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Effects of Controlled Cortical Impact and Docosahexaenoic Acid on Rat Pup Fatty Acid Profiles

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Abstract

Traumatic brain injury (TBI) is the leading cause of acquired neurologic disability in children, particularly in those under four years old. During this period, rapid brain growth demands higher Docosahexaenoic Acid (DHA) intake. DHA is an essential fatty acid and brain cell component derived almost entirely from the diet. DHA improved neurologic outcomes and decreased inflammation after controlled cortical impact (CCI) in 17-day old (P17) rats, our established model of pediatric TBI. In adult rodents, TBI decreases brain DHA. We hypothesized that CCI would decrease rat brain DHA at post injury day (PID) 60, blunted by 0.1% DHA diet. We quantitated fatty acids using Gas Chromatography–Mass Spectrometry. We provided 0.1% DHA before CCI to ensure high DHA in dam milk. We compared brain DHA in rats after 60 days of regular (REG) or DHA diet to SHAM pups on REG diet. Brain DHA decreased in REGCCI, not in DHACCI, relative to SHAMREG. In a subsequent experiment, we gave rat pups DHA or vehicle intraperitoneally after CCI followed by DHA or REG diet for 60 days. REG increased brain Docosapentaenoic Acid (n-6 DPA, a brain DHA deficiency marker) relative to SHAMDHA and DHACCI pups ($p<0.001$, diet effect). DHA diet nearly doubled DHA and decreased n-6 DPA in blood but did not increase brain DHA content ($p<0.0001$, diet effect). We concluded that CCI or craniotomy alone induces a mild DHA deficit as shown by increased brain DPA.

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Keywords

developing brain; traumatic brain injury; nutrition; polyunsaturated fatty acids; neuroprotection

1. Introduction

Nearly 40,000 children sustain severe TBI annually in the United States, adversely affecting as many as 1.3 million life-years. [1] Half of all children who survive sustain major neurologic disability.[2, 3] Oxidative stress and inflammation exacerbate the damage induced by mechanical forces alone. Children younger than age four are at greater risk for poor neurologic outcome after severe TBI than are older children and young adults. [4–6] These worse outcomes may relate to the immature brain's low antioxidant reserve [7, 8] and vigorous inflammatory response.[9–13]

Effective therapies for TBI at any age are lacking. DHA, or Docosahexaenoic Acid (C22:6n-3) is a candidate therapy for childhood TBI for several reasons. First, it is a direct antioxidant and anti-inflammatory agent that is already widely available as a nutritional supplement for infants and children. [14–17]. Second, in adult and immature rats after experimental TBI, DHA improved neurologic outcomes. [18–24] Third, DHA intake may be particularly relevant to young children after TBI since baseline DHA needs for a rapidly growing brain are already high. Indeed, DHA is the most abundant polyunsaturated fatty acid in the brain, second only to Arachidonic Acid (AA, C20:4n-6). To our knowledge, data on brain DHA content after TBI incurred during the developmental brain growth period are lacking.

Under normal conditions, DHA is not present as a free fatty acid in the brain but instead is found esterified into membrane phospholipids of neural cells (neurons, astrocytes, microglia and oligodendrocytes), synaptosomes, myelin, and mitochondria thus constituting a critical "building block" of the brain. [25–30] Postnatal DHA accrual depends almost entirely upon dietary intake because endogenous synthesis of DHA from alpha-linoleic acid (ALA), another essential fatty acid, is very limited in mammals.[25, 31] In humans, less than 0.05% of absorbed ALA is used to synthesize DHA. [32] DHA is available in specific foods, such as fatty fish. The greatest DHA accrual into the brain occurs in utero and during rapid postnatal brain growth. [30] Between 30 weeks of gestation to 18 months of life, human brain DHA content increases from 90 mg to 3300mg. [30, 33] While greater prematurity is associated with larger deficits in DHA accumulation in the fetus [34], a relative DHA deficiency can occur in full term infants not fed human milk. [35]

Dietary DHA deficiency alters membrane composition first by increasing the contribution of Docosapentenoic Acid or n-6DPA (C22:5n-6) as a substitute for DHA, suggesting a requirement for very long chain highly unsaturated fatty acids in the brain. [30, 36, 37] Relative to n-6DPA, DHA with its additional double bond is more flexible and isomerizes with shorter correlation times, a difference that may be of particular importance to the function of integral membrane proteins. [38]

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In adult mice and juvenile rats, a pre-existing DHA deficiency is associated with worse outcome after experimental TBI.[39, 40] In light of the importance of adequate dietary DHA to support rapid brain growth during development, DHA administration may be particularly relevant to the immature brain after TBI. Further, experimental evidence suggesting that rats have a greater capacity to synthesize DHA from ALA than do humans heightens the potential importance of dietary DHA in human TBI. [32, 41] TBI itself could result in an acquired DHA deficiency, because it creates low ATP conditions such that reutilization of DHA via re-esterification, a high-energy process, is not possible. [42] In the face of low ATP reserves after TBI, DHA released from disrupted neural membranes would be lost. [43] High cerebrospinal fluid DHA levels in patients after TBI support the possibility that DHA loss accrues after TBI in humans.[44] In rats, TBI decreases long-term brain DHA content. [45]

The objective of the present study was to test the hypothesis that TBI during development will decrease DHA content, expressed as a percentage of total fatty acids in the injured hemisphere at adulthood, blunted by daily DHA supplementation. To test this hypothesis, we used our established model of pediatric TBI using controlled cortical impact (CCI) to the left parietal cortex or SHAM injury as described by our group. [19, 46–51] This is a classic, well-characterized model of pediatric TBI. [52–56] We collected brain hemispheres ipsilateral to injury and blood at PID60. CCI We measured DHA, ALA, AA, n-6DPA and total fatty acids from rats randomized to DHA-supplemented or regular diet, as outlined in the Methods section.

2. Experimental Procedure

2.1 Animals

All experimental protocols were approved by the Animal Care and Use Committees at the University of Utah, in accordance with US NIH guidelines and carried out at the University of Utah.

Male Sprague-Dawley rats were obtained from Charles Rivers Laboratories (Raleigh, NC) on post-natal day (P) 7–10. To minimize litter effects in the experimental groups, Charles River culled rats from different litters for shipment to our laboratory to generate groups of ten male pups per dam, which we housed in litters of 10 with the lactating dam upon their arrival until weaning on P 21—23. We further minimized litter effects on the impact of diet or injury by randomizing rat pups in the same litter to either CCI or SHAM surgery within the same diet. To decrease the effect of a common dam for rats on a same diet, for Experiment 2 we used 2 dams to generate the rat pups in each of the study groups. For experiments 1 and 2, we used 7 and 9 rats per group, respectively. For experiment 1, we used a total of 3 litters. For experiment 2, we used a total of 4 litters. Rats were housed in groups of 3–5 rats per cage after weaning. All cages were kept in a temperature- and light-controlled (12 h on/12 h off) environment.

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2.2 DHA Administration

All dams and pups received REG diet (Envigo Teklad 2920X), with the exception of rats in the DHA groups. The DHA rodent diet (TD.150715, Envigo Teklad, WI) substitutes 0.1% of the soybean oil in Envigo Teklad 2920X with purified DHA (U-84-A, Nu-Chek Prep, MN). This substitution results in the same macronutrient content and caloric density (3.2 kcal/g and 15% kcal derived from fat) as standard rat chow. This 0.1% DHA diet provides DHA as 3.3% of total fat and is within the reported safe intake for rats and humans (Lien, 2009). Nutritional content is listed in Table 3 for the two diets.

P17 rats depend exclusively on dam milk for their intake. We chose not to gavage rat pups right after surgery to avoid the risk of pulmonary aspiration. We fed dams the DHA diet one day before CCI or SHAM surgery guided by human data that breast milk DHA peaked at 10hr and lasted 24hrs after ingestion of a DHA supplement.[57]. For Experiment 1, we implemented DHA diet on the day before surgery and used three groups: DHACCI, REGCCI and SHAMREG. To increase translational relevance by starting DHA on the day of injury, while still avoiding aspiration risk, we later conducted a pilot study and then Experiment 2 in which we gave rat pups intraperitoneal DHA on the day of injury. For Experiment 2, we gave one dose of DHA or Vehicle (VEH) intraperitoneally (IP) to rat pups 30 minutes after CCI or SHAM injury, to create four groups: DHACCI, REGCCI, SHAMDHA and SHAMREG.

For Experiment 1, all litters remained on REG or DHA diet, as received on the day before CCI, until PID 60. For Experiment 2, the REG diet was replaced with DHA diet for half the dams and their respective litters after surgeries ended that day. All litters remained on REG or DHA diet, as received on the day of CCI. For both Experiments, once rats were weaned (at P21) they consumed exclusively the DHA/REG chow for the duration of each experiment.

To determine the dose of IP DHA, we first conducted a pilot study to compare DHA blood levels between rat pups receiving 1, 3 or 7 mg DHA or vehicle IP 15 min after surgery. These pilot DHA doses were extrapolated to equal the estimated DHA intake in our rat pups, based on average rat milk intake at that age and published data on milk DHA content from dams with and without DHA supplementation. [58] DHA or vehicle (VEH) was prepared for injection as follows: DHA (0.3 mL of 1ml vial containing 250mg DHA in ethanol, Cayman Chemical Company, MI) and VEH (Intralipid, a 20% phospholipid-stabilized soybean oil emulsion, Sigma and 70% ethanol) to deliver desired dose in a volume of 0.1 mL per rat pup. The ≈7mg dose of DHA raised DHA blood levels (to 141% of VEH-treated rat pups) so we elected not to use the lower doses for Experiment 2.

Based on the pilot results, P17 rat pups randomized to DHA received 0.1 mL IP of a 75 mg/mL DHA solution (7.5 mg DHA) and those randomized to Regular Diet (REG) received 0.1 mL vehicle IP 15 minutes after either controlled cortical impact (CCI) or control (SHAM) procedure ended. The IP dose is approximately 185 mg/kg, close to the expected 100 –150 mg/kg/day DHA intake by rats consuming 0.1% DHA diet.

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2.3 CCI procedure

CCI was carried out as previously described [48]. 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±0.5 °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×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 not to 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/s velocity and 100 ms 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.

2.4 DHA brain content at PID 60 (Experiment 1 and 2) and DHA blood content at PID60 (Experiment 2)

At PID 60, rats were euthanized using isoflurane anesthesia and transcardially perfused with PBS. For Expt. 2, blood was collected into EDTA-containing tubes. Brain hemispheres were divided and each side (left, or ipsilateral to injury, and right, or contralateral to injury) was placed in 2ml bead mill tubes containing 1.4 mm diameter ceramic beads (MoBio Quiagen, CA). Brain tissue and blood samples were snap frozen and stored at −80C for later use.

Red cell membrane samples were obtained by thawing frozen blood on ice, followed by centrifugation of whole at 500×g for 10 min at 4 °C. The supernatant (plasma) was aspirated, then phosphate-buffered saline (PBS) was added as a cell wash buffer to the red cell pellet at a v: v ratio of 4:1. The resulting solution was transferred to a 15ml falcon tube. The tube was then centrifuged at 500xg for 10 min at 4 degrees C, again the supernatant was aspirated, and the process repeated until the pellet was clean. Cell wash buffer 1ml was added to the red cell pellet to make a final volume of approx. 2ml, then the tubes were frozen at −80C until ready for processing.

Lipids were extracted by transferring frozen tissue into bead-mill tubes then adding 1 mL of 5% HCl in methanol containing internal standards (FA 13:0 and FA 21:0 at 50 μg/mL) and homogenized in one 30-sec cycle using a bead homogenizer (Powerlyzer 24, Quiagen). Homogenates were centrifuged at 20,000*g* for 10 min and the supernatant was transferred to 13 × 100 mm culture tubes with PTFA lined cap. For red blood cells, lipids were extracted by transferring 100 μL of sample into 13 × 100 mm culture tubes with PTFA lined cap and then adding 1 mL of 5% HCl in methanol containing internal standards (FA 13:0 and FA 21:0 at 5 μg/mL). Extracts were derivatized to their methyl esters by heating in a sand bath

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at 80 °C for a minimum of 4 hrs. The resulting methanolysis reaction products were extracted with hexanes (2 × 2 mL), vortexed for 15 sec and then centrifuged for 4 min at 4,000*g*. Supernatants were transferred to new culture tube and dried under a gentle nitrogen stream. Samples were resuspended in 250 μL ethyl acetate and transferred to GC-MS vials with insert. Concurrently a process blank and pooled QC sample were prepared. Samples were extracted in randomized order.

GC-FID analyses were conducted using an HP6890 instrument interfaced with a flame ionization (FID) detector and equipped with a DB-23 (60 m × 0.25 mm ID, 0.15 μm film thickness; Agilent Technologies, Inc.) column and an HP7682 injector. Helium is used as a carrier gas with a 10:1 split ratio at an injection volume of 1 μL in a randomized injection sequence. The injector temperature is 250 °C. The oven temperature gradient was programmed as follows: 50 °C held for 1 min, increased at a rate of 25 °C/min to 175 °C, increased at a rate of 4 °C/min to 230 °C and held for 10 min. Detector temperature set at 280 °C. Hydrogen: 40 mL/min; Air: 450 mL/min; Helium make-up gas: 30 mL/min. The fatty acid methyl esters were identified by comparison of retention times with authentic standards (Supelco 37 component FAME mix (Sigma), DHA C22:5n3 and n-6DPA C22:5n6 (Cayman)). Results from GC-FID experiments were collected using Chemstation and analyzed using the software packages Agilent GC-MS Translator and Agilent Masshunter Quantitative Analysis B.07.00 (Agilent Technologies, Inc.). FAMES were quantitated based on peak area ratios relative to spiked internal standard FA 13:0.

2.5 Statistics

Investigators blinded to experimental groups performed all data acquisition and analyses. Analyses for enteral only administration were limited to three groups, so we used one-way analysis of variance (ANOVA) and n=6 per group. For the parenteral followed by enteral administration, we used two-way ANOVA and n=8 per group. For both studies, we followed ANOVA by the Holm-Sidak test for multiple comparisons using GraphPad® Prism 6.0 (GraphPad® Software, CA).

3. Results

In Experiment 1, brain DHA content of the injured hemisphere did not differ between CCI rats treated with DHA diet before and after injury and SHAM rats on a regular diet (DHACCI 17.2 ± 2.9 vs SHAMREG 16.5 ± 1.8% total fat, p=0.06) at PID 60. In contrast, DHA content decreased in CCI rats on a regular diet compared to SHAM rats on the same diet (REGCCI 15.95 ± 1.2% total fat vs SHAMREG 16.5 ± 1.8%, p=0.03) and to CCI rats treated with DHA diet before and after injury (REGCCI 15.95 ± 1.2% total fat vs DHACCI 17.2 ± 2.9 %, p=0.002). Neither brain DPA (DPA5n-6, or C22–5n-6), Arachidonic Acid (C20–4n-6) nor the n-3/n-6 ratios differed between these three groups.

In Experiment 2, brain DHA content differed between the four groups at PID 60 (DHACCI 18.9 ± 0.24, REGCCI 17.5 ± 0.16, SHAMDHA 19.8 ± 0.3 and SHAMREG 18.1 ± 0.19% total fat) largely as a factor of diet (p<0.0001) but also as a function of injury (p=0.004) driven by differences between SHAMDHA and DHACCI (adjusted p value =0.02) while SHAMREG and REGCCI did not differ significantly (adjusted p value=0.2) as shown in

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Table 1. DHA diet decreased brain n-6 DPA in both SHAMDHA and DHACCI rats relative to REGCCI and SHAMREG (DHACCI 0.98 ± 0.13 and SHAMDHA 0.6 ± 0.07 vs REGCCI 1.37 ± 0.09 and SHAMREG 1.47 ± 0.11% total fat, $p<0.001$ effect of diet, $p=0.03$ effect of injury). Similarly, DHA diet increased brain n-3/n-6 ratio (DHACCI 1.13 ± 0.02 and SHAMDHA 1.21 ± 0.01 vs REGCCI 1.00 ± 0.02 and SHAMREG 1.03 ± 0.01 total fat, $p<0.0001$ effect of diet, $p=0.0007$ effect of injury). Wet brain weights did not differ between groups DHACCI (474 ± 10 and SHAMDHA 482 ± 14 vs REGCCI 459 ± 13 and SHAMREG 457 ± 19 mg)

Red blood cell membrane fatty acid profiles in Experiment 2 are shown in Table 2. PID 60 red blood cell membrane DHA in rats treated after CCI or SHAM surgery with either vehicle or DHA increased as a result of diet (DHACCI 3.9 ± 0.25 and SHAMDHA 4.0 ± 0.1 vs REGCCI 2.1 ± 0.1 and SHAMREG 2.3 ± 0.1 % total fat, $p<0.0001$ effect of diet, $p=0.8$ effect of injury). Red blood cell membrane DPA content in rats treated after CCI or SHAM surgery with either vehicle or DHA decreased as a result of diet and injury (DHACCI 0.52 ± 0.05 and SHAMDHA 0.56 ± 0.03 vs REGCCI 0.59 ± 0.03 and SHAMREG 0.8 ± 0.06 % total fat, $p=0.0007$ effect of diet, $p=0.007$ effect of injury). DHA, but not injury, increased red blood cell n-3/n-6 (DHACCI 0.35 ± 0.01 and SHAMDHA 0.36 ± 0.01 vs REGCCI 0.3 ± 0.01 and SHAMREG 0.3 ± 0.02, $p=0.001$ effect of diet, $p=0.6$ effect of injury). Red blood cell Arachidonic Acid levels did not differ between the four groups.

4. Discussion

In rat pups on a regular (REG) diet, CCI decreased brain DHA content at PID60 relative to SHAM rats. In contrast, CCI did not decrease brain DHA in pups fed DHA-rich maternal milk and a 0.1%DHA (DHA) diet one day before injury. These results prompted us to conduct Experiment 2, in which we used IP DHA to approximate the estimated daily DHA intake of DHA-enriched dam milk. Experiment 2 was geared at increasing translational relevance of our model by providing DHA on the day of injury, while still avoiding aspiration risk. In both experiments, rat pups returned to the dam after CCI or SHAM surgery and were fed either DHA or REG diet daily until time of outcome measure.

In Experiment 2, relative to SHAM and CCI rats on DHA diet, brain n-6 DPA increased in SHAM and CCI rats on REG diet without an associated significant decrease in DHA content, signaling the presence of a mild brain DHA deficit in both REG groups. Dietary DHA deficiency alters membrane composition first by increasing the contribution of n-6DPA (C22:5n-6) as a substitute for DHA. [30] [36, 37]

In rats, severe DHA depletion decreased rat pup brain DHA and increased the relative contributions of AA and n-6DPA, thus maintaining total unsaturated fat brain content. [36] Unsaturated fats encompass fatty acids with any double bonds: monounsaturated fats (found in olive and peanut oil, for example) have one double bond while polyunsaturated fats have more than one double bond. Polyunsaturated fats are further subclassified based on the number of double bonds and their location relative to the tail (n-3, n-6 and n-9 groups), the number of double bonds (2–6) and the length of the carbon chain. Compared to saturated fat, unsaturated fats increase fluidity and other biophysical parameters of the membrane bilayer,

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such as curvature and permeability. [59] Relative to n-6DPA,DHA with its additional double bond is more flexible and isomerizes with shorter correlation times, a difference that may be of particular importance to the function of integral membrane proteins. [38] The last of the 4 double bonds in AA and the last of the 5 double bonds in n-6DPA are located 6 carbons away from the tail, hence the categorization of both these PUFAs as n-6 fatty acids. In contrast, the last of the 6 double bonds in DHA is located 3 carbons away from the tail and thus DHA is categorized as an n-3 fatty acid. A lower dietary n-3/n-6 ratio is associated with a greater risk of cardiovascular and inflammatory diseases [60] and with cognitive impairment in humans across the life span. [61, 62]

In Experiment 2, diet, but not injury, increased PID 60 red blood cell DHA and omega 3 fatty acid content in the DHA fed SHAM and CCI rats relative to those on REG diet. In contrast to our findings, experimental mild TBI alone altered blood fatty acid profiles in adult mice: both AA and DHA-containing plasma phospholipids decreased at chronic time points after injury. [63] In humans, a cross-sectional analysis of blood samples from demographically matched soldiers classified as having mild TBI, post-traumatic stress disorder, or both showed that ratios of AA- to DHA-containing species within phosphatidyl choline and phosphatidyl ethanolamine (PC and PE) classes decreased relative to those considered cognitively and psychologically normal. [64] We speculate that, in our model, the dietary effects were greater than those of injury, as further outlined in reference to the brain fatty acid results below.

Similar to our findings in brain, CCI in adult mice decreased cortical and hippocampal DHA/AA ratios in PC and PE fractions relative to controls at 3 months after injury. [45] In another study, repetitive mild TBI in adult mice also decreased cortical and hippocampal n-3 (DHA) to n-6 (AA) ratios for PC and PE species late after injury. [65] We cannot compare our findings to those in immature brain because, to our knowledge, reports on DHA content after experimental TBI in the developing brain are lacking.

DHA deficiency stimulates increased brain accumulation of DPA, a 22-carbon n-6 fatty acid derived from AA. [36, 66] In our study, brain n-6DPA increased in SHAM and CCI rats on REG diet relative to their DHA-treated counterparts. All four groups received a 20% soybean emulsion IP either as a control (vehicle given to SHAMREG and SHAMCCI rats) or together with DHA to SHAMDHA and DHACCI rats. We speculate that soybean oil's high content of ALA, an essential fatty acid that can be used by the liver to synthesize DHA, may have blunted brain DHA losses such that the DHA deficit was mild, manifest only as increased n-6DPA in the REG treated rats. Safflower oil, an oil devoid of n- 3 fatty acids, is the primary source of fat in the REG diet though some soybean oil is present to ensure adequacy of n-3 fatty acid content. Of note, we did not find that CCI induced a statistically significant decrease in DHA nor an increase in n-6DPA relative to SHAM. Injury induced by the craniotomy may have sufficed to blunt differences between CCI and SHAM. Indeed, craniotomy alone produced a mild TBI in rats, shown by abnormal magnetic resonance imaging, increased inflammatory cytokines and impaired motor and cognitive function. Controls exposed to anesthesia without craniotomy, on the other hand, did not have any such injury. [67]

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As mentioned in the introduction, DHA may improve outcomes after TBI via several mechanisms other than DHA repletion. In adult rats after experimental TBI, dietary DHA improved cognitive function associated with decreased brain histologic injury, oxidative stress and inflammation. [21–24] We used our established model of severe pediatric TBI, controlled cortical impact (CCI) in male 17-day old (P17) rats to test effects of DHA before and after injury. [48, 68] The P17 rat brain is developmentally similar to the 2–4 year old human, the pediatric age at greatest risk of poor TBI outcomes, in terms of key indices such as synaptogenesis, synaptic maturation and brain growth velocity.[69–71]. Comparing rat pups given a 0.1% DHA diet before CCI (DHACCI pups) to pups on regular diet (REGCCI), DHA decreased oxidative stress, edema, white matter injury and lesion volume, associated with improved cognitive function.[19] We subsequently evaluated post-CCI DHA initiation, a more translationally relevant paradigm, by administering intraperitoneal DHA or vehicle at 30 minutes after CCI followed by allocation to the 0.1%DHA or REG diet. We found that post-CCI DHA administration decreased oxidative stress and improved cognitive function. [72] DHA decreased pro-inflammatory markers in histologic sections and in flow-sorted cells during the first week after CCI.[72] In summary, DHA improved neurologic outcomes and decreased brain oxidative stress and inflammation after experimental TBI in adult and immature rats.

Our findings are limited to 17- day old male rat pups. While no animal model can replicate the human condition, the results of our study provide useful information for further work. For example, our future research will explore effects of sex on DHA content and neurologic outcomes after CCI. DHA deficiency affects the developing brain in a sex-dependent manner: exposure to a low-DHA diet during development decreased brain DHA/ n-6DPA ratio in both sexes, but locomotor deficits at particular developmental periods developed in male, but not female, rats on the low DHA diet. [66] Differences in endogenous DHA synthesis and recycling between rats and humans pose another limitation. However, while estimated dietary needs for DHA in adult humans appear to be lower than those inferred from rat studies, inferences from developing rodents are more relevant to the developing human because both species share similarly high DHA accrual during rapid brain growth [73]. Further, in a low ATP environment after TBI, extrapolation of DHA recycling in the healthy rat likely overestimates the capacity to conserve brain DHA after injury. [43]

Our results do not account for regional variations in DHA content. We used whole hemispheres rather than dissected tissues. Joffre et al. studied adult mouse brain fatty acid content differences between the hippocampus, prefrontal cortex, cortex and hypothalamus, among other regions. They found that pre-frontal cortex and hippocampus had higher DHA content than did the frontal cortex. [74] However, we chose to collect the entire injured hemisphere to minimize the greater variability that would result from using dissected tissue at a late time point after CCI. Variable amounts of region-specific tissue loss over time would be expected to occur between rats despite a consistent delivery of impact to the frontoparietal cortex and underlying hippocampus. [75]

To our knowledge, ours is the first study to report DHA content after experimental TBI incurred during development. We speculate that the significance of a DHA deficit would be even greater in the developing than in the mature brain after TBI. In humans, autopsy

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findings show that DHA content of PC and PE increases with brain maturation, supporting the importance of neural membrane DHA in normal development. [76] While greater prematurity is associated with larger deficits in DHA accumulation in the fetus [34], a relative DHA deficiency with functional implications can occur in full term infants not fed human milk. Healthy infants fed formula without DHA during the period of rapid brain growth had poor visual evoked potentials compared to DHA-supplemented infants.[35] Indeed, post-mortem studies in humans showed that brain DHA was significantly higher in breast-fed infants than in those fed formula containing ALA but not DHA. [77] In a rat model of premature birth and post-natal artificial feeding, rat pups raised on a DHA-free diet performed poorly on a test of spatial memory and learning, the Morris Water Maze, at 6 weeks of age compared to rat pups raised on a DHA sufficient (100mg/kg/day) or DHA enriched (300mg/kg/day) diet. [78]

4.1 Conclusions

DHA improves functional and anatomic outcomes after experimental TBI. Using our model of pediatric TBI, CCI in the 17-day old rat, we found that TBI induces a mild DHA deficit. While the deficit is small, it may nevertheless be significant in light of the importance of DHA in normal brain development. Future research on rats of both sexes including controls without craniotomy will increase understanding of the potential magnitude of brain DHA deficit after developmental TBI. This study further supports the need for pre-clinical research to explore the potential of DHA as a neuroprotectant in childhood TBI.

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REFERENCES

[1]. Popernack ML, Gray N, Reuter-Rice K, Moderate-to-Severe Traumatic Brain Injury in Children: Complications and Rehabilitation Strategies, J Pediatr Health Care (2014).

[2]. Vavilala MS, Kernic MA, Wang J, Kannan N, Mink RB, Wainwright MS, Groner JI, Bell MJ, Giza CC, Zatzick DF, Ellenbogen RG, Boyle LN, Mitchell PH, Rivara FP, Pediatric Guideline A, Outcomes S, Acute care clinical indicators associated with discharge outcomes in children with severe traumatic brain injury, Crit Care Med 42(10) (2014) 2258–66. [PubMed: 25083982]

[3]. Bennett TD, Dixon RR, Kartchner C, DeWitt PE, Sierra Y, Ladell D, Kempe A, Runyan DK, Dean JM, Keenan HT, Functional Status Scale in Children With Traumatic Brain Injury: A Prospective Cohort Study, Pediatr Crit Care Med 17(12) (2016) 1147–1156. [PubMed: 27753754]

[4]. Anderson V, Catroppa C, Morse S, Haritou F, Rosenfeld J, Functional plasticity or vulnerability after early brain injury?, Pediatrics 116(6) (2005) 1374–82. [PubMed: 16322161]

[5]. Slovis JC, Gupta N, Li NY, Kernie SG, Miles DK, Assessment of Recovery Following Pediatric Traumatic Brain Injury, Pediatr Crit Care Med 19(4) (2018) 353–360. [PubMed: 29419604]

[6]. Ryan NP, Catroppa C, Beare R, Coleman L, Ditchfield M, Crossley L, Beauchamp MH, Anderson VA, Predictors of longitudinal outcome and recovery of pragmatic language and its relation to externalizing behaviour after pediatric traumatic brain injury, Brain Lang 142 (2015) 86–95. [PubMed: 25677376]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

[7]. Bayir H, Kochanek PM, Kagan VE, Oxidative stress in immature brain after traumatic brain injury, Dev Neurosci 28(4–5) (2006) 420–31. [PubMed: 16943665]

[8]. Ikonomidou C, Kaindl AM, Neuronal death and oxidative stress in the developing brain, Antioxid Redox Signal 14(8) (2011) 1535–50. [PubMed: 20919934]

[9]. Anthony D, Dempster R, Fearn S, Clements J, Wells G, Perry VH, Walker K, CXC chemokines generate age-related increases in neutrophil-mediated brain inflammation and blood-brain barrier breakdown, Curr Biol 8(16) (1998) 923–6. [PubMed: 9707404]

[10]. Anthony DC, Bolton SJ, Fearn S, Perry VH, Age-related effects of interleukin-1 beta on polymorphonuclear neutrophil-dependent increases in blood-brain barrier permeability in rats, Brain 120 (Pt 3) (1997) 435–44. [PubMed: 9126055]

[11]. Hagberg H, Mallard C, Ferriero DM, Vannucci SJ, Levison SW, Vexler ZS, Gressens P, The role of inflammation in perinatal brain injury, Nat Rev Neurol 11(4) (2015) 192–208. [PubMed: 25686754]

[12]. Potts MB, Koh SE, Whetstone WD, Walker BA, Yoneyama T, Claus CP, Manvelyan HM, Noble-Haeusslein LJ, Traumatic injury to the immature brain: inflammation, oxidative injury, and iron-mediated damage as potential therapeutic targets, NeuroRx 3(2) (2006) 143–53. [PubMed: 16554253]

[13]. Claus CP, Tsuru-Aoyagi K, Adwanikar H, Walker B, Manvelyan H, Whetstone W, Noble-Haeusslein LJ, Age is a determinant of leukocyte infiltration and loss of cortical volume after traumatic brain injury, Dev Neurosci 32(5–6) (2010) 454–65. [PubMed: 20847543]

[14]. Yavin E, Glozman S, Green P, Docosahexaenoic acid accumulation in the prenatal brain: prooxidant and antioxidant features, J Mol Neurosci 16(2–3) (2001) 229–35; discussion 279–84. [PubMed: 11478378]

[15]. Antonietta Ajmone-Cat M, Lavinia Salvatori M, De Simone R, Mancini M, Biagioni S, Bernardo A, Cacci E, Minghetti L, Docosahexaenoic acid modulates inflammatory and antineurogenic functions of activated microglial cells, J Neurosci Res 90(3) (2012) 575–87. [PubMed: 22057807]

[16]. Chang HY, Lee HN, Kim W, Surh YJ, Docosahexaenoic acid induces M2 macrophage polarization through peroxisome proliferator-activated receptor gamma activation, Life Sci 120 (2015) 39–47. [PubMed: 25445227]

[17]. Rogers LK, Valentine CJ, Keim SA, DHA supplementation: current implications in pregnancy and childhood, Pharmacol Res 70(1) (2013) 13–9. [PubMed: 23266567]

[18]. Russell KL, Berman NE, Gregg PR, Levant B, Fish oil improves motor function, limits blood-brain barrier disruption, and reduces Mmp9 gene expression in a rat model of juvenile traumatic brain injury, Prostaglandins Leukot Essent Fatty Acids 90(1) (2014) 5–11. [PubMed: 24342130]

[19]. Schober ME, Requena DF, Abdullah OM, Casper TC, Beachy J, Malleske D, Pauly JR, 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, J Neurotrauma 33(4) (2016) 390–402. [PubMed: 26247583]

[20]. Schober ME RD, Casper TC, Veldhorst AK, Lolofie A, McFarlane KE, Otto TE, Terry C, Gensel JC, Docosahexaenoic Acid Decreased Neuroinflammation in Rat Pups after Controlled Cortical Impact Experimental Neurology, Exp Neurol (Special Issue on Pediatric TBI: "Defining the terrains that support recovery after traumatic injury to the developing brain") (2019).

[21]. Wu A, Ying Z, Gomez-Pinilla F, The salutary effects of DHA dietary supplementation on cognition, neuroplasticity, and membrane homeostasis after brain trauma, J Neurotrauma 28(10) (2011) 2113–22. [PubMed: 21851229]

[22]. Bailes JE, Mills JD, Docosahexaenoic acid reduces traumatic axonal injury in a rodent head injury model, J Neurotrauma 27(9) (2010) 1617–24. [PubMed: 20597639]

[23]. Wu A, Ying Z, Gomez-Pinilla F, Dietary Strategy to Repair Plasma Membrane After Brain Trauma: Implications for Plasticity and Cognition, Neurorehabil Neural Repair (2013).

[24]. Harvey LD, Yin Y, Attarwala IY, Begum G, Deng J, Yan HQ, Dixon CE, Sun D, Administration of DHA Reduces Endoplasmic Reticulum Stress-Associated Inflammation and Alters Microglial or Macrophage Activation in Traumatic Brain Injury, ASN Neuro 7(6) (2015).

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

[25]. Innis SM, Dietary (n-3) fatty acids and brain development, J Nutr 137(4) (2007) 855–9. [PubMed: 17374644]

[26]. Zarate R, El Jaber-Vazdekis N, Tejera N, Perez JA, Rodriguez C, Significance of long chain polyunsaturated fatty acids in human health, Clin Transl Med 6(1) (2017) 25. [PubMed: 28752333]

[27]. Stillwell W, Wassall SR, Docosahexaenoic acid: membrane properties of a unique fatty acid, Chem Phys Lipids 126(1) (2003) 1–27. [PubMed: 14580707]

[28]. Stillwell W, Shaikh SR, Zerouga M, Siddiqui R, Wassall SR, Docosahexaenoic acid affects cell signaling by altering lipid rafts, Reprod Nutr Dev 45(5) (2005) 559–79. [PubMed: 16188208]

[29]. Sugasini D, Lokesh BR, Rats given linseed oil in microemulsion forms enriches the brain synaptic membrane with docosahexaenoic acid and enhances the neurotransmitter levels in the brain, Nutr Neurosci 18(2) (2015) 87–96. [PubMed: 24621059]

[30]. Kim HY, Novel metabolism of docosahexaenoic acid in neural cells, J Biol Chem 282(26) (2007) 18661–5. [PubMed: 17488715]

[31]. Lauritzen L, Brambilla P, Mazzocchi A, Harslof LB, Ciappolino V, Agostoni C, DHA Effects in Brain Development and Function, Nutrients 8(1) (2016).

[32]. Attar-Bashi NM, Weisinger RS, Begg DP, Li D, Sinclair AJ, Failure of conjugated linoleic acid supplementation to enhance biosynthesis of docosahexaenoic acid from alpha-linolenic acid in healthy human volunteers, Prostaglandins Leukot Essent Fatty Acids 76(3) (2007) 121–30. [PubMed: 17275274]

[33]. Lauritzen L, Hansen HS, Jorgensen MH, Michaelsen KF, The essentiality of long chain n-3 fatty acids in relation to development and function of the brain and retina, Prog Lipid Res 40(1–2) (2001) 1–94. [PubMed: 11137568]

[34]. Najm S, Lofqvist C, Hellgren G, Engstrom E, Lundgren P, Hard AL, Lapillonne A, Savman K, Nilsson AK, Andersson MX, Smith LEH, Hellstrom A, Effects of a lipid emulsion containing fish oil on polyunsaturated fatty acid profiles, growth and morbidities in extremely premature infants: A randomized controlled trial, Clin Nutr ESPEN 20 (2017) 17–23. [PubMed: 29072164]

[35]. Birch EE, Carlson SE, Hoffman DR, Fitzgerald-Gustafson KM, Fu VL, Drover JR, Castaneda YS, Minns L, Wheaton DK, Mundy D, Marunycz J, Diersen-Schade DA, The DIAMOND (DHA Intake And Measurement Of Neural Development) Study: a double-masked, randomized controlled clinical trial of the maturation of infant visual acuity as a function of the dietary level of docosahexaenoic acid, Am J Clin Nutr 91(4) (2010) 848–59. [PubMed: 20130095]

[36]. Moriguchi T, Loewke J, Garrison M, Catalan JN, Salem N Jr., Reversal of docosahexaenoic acid deficiency in the rat brain, retina, liver, and serum, J Lipid Res 42(3) (2001) 419–27. [PubMed: 11254754]

[37]. Moriguchi T, Harauma A, Salem N Jr., Plasticity of mouse brain docosahexaenoic acid: modulation by diet and age, Lipids 48(4) (2013) 343–55. [PubMed: 23460301]

[38]. Eldho NV, Feller SE, Tristram-Nagle S, Polozov IV, Gawrisch K, Polyunsaturated docosahexaenoic vs docosapentaenoic acid-differences in lipid matrix properties from the loss of one double bond, J Am Chem Soc 125(21) (2003) 6409–21. [PubMed: 12785780]

[39]. Desai A, Park T, Barnes J, Kevala K, Chen H, Kim HY, Reduced acute neuroinflammation and improved functional recovery after traumatic brain injury by alpha-linolenic acid supplementation in mice, J Neuroinflammation 13(1) (2016) 253. [PubMed: 27663791]

[40]. Russell KL, Berman NE, Levant B, Low brain DHA content worsens sensorimotor outcomes after TBI and decreases TBI-induced Timp1 expression in juvenile rats, Prostaglandins, leukotrienes, and essential fatty acids 89(2–3) (2013) 97–105.

[41]. Rodriguez A, Sarda P, Nessmann C, Boulot P, Leger CL, Descomps B, Delta6- and delta5-desaturase activities in the human fetal liver: kinetic aspects, J Lipid Res 39(9) (1998) 1825–32. [PubMed: 9741695]

[42]. Rapoport SI, Translational studies on regulation of brain docosahexaenoic acid (DHA) metabolism in vivo, Prostaglandins Leukot Essent Fatty Acids 88(1) (2013) 79–85. [PubMed: 22766388]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

[43]. Frenguelli BG, The Purine Salvage Pathway and the Restoration of Cerebral ATP: Implications for Brain Slice Physiology and Brain Injury, Neurochem Res 44(3) (2019) 661–675. [PubMed: 28836168]

[44]. Farias SE, Heidenreich KA, Wohlauer MV, Murphy RC, Moore EE, Lipid mediators in cerebral spinal fluid of traumatic brain injured patients, J Trauma 71(5) (2011) 1211–8. [PubMed: 21427623]

[45]. Abdullah L, Evans JE, Ferguson S, Mouzon B, Montague H, Reed J, Crynen G, Emmerich T, Crocker M, Pelot R, Mullan M, Crawford F, Lipidomic analyses identify injury-specific phospholipid changes 3 mo after traumatic brain injury, FASEB J 28(12) (2014) 5311–21. [PubMed: 25208845]

[46]. Schober ME, Traumatic brain injury increased IGF-1B mRNA and altered IGF-1 exon 5 and promoter region epigenetic characteristics in the rat pup hippocampus, 29(11) (2012) 2075–85.

[47]. Schober ME, Pauly JR, Requena D and Cusick M, Neuroprotective Mechanisms of Docosahexaenoic Acid in Rat Pups after Traumatic Brain Injury, Journal of Neurotrauma. 31(12) (2014) A1–A126.

[48]. Schober ME, Block B, Beachy JC, Statler KD, Giza CC, Lane RH, Early and sustained increase in the expression of hippocampal IGF-1, but not EPO, in a developmental rodent model of traumatic brain injury, J Neurotrauma 27(11) (2010) 2011–20. [PubMed: 20822461]

[49]. Schober ME, Block B, Requena DF, Hale MA, Lane RH, Developmental traumatic brain injury decreased brain derived neurotrophic factor expression late after injury, Metab Brain Dis 27(2) (2012) 167–73. [PubMed: 22527999]

[50]. Schober ME, Requena DF, Block B, Davis LJ, Rodesch C, Casper TC, Juul SE, Kesner RP, Lane RH, Erythropoietin improved cognitive function and decreased hippocampal caspase activity in rat pups after traumatic brain injury, J Neurotrauma 31(4) (2014) 358–69. [PubMed: 23972011]

[51]. Schober ME, Requena DF, Davis LJ, Metzger RR, Bennett KS, Morita D, Niedzwecki C, Yang Z, Wang KK, Alpha II Spectrin breakdown products in immature Sprague Dawley rat hippocampus and cortex after traumatic brain injury, Brain Res 1574 (2014) 105–12. [PubMed: 24929209]

[52]. Adelson PD, Fellows-Mayle W, Kochanek PM, Dixon CE, Morris water maze function and histologic characterization of two age-at-injury experimental models of controlled cortical impact in the immature rat, Childs Nerv Syst 29(1) (2013) 43–53. [PubMed: 23089934]

[53]. Dixon CE, Clifton GL, Lighthall JW, Yaghmai AA, Hayes RL, A controlled cortical impact model of traumatic brain injury in the rat, J Neurosci Methods 39(3) (1991) 253–62. [PubMed: 1787745]

[54]. Dixon CE, Kochanek PM, Yan HQ, Schiding JK, Griffith RG, Baum E, Marion DW, DeKosky ST, One-year study of spatial memory performance, brain morphology, and cholinergic markers after moderate controlled cortical impact in rats, J Neurotrauma 16(2) (1999) 109–22. [PubMed: 10098956]

[55]. Lighthall JW, Controlled cortical impact: a new experimental brain injury model, J Neurotrauma 5(1) (1988) 1–15. [PubMed: 3193461]

[56]. Mao H, Guan F, Han X, Yang KH, Strain-based regional traumatic brain injury intensity in controlled cortical impact: a systematic numerical analysis, J Neurotrauma 28(11) (2011) 2263–76. [PubMed: 21488718]

[57]. Lauritzen L, Jorgensen MH, Hansen HS, Michaelsen KF, Fluctuations in human milk long-chain PUFA levels in relation to dietary fish intake, Lipids 37(3) (2002) 237–44. [PubMed: 11942473]

[58]. Valenzuela A, Nieto S, Sanhueza J, Nunez MJ, Ferrer C, Tissue accretion and milk content of docosahexaenoic acid in female rats after supplementation with different docosahexaenoic acid sources, Ann Nutr Metab 49(5) (2005) 325–32. [PubMed: 16088098]

[59]. Ibarguren M, Lopez DJ, Escriba PV, The effect of natural and synthetic fatty acids on membrane structure, microdomain organization, cellular functions and human health, Biochim Biophys Acta 1838(6) (2014) 1518–28. [PubMed: 24388951]

[60]. Simopoulos AP, The importance of the omega-6/omega-3 fatty acid ratio in cardiovascular disease and other chronic diseases, Exp Biol Med (Maywood) 233(6) (2008) 674–88. [PubMed: 18408140]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

[61]. Andruchow ND, Konishi K, Shatenstein B, Bohbot VD, A lower ratio of omega-6 to omega-3 fatty acids predicts better hippocampus-dependent spatial memory and cognitive status in older adults, Neuropsychology 31(7) (2017) 724–734. [PubMed: 28394147]

[62]. Kim H, Kim H, Lee E, Kim Y, Ha EH, Chang N, Association between maternal intake of n-6 to n-3 fatty acid ratio during pregnancy and infant neurodevelopment at 6 months of age: results of the MOCEH cohort study, Nutr J 16(1) (2017) 23. [PubMed: 28420388]

[63]. Emmerich T, Abdullah L, Ojo J, Mouzon B, Nguyen T, Laco GS, Crynen G, Evans JE, Reed J, Mullan M, Crawford F, Mild TBI Results in a Long-Term Decrease in Circulating Phospholipids in a Mouse Model of Injury, Neuromolecular Med 19(1) (2017) 122–135. [PubMed: 27540748]

[64]. Emmerich T, Abdullah L, Crynen G, Dretsch M, Evans J, Ait-Ghezala G, Reed J, Montague H, Chaytow H, Mathura V, Martin J, Pelot R, Ferguson S, Bishop A, Phillips J, Mullan M, Crawford F, Plasma Lipidomic Profiling in a Military Population of Mild Traumatic Brain Injury and Post-Traumatic Stress Disorder with Apolipoprotein E varepsilon4-Dependent Effect, J Neurotrauma 33(14) (2016) 1331–48. [PubMed: 26714394]

[65]. Ojo JO, Algamal M, Leary P, Abdullah L, Mouzon B, Evans JE, Mullan M, Crawford F, Disruption in Brain Phospholipid Content in a Humanized Tau Transgenic Model Following Repetitive Mild Traumatic Brain Injury, Front Neurosci 12 (2018) 893. [PubMed: 30564087]

[66]. Levant B, Ozias MK, Carlson SE, Sex-specific effects of brain LC-PUFA composition on locomotor activity in rats, Physiol Behav 89(2) (2006) 196–204. [PubMed: 16875705]

[67]. Cole JT, Yarnell A, Kean WS, Gold E, Lewis B, Ren M, McMullen DC, Jacobowitz DM, Pollard HB, O'Neill JT, Grunberg NE, Dalgard CL, Frank JA, Watson WD, Craniotomy: true sham for traumatic brain injury, or a sham of a sham?, J Neurotrauma 28(3) (2011) 359–69. [PubMed: 21190398]

[68]. Schober ME, Requena D, Block BP, Davis LJ, Rodesch C, Casper TC, Juul SE, Kesner RP, Lane RH, Erythropoietin Improved Cognitive Function and Decreased Hippocampal Caspase Activity in Rat Pups after Traumatic Brain Injury, J Neurotrauma (2013).

[69]. Fink EL, Alexander H, Marco CD, Dixon CE, Kochanek PM, Jenkins LW, Lai Y, Donovan HA, Hickey RW, Clark RS, Experimental model of pediatric asphyxial cardiopulmonary arrest in rats, Pediatr Crit Care Med 5(2) (2004) 139–44. [PubMed: 14987343]

[70]. Rice D, Barone S Jr., Critical periods of vulnerability for the developing nervous system: evidence from humans and animal models, Environ Health Perspect 108 Suppl 3 (2000) 511–33. [PubMed: 10852851]

[71]. Dobbing J, Sands J, Comparative aspects of the brain growth spurt, Early Hum Dev 3(1) (1979) 79–83. [PubMed: 118862]

[72]. Schober ME RD, Casper TC, Veldhorst AK, Lolofie A, Otto TE, Terry C, Gensel JC, Docosahexaenoic Acid Decreased Neuroinflammation in Rat Pups after Controlled Cortical Impact, Exp Neurol (Special Issue on Pediatric TBI: "Defining the terrains that support recovery after traumatic injury to the developing brain") (2019).

[73]. Domenichiello AF, Kitson AP, Bazinet RP, Is docosahexaenoic acid synthesis from alpha-linolenic acid sufficient to supply the adult brain?, Prog Lipid Res 59 (2015) 54–66. [PubMed: 25920364]

[74]. Joffre C, Gregoire S, De Smedt V, Acar N, Bretillon L, Nadjar A, Laye S, Modulation of brain PUFA content in different experimental models of mice, Prostaglandins Leukot Essent Fatty Acids 114 (2016) 1–10. [PubMed: 27926457]

[75]. Kochanek PM, Hendrich KS, Dixon CE, Schiding JK, Williams DS, Ho C, Cerebral blood flow at one year after controlled cortical impact in rats: assessment by magnetic resonance imaging, J Neurotrauma 19(9) (2002) 1029–37. [PubMed: 12482116]

[76]. Martinez M, Mougan I, Fatty acid composition of human brain phospholipids during normal development, J Neurochem 71(6) (1998) 2528–33. [PubMed: 9832152]

[77]. Makrides M, Neumann MA, Byard RW, Simmer K, Gibson RA, Fatty acid composition of brain, retina, and erythrocytes in breast- and formula-fed infants, Am J Clin Nutr 60(2) (1994) 189–94. [PubMed: 7913291]

[78]. Wang Q, Jia C, Tan X, Wu F, Zhong X, Su Z, Sun W, Cui Q, Different concentrations of docosahexanoic acid supplement during lactation result in different outcomes in preterm Sprague-Dawley rats, Brain Res 1678 (2018) 367–373. [PubMed: 29146112]

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Highlights

- DHA decreased injury, oxidative stress and inflammation in rat pup brain after CCI
- In rat pups, CCI and craniotomy each induce a mild brain DHA deficiency
- Diet, but not injury, affected rat pup blood lipid profiles
- DHA treatment blunted brain DHA deficiency after CCI
- DHA neuroprotection likely rests upon effects on inflammation and oxidative stress

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Table 1.

Fatty Acid Profiles in Injured Brain (Left Parietal Cortex) 60 days after Injury in Experiment 2.

Fatty Acid	DHACCI *(Mean ± SEM)*	REGCCI *(Mean ± SEM)*	SHAMDHA *(Mean ± SEM)*	SHAMREG *(Mean ± SEM)*
Saturates	39.27 ± 0.42%	40.24 ± 0.44%	38.91 ± 0.7%	39.46 ± 0.56%
Monounsaturates	21.77 ± 0.21%	21.23 ± 0.27%	21.77 ± 0.22%	21.65 ± 0.1%
Total n-6 PUFAs	16.76 ± 0.20%	17.63 ± 0.28%	16.36 ± 0.27%	17.64 ± 0.29%
20:4n-6 (AA)	14.25 ± 0.11%	14.77 ± 0.21%	14.09 ± 0.23 %	14.67 ± 0.17%
20:3n-6	0.53 ± 0.02	0.49 ± 0.01%	0.49 ± 0.01%	0.49 ± 0.01%
18:2n-6c (LA)	0.79 ± 0.02%	0.77 ± 0.03%	0.80 ± 0.02%	0.78 ± 0.01%
22:5n-6 (n-6 DPA)	0.98 ± 0.13 % [a,b]	1.37 ± 0.09 %	0.6 ± 0.07 % [a,b]	1.47 ± 0.11%
Total n-3 PUFAs	18.91± 0.24%	17.56 ± 0.16%	19.82 ± 0.31%	18.09 ± 0.19%
22:6n-3 (DHA)	18.9 ± 0.24% [a,b]	17.5 ± 0.16%	19.8 ± 0.3 % [a]	18.1 ± 0.19 %
20:3n-3	0.03 ± 0.003%	0.03 ± 0.002%	0.03 ± 0.003%	0.03 ± 0.002%

Fatty acids are expressed in % of total fat ± SEM.

[a]Significance denoted for $p<0.05$ for the significance of diet while

[b]significance denoted for $p<0.05$ for the significance of injury as described in the results.

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Table 2.

Fatty Acid Profiles in Red Blood Celi Membrana 60 uays after Injury in Experiment 2.

Fatty Acid	DHACCI *(Mean ± SEM)*	REGCCI *(Mean ± SEM)*	SHAMDHA *(Mean ± SEM)*	SHAMREG *(Mean ± SEM)*
Saturates	49.91 ± 0.42%	51.11 ± 0.63%	49.67 ± 0.68%	52.67 ± 0.63%
Monounsaurates	8.39 ± 0.51 %	8.62 ± 0.49 %	8.76 ± 0.54 %	7.63 ± 0.46 %
Total n-6 PUFAs	35.52 ± 0.46 %	35.71 ± 0.47 %	35.33 ± 0.45%	34.8 ± 0.67 %
20:4n-6 (AA)	20.22 ± 0.78 %	20.35 ± 0.1 %	20.02 ± 0.81 %	21.28 ± 0.72 %
18:2n-6c (LA)	13.46 ± 0.68%	13.34 ± 0.86%	13.07 ± 0.69%	11.40 ± 0.68%
22:5n-6 (n-6 DPA)	0.52 ± 0.05 %	0.59 ± 0.03 %	0.56 ± 0.03 %	0.8 ± 0.06 % [b]
Total n-3 PUFAs	6.19 ± 0.23 % [a]	4.55 ± 0.13 %	6.33 ± 0. 11% [a]	4.91 ± 0.23 %
22:6n-3 (DHA)	3.89 ± 0.25 % [a]	2.08 ± 0.09 %	4.02 ± 0.12 % [a]	2.29 ± 0.13 %

Fatty acids are expressed in % of total fat ± SEM.

[a]Significance denoted for p<0.05 for the significance of diet, while

[b]significance denoted for p<0.05 for the significance of injury as described in the results.