; ROI, region of interest; DHACCI, docosahexaenoic acid controlled cortical impact group; REGCCI, standard rat chow controlled cortical impact group; Ipsi, ipsilateral; Con, contralateral; Splen, splenium; EC, external capsule.

TABLE 3. RADIAL DIFFUSIVITY

ROI	PID12			PID28		
	DHACCI	REGCCI	SHAM	DHACCI	REGCCI	SHAM
Body Ipsi	0.577±0.020	0.625±0.020	0.587±0.031	0.627±0.038*	0.636±0.040*	0.527±0.014
Body Con	0.553±0.014*&	0.632±0.025*	0.602±0.035	0.567±0.017	0.552±0.019	0.525±0.015
Genu Ipsi	0.508±0.021	0.577±0.019	0.568±0.025	0.493±0.035	0.562±0.069	0.516±0.013
Genu Con	0.526±0.033	0.526±0.033	0.526±0.033	0.513±0.022	0.606±0.081	0.514±0.016
Splen Ipsi	0.508±0.034	0.538±0.031	0.473±0.019	0.552±0.036*	0.671±0.059*	0.378±0.020
Splen Con	0.500±0.016	0.545±0.016	0.472±0.023	0.557±0.020*	0.525±0.060*	0.387±0.013
EC Ipsi	0.630±0.043	0.696±0.037	0.6510±0.052	0.643±0.034	0.781±0.103	0.553±0.008
EC Con	0.601±0.020	0.665±0.025	0.617±0.024	0.626±0.018*	0.625±0.017*	0.544±0.022

*PID, post-injury Day; ROI, region of interest; DHACCI, docosahexaenoic acid controlled cortical impact group; REGCCI, standard rat chow controlled cortical impact group; Ipsi, ipsilateral; Con, contralateral; Splen, splenium; EC, external capsule.

by acting as a free radical scavenger, as it does in retinal cells, and/or indirectly by decreasing inflammation and microglial nitric oxide production as supported by in vitro studies.^{62,68,69} By PID2, increased NO_x levels in CCI brains were no longer statistically different from that in SHAM brains. The short-lived nature of NO_x elevation in our model is similar to that reported in adult rats after experimental TBI.^{70,71} Future studies on potential mechanisms for DHA's antioxidant activity after developmental TBI are warranted.

Cytokine results suggest that DHA had anti-inflammatory and potentially neuroprotective immunomodulatory effects in the rat pup brain after CCI. DHA did not change post-CCI increases in TNF α , IL-1 β , or CCL2. DHA increased IL-10, IL-6, and IL-2 in rat hippocampus at PID2 relative to REGCCI and SHAM rats. IL-10 limits the inflammatory response and regulates immune cell differentiation and proliferation.⁷² A study showing that IL-10 administration improved outcome in adult rats after TBI suggests that DHA's effect on IL-10 levels could be neuroprotective.⁷³

Interpretation of IL-6 and IL-2 levels after TBI is complicated, in part because each has complex actions on the immune response. IL-6 is a pleiotropic cytokine with "context-dependent pro-and anti-inflammatory properties."⁷⁴ Increased IL-6 levels in blood or cerebrospinal fluid is associated with poor outcome after TBI in adult patients.^{75,76} On the other hand, a cerebral microdialysis study in human patients showed that increased parenchymal IL-6 levels correlated with improved outcome after TBI and, in a mouse focal brain injury model, astrocyte-targeted increased expression of IL-6 reduced lesion volume.^{77,78} In summary, increased IL-6 expression is associated with neuroprotection, as well as with increased severity of TBI.⁷⁹ Little is known about the role of IL-2 after TBI. Brain-derived IL-2 is a neurotrophic factor that is required for immune homeostasis in the brain.⁸⁰ In a traumatic nerve injury model using axotomy, transgenic mice lacking brain-derived IL-2 showed increased microglial activation.⁸¹ We did not find any published studies on the effect of either increasing IL-2 brain expression or administering IL-2 on TBI outcomes.

We assessed the effects of DHA on DTI indices of white matter injury after CCI. To our knowledge, DHA effects on DTI or T2 imaging after brain injury have not been reported previously. DTI changes correlate well with axonal injury in adult mice after experimental TBI and in neonatal rats after experimental stroke. In both stroke models, histologic axonal damage correlated with decreased FA and increased RD.^{40,41} DTI indices of white matter injury, including decreased FA and increased RD, correlated with poor neurologic outcome in adult humans after TBI and in neonates after hypoxic ischemic injury.^{82,83} Interestingly, increased RD and decreased FA also correlated with poor neurologic outcomes in

children after TBI.⁸⁴ We found that DHA decreased the CCI-induced increment in RD at PID12, suggesting that DHA decreased axonal injury in rat pups after CCI. Further study is needed to elucidate if DHA modulates white matter injury after developmental TBI.

Our study has several limitations. First, our results are limited by the pre-treatment algorithm and by the exclusion of female and DHA-treated SHAM rats. In the absence of any published data on DHA and developmental TBI at the time of study inception, we designed exploratory studies to determine if DHA had any effect on outcomes in our existing rat pup CCI model. As explained in the Methods, we used a pre-injury treatment algorithm to avoid the risk of aspiration and we limited our study to male rats to avoid confounding effects of sex. In addition, based on literature showing that DHA had no effect on naïve rat pup performance on MWM testing, we excluded a DHA-treated sham group to minimize the number of rats needed to answer the experimental question.⁸⁵ We recently modified our algorithm to include parenteral DHA to allow exclusive post-CCI DHA administration. Thus far, our preliminary data supports equivalent effects on cognitive outcome using this modified DHA treatment algorithm. Future studies will incorporate female and DHA-treated SHAM rats.

Second, our DTI results are not directly comparable to humans because rat brains have significantly less white matter than do human brains.⁸⁶ We speculate that DHA would have more dramatic effects on DTI indices in a gyrencephalic animal model of TBI. DTI results are limited also by the type of injury. While CCI is a validated and useful model of pediatric TBI, it induces anatomic distortions that complicate DTI analyses. While tractography analyses mitigate these anatomic distortions, such analyses lose region-specific sensitivity to the effects of CCI and DHA. Our results support the utility of complementary studies to examine DHA effects on DTI outcomes after diffuse TBI.

A third limitation is our use of a DHA-enriched diet rather than a fixed dose of DHA. However, based on an extrapolation to humans, our dose is comparable to the dosing regimens being tested in clinical trials (1.5-2 g/d of DHA) for adults and adolescents after concussion (NCT01814525 and NCT 01903525).

We report for the first time that dietary DHA, given before CCI and continued daily, improved long-term rat pup outcomes after CCI. Specifically, DHA improved cognitive function, tissue sparing, and DTI indices of white matter injury after CCI, associated with decreased edema and oxidative stress. DHA also modulated the cytokine response in a manner suggesting anti-inflammatory and neuroprotective activity. Our results are important because they will guide future preclinical studies and suggest avenues for

research into neuroprotective mechanism(s) of DHA after TBI in the immature brain. Based on our findings, DTI can be a useful tool to study DHA effects after experimental TBI, particularly if a gyrencephalic animal and/or a diffuse injury model is used. Building on these initial results, we are using a more translationally-relevant algorithm in which DHA is given post-injury to glean information on DHA dosing, timing window and measures of efficacy. Finally, our results suggest that putative mechanisms for DHA neuroprotection in the developing brain after TBI include free-radical scavenging and/or immunomodulatory effects after injury.

DHA is an appealing candidate therapy for pediatric TBI. It has an excellent safety record in non-TBI pediatric studies that span the developmental spectrum between infancy and adolescence.^{18,19,87} Our results complement those of Russell and colleagues²⁷ in showing that DHA is safe and neuroprotective after experimental TBI in rat pups, and pave the way for more research to enable clinical testing. In light of a recent report of dramatic improvement following DHA administration in a teenager after severe TBI, such research is sorely needed.⁸⁸

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Author Disclosure Statement

No competing financial interests exist.

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