Current Neurology and Neuroscience Reports (2021) 21:45
https://doi.org/10.1007/s11910-021-01132-z

NEUROTRAUMA (D. SANDSMARK, SECTION EDITOR)

The More, the Better: High-Dose Omega-3 Fatty Acids Improve Behavioural and Molecular Outcomes in Preclinical Models in Mild Brain Injury

Craig S. Patch[1] · Elisa L. Hill-Yardin[2] · Michael Lewis[3] · Lisa Ryan[4] · Ed Daly[4] · Alan J. Pearce[1]

Accepted: 7 June 2021

Abstract

Purpose of Review Mild traumatic brain injury (mTBI) is a continuing healthcare concern worldwide contributing to significant cognitive and neurological impairment, consequently affecting activities of daily living. While mTBI recovery is becoming well studied, there are no interventions to reduce the known impairments of mTBI. Omega-3 fatty acids (N-3FA) are safe and beneficial for brain health; however, their potential effects in a pathophysiological environment such as that seen post-mTBI are unknown.

Recent Findings Preclinical studies using rodent models are key to understanding molecular mechanisms underlying improvements post-injury. Studies to date have shown improved outcomes in rodent models following mTBI protocols, but these data have not been quantified using a systematic review and meta-analysis approach.

Summary Our systematic review assessed 291 studies identified from the literature. Of these studies, 18 studies met inclusion criteria. We conducted a meta-analysis examining the effect of high-dose n-3FA vs placebo on neurological, cognitive and molecular changes following mTBI. Quality of studies was rated as moderate to high quality, and while mostly compliant, some areas of risk of bias were identified. Results showed that preclinical doses of 10–370 mg/kg/day of n-3FA per day in rodents (equivalent to high clinical doses) resulted in improvements in neurological and cognitive performance (pooled effect sizes ranging between 1.52 and 3.55). Similarly, improvements in molecular and inflammatory markers were observed in treated rodents vs control (pooled effect sizes: 3.73–6.55). Overall, these findings highlight the potential for high-dose n-3FA for human clinical studies following mTBI.

Keywords Mild traumatic brain injury · Omega-3 fatty acids · Neurological recovery · Systematic review

Introduction

Mild traumatic brain injury (mTBI) is recognised as one of the most common neurological disorders and the most common type of traumatic brain injury [1, 2]. Across the USA, Europe and Australia, epidemiological studies report an incidence of mTBI ranging between 100 and 300 cases per 100,000 people [3, 4]. The reality of incidence, however, may be as high as 600–790 per 100,000 due to poor reporting practices and lack of acknowledgement by individuals on the severity of the injury [3, 5].

Mild TBI is characterised by an impact, directly or indirectly, to the head without evidence of skill fracture, a Glasgow Coma Scale score between 13 and 15 [6] and including but not limited to a range of symptoms such as nausea, confusion, transient amnesia and aggression. Moreover, clinical neuroimaging shows negative findings [7]. While terms have been used interchangeably, concussion has been described as reflecting a 'functional' disturbance following injury [8]. Although concussion may be seen as a sub-set of mTBI, it has been argued that mTBI cannot be a sub-set of concussion [9].

This article is parts of the Topical Collection on *Neurotrauma*

✉ Alan J. Pearce
alan.pearce@latrobe.edu.au

[1] College of Science, Health and Engineering, La Trobe University, Melbourne, Australia

[2] School of Health and Biomedical Sciences, STEM College, RMIT University, Melbourne, Australia

[3] Brain Health Education and Research Institute, Potomac, MD, USA

[4] School of Science and Computing, Galway-Mayo Institute of Technology, Dublin, Ireland

Published online: 06 July 2021

The functional disturbance described usually includes a range of pathophysiological outcomes suggesting a disruption of neurological homeostasis. These negative outcomes include dysregulation of ionic and neurometabolic processing, neurovascular and autonomic uncoupling, impaired synaptic transmission, and reduced cerebral blood flow creating an anaerobic cellular environment that Giza and Hovda [10, 11] describe as a 'cellular crisis'. Further, Romeu-Mejia et al [12] describe neuroflammation and micropathological damage including disruption of blood-brain barrier permeability, damaged cellular membranes, and neuronal apoptosis following brain injury.

The majority of people who suffer a mTBI will recover. However, timelines for recovery vary between individuals. A recent New Zealand study by Kara et al [13] reported that less than half of patients from their general concussion clinic recovered in less than 14 days post-concussion, three-quarters of patients recovered within 4 weeks of injury and 96% recovering by 2 months post-injury. While most will recover, there is growing concern that chronic exposure to repeated mTBI, or even exposure to sub-concussive impacts (where no signs or symptoms are observed following an impact to the head), is associated with long-term negative outcomes including neurodegenerative disorders and dementias [14, 15]. Indeed, the 2020 *The Lancet* commission report on dementia prevention, intervention and care highlighted new evidence suggesting that head injury is a modifiable risk factor for dementia [15]. While the risk of dementia increases with the severity or number of brain injuries, it has been reported that a single TBI event increases the risk of neurodegenerative disease by as much as 60% [16].

While it is acknowledged that not every impact to the head leads to neurodegeneration, evidence suggests that mTBI can give rise to progressive brain atrophy and enduring cognitive impairments [17, 18]. Studies investigating repeated mild brain injuries in sports and military environments have shown significant neurological impairments over time in these populations. For example, American football players and military veterans who have experienced three or more concussions are more likely to develop mild cognitive impairment, and the onset of Alzheimer's disease (AD) is earlier than in the general population [16, 19]. Retrospective epidemiological studies have shown that while former contact sport athletes (e.g. the football codes) lived longer than the general population, their risk of neurodegenerative diseases (including AD, Parkinson's disease, motor neurone disease) is three to five times higher [20, 21].

Consequently, it is important to understand both the acute response profile following acute mTBI and long-term risk in affected individuals. Clarifying response profiles may assist in providing targeted therapeutic interventions to improve recovery outcomes and potentially reduce long-term risks [22••]. However, current treatments and therapies are limited. The most recent consensus statement suggests that there is little evidence to support the use of pharmacotherapy [8], whereas the use of individualised and graduated exercise that does not exacerbate symptoms is supported in the acute rehabilitation period post-concussion. For those with specific vestibular dysfunction post-concussion, physical therapy is recommended. Conversely, for post-concussion patients with persistent mood or behavioural issues, cognitive behaviour therapy is frequently prescribed [8]. Interestingly, because it has not been discussed in any consensus statement, an area of therapeutic potential for neural repair and inflammation modulation following injury is omega-3 fatty acid (n-3FA) supplementation [23]. n-3FA acid supplementation post-brain injury may act on neurophysiological pathways and reduce pathophysiological effects of impact to the brain [24]. However, previous brain injury reviews have not focussed on nutritional interventions that target observable clinical symptoms or outcomes in mTBI [22••, 25••].

The objective of this systematic review and meta-analysis was to address this gap in the literature. Specifically, our study aimed to focus on the efficacy of high-dose, long-chain n-3FA, specifically docosahexaenoic acid (DHA) and/or eicosapentaenoic acid (EPA) supplementation post-mild brain injury. Based on previous systematic reviews that showed no interventional studies in humans [22••, 25••], this review has focused on neurological, cognitive, and molecular outcomes in animal models.

Methods

This systematic review and meta-analysis was conducted in accordance with the recommendations of the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) [26].

Study Search

Our search strategy used the following electronic databases: PubMed/MEDLINE, EMBASE, Science Direct, and Web of Science from 1 January 1980 until 31 December 2020. Medical Subject Headings (MeSH) or keywords and matching synonyms were combined (keywords and search strategy can be found in Table 1).

Study Selection

The inclusion and exclusion criteria followed the Population–Indicator–Comparator–Outcome (PICO) principle (Table 1) [27] to identify studies relevant to our research hypothesis. Inclusion criteria were (1) studies in English, (2) studies involving mature rodents (minimum age range 6 months), (3) studies involving intervention of n-3FA with

Table 1. PICO model and MEDLINE search strategy in accordance with PRISMA statement [9].

Population (P)	Mature rodents (minimum 6 months); male/female		
Intervention (I)	Post-mTBI diet rich in LC n-3FA, specifically eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) versus placebo		
Comparator (C)	Placebo controlled studies		
Outcomes (O)	Improved scores on neurological, motor and cognitive functions and molecular outcomes compared to control rodents		
Nutrition terms	Neurological signs terms	Brain injury terms	Animal terms
Fatty Acids, Omega-3 (MeSH*) OR Omega 3 Fatty Acids OR n-3 Fatty Acids OR n-3 Oils OR n-3 PUFA OR n-3 Polyunsaturated Fatty Acid OR eicosapentaenoic acid (EPA) OR docosahexaenoic acid (DHA)	Signs and Symptoms, Neurobehavioral (MeSH) OR Neurobehavioral Signs and Symptoms OR Behavior* Disorder* OR Cognitive Manifestation* OR Cognitive Symptom*	Traumatic brain injury OR Mild traumatic brain injury OR concussion	Rodent

*MeSH terms were exploded to include more specific terms; MeSH terms were translated into the appropriate subject headings for other databases. Keywords were the same for each database searched

eicosapentaenoic acid (EPA) and/or docosahexaenoic acid (DHA) (versus placebo) and (4) reporting of pre- and post-mTBI neurological or cognitive outcomes and/or molecular outcomes (see "Data analysis" section). Exclusion criteria included (1) non-rodent or human studies; (2) case studies, case reports and study protocols; non-peer-reviewed articles, conference abstracts, undergraduate (e.g. honours) or post-graduate (e.g. masters/PhD) theses, narrative reviews and basic studies investigating mechanisms of n-3 without presentation of mTBI data; and (3) disease models other than mTBI.

Data Extraction and Quality Assessment

Two authors (EH and AJP) evaluated the title and abstracts of the articles against inclusion and exclusion criteria. Further, any titles not relating to the study topic were also excluded. Lack of agreement regarding title and abstracts from the first pass of the review was resolved by mutual agreement with the third author (CSP). Following duplication, article full texts were then obtained for data extraction and analysis.

Study quality was assessed by two authors (EH and AJP) using the Animal Research: Reporting of In Vivo Experiments (ARRIVE 2.0) guidelines [28, 29]. Risk of bias was completed using the Cochrane risk of bias tool [30]. Lack of agreement about grading against study quality was reconciled by mutual agreement with a third author (CSP). Articles that satisfied the inclusion criteria were read, and eligible studies were then included in the meta-analysis.

Data Analysis

For all included articles, data extraction involved the retrieval of study characteristics (author, year, sample size and study design), animal demographics (age, gender) and variables analysed. This was completed by one author (AJP) with a sub-sample checked by two other authors (EH and CSP). Table 2 outlines a summary of included studies and variables extracted that were included in the meta-analysis. Data comparing the supplemented animals to non-supplemented controls (mean and SD) were extracted from eligible studies. If studies contained data in a graphical rather than in a tabulated format, Plot Digitizer (Version 4.4) [49] was used to extract visually presented data.

As systematic influences and random error were predicted to be present between study-level effect sizes, a random effects meta-analysis was performed to compare the overall pooled standardised mean differences (SMDs) for the main outcome measures [50]. SMDs with 95% confidence intervals (CIs) were used to measure mTBI effects on performance as the included studies presented outcome measures in a variety of ways. The absolute SMD values of < 0.5 (small), 0.50–0.79 (moderate) and ≥ 0.80 (large) were used to describe the magnitude of effects [51]. Heterogeneity was measured using the I^2 statistic, indicating the percentage variance between studies for low (25%), moderate (50%) and high (75%) heterogeneity [52]. All statistical analyses were conducted using RevMan (V5.3, Review Manager, The Cochrane Collaboration) using a significance level of $\alpha = 0.05$.

Results

Summary of Included Studies

Figure 1 outlines the PRISMA data flow of study search and selection. The initial search yielded 291 records based on title and abstract. Following removal of duplicates (n=139), the remaining 152 records were screened with 107 removed, as they did not meet the inclusion criteria. Forty-five full-text manuscripts were assessed for eligibility, with 27 of these being removed for reasons including spinal cord and penetrating brain injury, phospholipid profiling of n-3FA and prophylactic administration of n-3FA and no data presented for the meta-analysis. Further searching of reference lists and hand searching revealed no records meeting the inclusion criteria, making the final total of 18 studies.

Table 2. Summary of studies included in the systematic review and meta-analysis.

Study	Design	Animal cohort	Dosage	Main findings	ARRIVE score (max 38)
Bailes and Mills 2010 [31]	Between groups	Sprague–Dawley rats (n=40, male, 350–400 g)	10 mg/kg/d DHA (low dose) 40 mg/kg/d DHA (high dose)	Significantly decreased numbers of APP-positive axons in animals receiving n-3 FAs dietary supplementation vs control	27
Begum et al 2014 [32]	Between groups repeated measures	Sprague–Dawley rats (n=150, male, 250–275 g)	16 mg/kg/d DHA	Administration of n-3 FAs reduced the accumulation of both ubiquitinated proteins and APP/p-Tau proteins. DHA-treated animals exhibited early recovery of their sensorimotor function after TBI vs control	25
Chen et al 2017 [33]	Between groups repeated measures	Sprague–Dawley rats (n=108, male, 230–260 g)	2 ml/kg/d including 28.8–61.8mg DHA/kg/day 25–56.4mg EPA/kg/day	n-3 FAs supplementation inhibited TBI-induced microglial activation and expression of inflammatory factors (TNF-α, IL-6), decreased neuronal apoptosis and improved neurological functions after TBI vs control	29
Chen et al 2018 [34]	Between groups repeated measures	Sprague–Dawley rats (n=48, male, 230–260 g)	2 ml/kg/d 28.8–61.8mg DHA/kg/day 25–56.4mg EPA/kg/day	n-3 FAs supplementation protected neurons against TBI-induced neuronal apoptosis. Supplementation significantly increased the SIRT1 activity following TBI	29
Chen et al 2018 [35]	Between groups repeated measures	Sprague–Dawley rats (n=48, male, 230–260 g)	2 ml/kg/d 28.8–61.8mg DHA/kg/day 25–56.4mg EPA/kg/day	n-3 FAs supplementation reduced TBI-induced inflammatory factors. Treatment with DHA/EPA inhibited HMGB1 acetylation and induced direct interactions between SIRT1 and HMGB1 by elevating SIRT1 activity following TBI via the NF-kB pathway	29
Ghazale et al 2018 [36]	Between groups repeated measures	C57BL6 mice (n=31, male, 7–8 wk. old)	12 mg/kg/d DHA	n-3 FAs (DHA) injections significantly attenuated TBI-induced motor function deficits (pole climbing test) and promoted neurogenesis. Compared to neural stem cell injection alone, DHA induced neurogenesis when injected together with neural stem cells. Neurogenesis was coupled with an increase in glial reactivity (i.e. GFAP) at the cortical site of injury. Immunoblotting analysis indicated that DHA + NSCs-treated animals showed attenuated calpain/caspase activation	29
Harvey et al 2015 [37]	Between groups repeated measures	Sprague–Dawley rats (n=72, male, 250–275 g)	16 mg/kg/d DHA	n-3 FAs post-TBI decreased in nuclear translocation of activated nuclear factor kappa-light-chain-enhancer of activated B cells protein at 3 days in DHA-treated tissue and reduced neuronal degeneration in DHA-treated brains at 3, 7 and 21 days after TBI	27
Lin et al 2017 [38]	Between groups repeated measures	Sprague–Dawley rats (n=72, male, 220–260 g)	Not stated	Rats treated with n-3 FAs had significantly less TBI-induced caspase cleavage and IL-1β secretion than those with vehicle. Further, n-3 FAs markedly ameliorated neuronal death and behavioural deficits after TBI	30
Lucke-Wold et al 2016 [39]	Between groups repeated measures	Adult rats (n=88, male)	16 mg/kg/d DHA	DHA supplementation improved cognitive performance in the r at model 3 weeks after repetitive blast exposure	26.5
Mills et al 2011 [40]	Between groups	Sprague–Dawley rats (n=40, male, 350–400 g)	10 mg/kg/d (high dose) 40 mg/kg/d (low dose) EPA:DHA 2:1 ratio	Dietary supplementation with a fish oil concentrated rich in EPA and DHA for 30 days resulted in decreased numbers of APP-positive axons. Immunohistochemical analysis of caspase-3 expression demonstrated significant reduction compared to non-supplemented rats	28

Table 2. (continued)

Study	Design	Animal cohort	Dosage	Main findings	ARRIVE score (max 38)
Pu et al 2017 [41]	Between groups repeated measures	C57BL/6J mice (male, 10–12 wk. old)	7 mg/kg/d EPA and 3 mg/kg/d DHA	Mice receiving post-TBI n-3 FA treatments, the combined treatment of fish oil dietary supplement and n-3 PUFA injections demonstrated a eproducible beneficial effect in attenuating cognitive deficits although without reducing gross tissue loss. Post-TBI fish oil dietary supplementation alone as well as dietary supplementation combined with n-3 PUFA daily injections stimulates the generation of neural progenitor cells (stained for BrdU and doublecortin) in the cortex and striatum	30
Tang et al 2018 [42]	Between groups repeated measures	Sprague–Dawley rats (n=40, male, 280–300 g)	16 mg/kg/d DHA	DHA improved the neurological function and learning and memory ability of rats after TBI as well as reduced neuronal apoptosis in the hippocampus. TLR4 expression and the expression of the inflammatory mediator NF-Kappa B were also repressed by DHA treatment. Rats treated with DHA after TBI also showed reduced hippocampal glial reactivity (i.e. reduced GFAP staining)	29
Thau-Zuchman et al 2019 [43•]	Between groups repeated measures	C57BL/6 mice (n=20, male, 10–12 wk. old)	Dietary formulation of DHA oil 4.5 g/100 g; EPA 0.3 g/100 g animal feed. Dietary intake similar but no report on mean intakes	n-3 FA treatment led to a significantly improved sensorimotor outcome and cognition reduced lesion size and oligodendrocyte loss, and it restored myelin. It decreased microglia activation and the rise in ß-amyloid precursor protein and restored depressed neurogenesis resulting from TBI	30
Wu et al 2011 [44]	Between groups repeated measures	Sprague–Dawley rats (n=36, male, 200–240 g)	32.9 ± 1.9 g DHA diet (via food intake). Dietary intake similar but no report on mean intakes	DHA supplementation improved learning ability in injured rats compared to no supplementation	26
Wu et al 2014 [45]	Between groups repeated measures	Sprague–Dawley rats (n=25–30, male, 200–240 g)	Exact dosage not provided 'DHA-enriched diet'	DHA supplementation reduced cognitive impairments with injured rats compared to no supplementation	26
Yin et al 2018 [46]	Between groups repeated measures	Sprague–Dawley rats (n=152, male, 280–300 g)	16 mg/kg/d DHA	DHA-treated animals performed better than the non-supplemented control group on the Morris water maze test	27
Zhu et al 2017 [47]	Between groups repeated measures	Sprague–Dawley rats (n=80, male/female 1:1, 300–500 g)	370 mg/kg/d DHA 740 mg/kg/d DHA	Compared with the control and TBI-low DHA-supplemented group, the TBI-high DHA-supplemented group demonstrated shorter escape latency and swimming distances. DHA inhibited the expression of caspase-3, and the inhibition effect was dose dependent	24
Zhu et al 2018 [48]	Between groups repeated measures	Wistar rats (n=240, male, 7 wk. old, 200–250 g)	370 mg/kg/d DHA 740 mg/kg/d DHA	Rats in the DHA-supplemented group performed better than those of the TBI group in both neurological severity score (NSS) test and water maze experiment. DHA reduced the expression of caspase-3	27

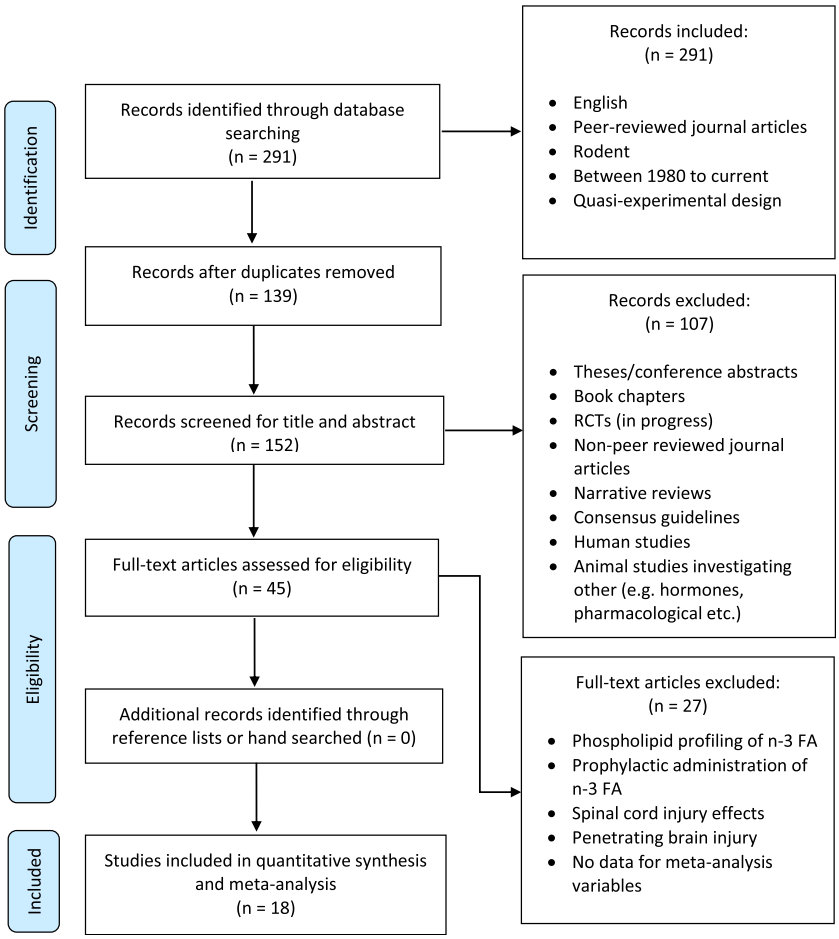


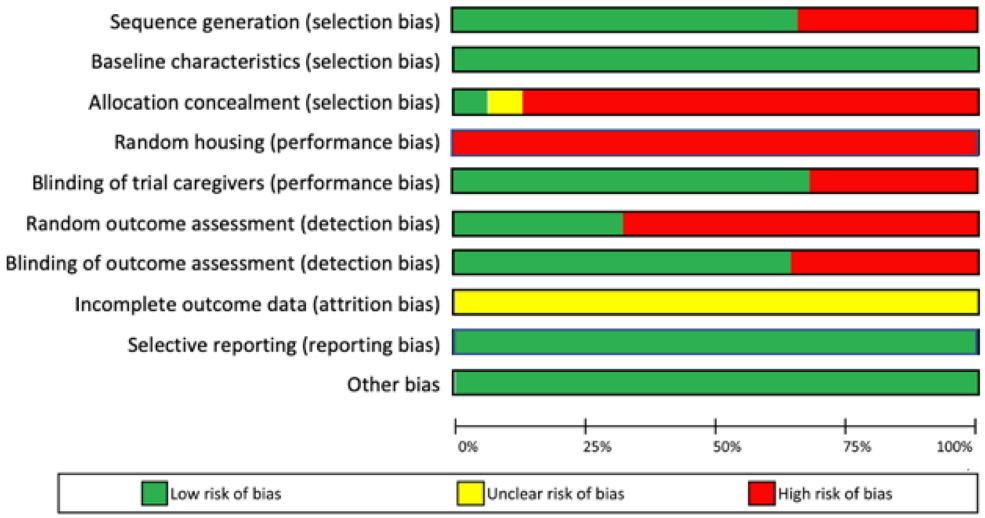
Fig 1 Flow of identification, screening, eligibility and study inclusion of previously published studies using the PRISMA guidelines.

Quality assessment scores using the ARRIVE 2.0 guidelines [28, 29]. Scores ranged from 24 to 30 suggesting moderate to high quality (Table 2). While studies were mostly compliant in addressing the ARRIVE criteria, common criteria were not properly addressed related to protocol registration, sample size calculation, blinding, justification of statistical methods and detailing animal care and monitoring. Risk of bias is presented in Figure 2.

Neurological and Cognitive Outcomes

Neurological scores and cognitive (water maze, rotarod, beam walking) performance are presented in Figures 2, 3, 4 and 5. Neurological severity score refers to a composite score of animal neurological function across motor, sensory, reflex and balance [53]. In the calculation of the severity scores of injury, one point is awarded for the *inability* to perform the test or the lack of a tested reflex. The higher score indicates a more severe injury. Neurological scores are presented as a time-course recovery post-injury, which are shown as subgroups in the analysis for 1, 7, 14 and 21 days post-injury (Figure 3). At 1 day post-injury, there were no significant differences in neurological scores between groups (SMD=0.81 [−0.24, 1.86]; P=0.13, I^2=84%). However, at 7, 14 and 21 days, there were significant differences in neurological function between rodents administered mTBI alone versus animals that received mTBI and n-3FA (SMD=2.66 [0.73, 4.59]; P=0.007, I^2=92%; SMD=2.75 [2.18, 3.32]; P<0.001, I^2=8%; SMD=1.66 [0.56, 2.76]; P=0.003, I^2=48%, respectively). The overall pooled data across all time points (SMD=1.98 [0.56, 2.77]; Z=4.92, P<0.001, I^2=91%) indicated significant decreases in both neurological scores and corresponding increases cognitive performance in rodents who received n-3FA compared to controls.

Water maze, rotarod and beam walking performance were quantified based on the time taken to complete the task. The Morris water maze test assesses learning and memory in rodents with a quicker time indicating improvements in learning and memory [54]. Water maze data is presented across 1, 2 and 4 days post-injury (Figure 4). At each time point, significant differences were seen between groups (SMD=1.47 [0.49, 2.45]; P=0.003, I^2=88%; SMD=2.35 [1.01, 3.69]; P<0.001, I^2=91%; SMD=1.52 [0.54, 2.49]; P=0.002, I^2=84%, respectively). The overall pooled data across all time points showed significant increases in the time taken to complete tasks post-injury compared to the duration to complete



	Sequence generation (selection risk)	Baseline characteristics (selection bias)	Allocation concealment (selection bias)	Random housing (performance bias)	Blinding of trial caregivers (performance bias)	Random outcome assessment (detection bias)	Blinding outcome assessment (detection bias)	Incomplete outcome data (attrition bias)	Selective reporting (reporting bias)	Other bias
Biles and Mills	+	+	X	X	X	X	X	-	+	+
Begum et al	X	+	X	X	+	X	+	-	+	+
Chen et al	+	+	X	X	+	X	+	-	+	+
Chen et al	+	+	X	X	+	X	+	-	+	+
Chen et al	+	+	X	X	+	X	+	-	+	+
Ghazaie et al	+	+	+	X	+	X	+	-	+	+
Harvey et al	X	+	-	X	+	X	+	-	+	+
Lin et al	+	+	X	X	X	X	X	-	+	+
Lucke-Wold et al	+	+	X	X	+	+	+	-	+	+
Mills et al	X	+	X	X	X	+	X	-	+	+
Pu et al	+	+	X	X	+	+	+	-	+	+
Tang et al	+	+	X	X	+	X	+	-	+	+
Thau-Zuchman et al	+	+	X	X	+	+	+	-	+	+
Wu et al	X	+	X	X	X	X	X	-	+	+
Wu et al	X	+	X	X	X	X	X	-	+	+
Yin et al	X	+	X	X	+	+	+	-	+	+
Zhu et al	+	+	X	X	X	X	X	-	+	+
Zhu et al	+	+	X	X	X	+	X	-	+	+

Fig 2 Risk of bias of included studies.

tasks for rodents who received mTBI and treatment with n-3FA (SMD=1.78 [1.16, 2.39]; Z= 5.66; P<0.001, I^2=88%).

The rotarod test measures locomotor ability by recording the duration that rodents remain on a rotating cylinder [55]. Similarly, the beam walking test assesses motor behaviour in a restricted area that requires forepaw and hind paw locomotor activity [56]. The rotarod data are presented at 3, 7 and 14 days post-injury (Figure 5). No differences were seen between rodents receiving mTBI alone and animals that received mTBI with n-3FA treatment at 3 days post-injury (SMD=1.29 [−0.05, 2.63]; P=0.06, I^2=72%). In contrast, significant

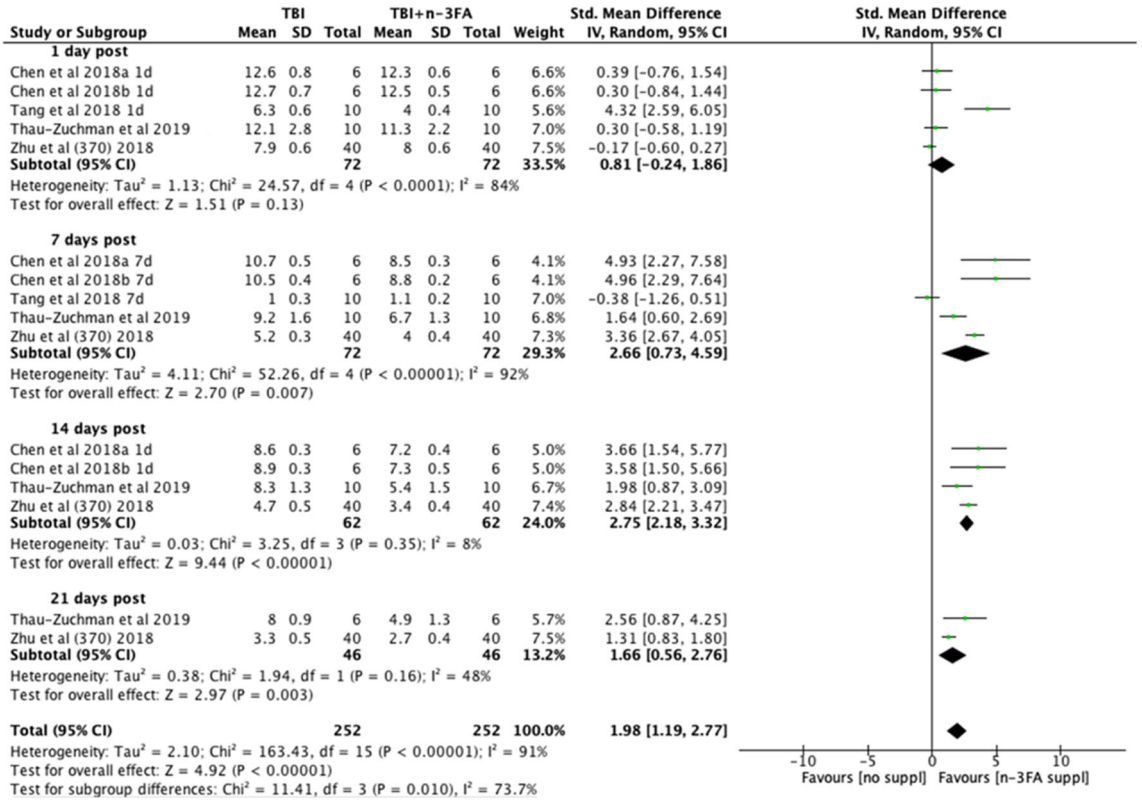


Fig 3 Neurological severity score

differences were found at 7 days (SMD=1.98 [0.75, 3.22]; P=0.002, I^2=71%) and 14 days (SMD=1.78 [0.07, 3.49]; P=0.04, I^2=78%) where rodents treated with n-3FA had longer durations on the rotarod post-injury compared with rodents that received mTBI without treatment. Overall pooled data showed (SMD=1.65 [0.96, 2.35]; Z= 4.67; P<0.001, I^2=67%) a significant improvement in locomotor activity in mTBI-treated rodents.

Beam walking time and score, where the rodent was scored on how far they progressed along the length of the beam [32, 42], is shown in Figure 6a and b, respectively. No significant differences in beam walking time were found between n-3FA-treated and untreated groups at 1 and 2 days post-injury (SMD=0.16 [−1.94, 2.25]; P=0.88, I^2=90%; SMD=1.59 [−1.87, 5.05]; P=0.37, I^2=94%, respectively). On day 4 post-injury, however, rodents treated with n-3FA showed an increased latency in beam walking (SMD=2.95 [0.93, 4.98]; P=0.004, I^2=76%). Overall pooled data showed a significant effect (SMD=1.52 [0.10, 2.94]; Z= 2.10; P=0.04, I^2=90%). Beam walking score showed no significant differences between groups at 1 day post-injury (SMD=0.2.55 [−0.73, 5.83]; P=0.13, I^2=91%). However, at 2 and 4 days post-injury, significant differences were observed between groups (SMD=3.13 [1.72, 4.55]; P<0.001, I^2=84%; SMD=4.48 [2.34, 6.63]; P<0.001, I^2=89%, respectively). Overall pooled data showed a significant effect indicating an increased beam walking score with n-3FA treatment (SMD=3.55 [2.42, 4.67]; Z= 6.19; P<0.001, I^2=88%).

Molecular and Inflammatory Markers

Routinely used molecular markers (Caspase3 and TUNEL) and inflammatory markers (IL6, NFkB\ and TFNα) to quantify tissue pathology are presented in Figure 7a–e. Overall pooled data for each marker showed significantly decreased levels of each marker in rodents supplemented with n-3FA compared to control (SMD ranges 3.73–6.55; P<0.01). However, heterogeneity across variables was observed to be moderate to high (I^2 range 56–94%).

Discussion

This is the first systematic review and meta-analysis to quantify the efficacy of n-3FA supplementation on neurological, cognitive and molecular outcomes following mTBI in rodents. Our data indicate that following mTBI, rodents that were fed a diet rich in n-3FA (DHA and EPA), compared to control, significantly improved neurological and cognitive parameters including water maze, rotarod and beam walking performance test. The papers reviewed utilised both DHA only and EPA plus DHA interventions. While EPA and DHA have been shown to exert differential physiological effects, the relative

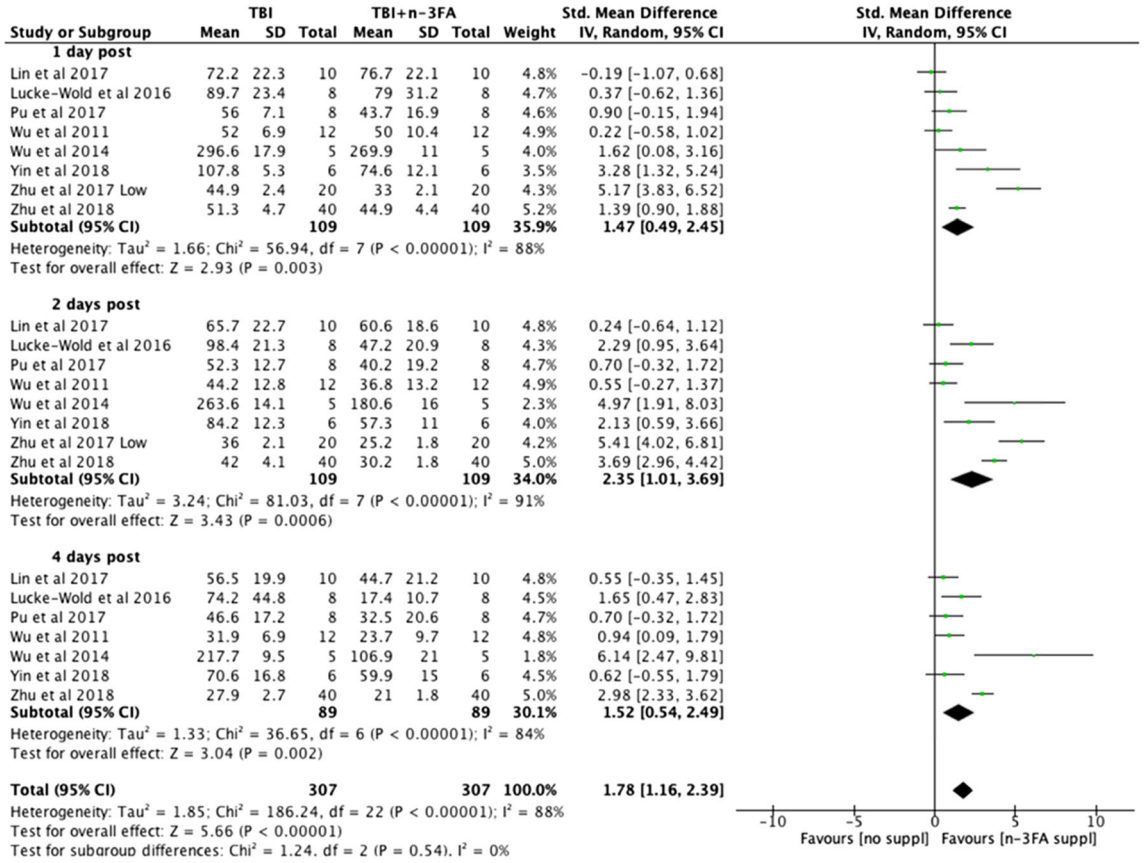


Fig 4 Water maze–latency time(s)

roles each fatty acid plays in modulating the effect of mTBI warrant further investigation.

The various physiological effects of n-3FA are well documented and offer a number of plausible mechanisms of action. Firstly, n-3FA exert a generalised anti-inflammatory effect which in turn acts to reduce micro-anatomical injury in improved post-TBI recovery [57]. Secondly, at the molecular level, post-injury n-3FA DHA supplementation has been shown to normalise levels of brain-derived neurotrophic factor (BDNF), synapsin 1

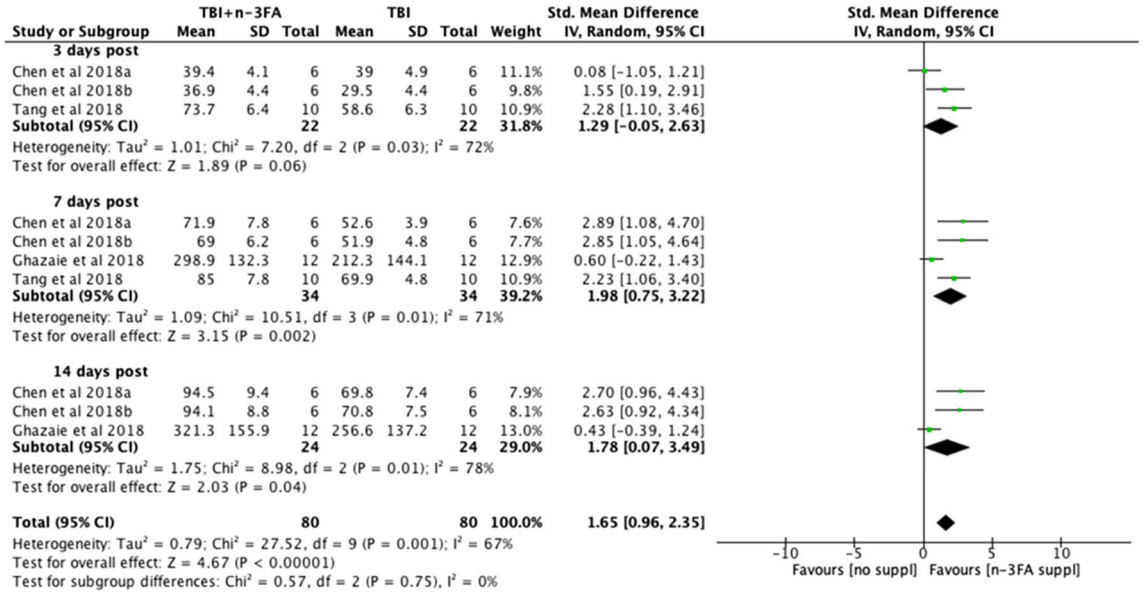


Fig 5 Rotarod–latency time(s)

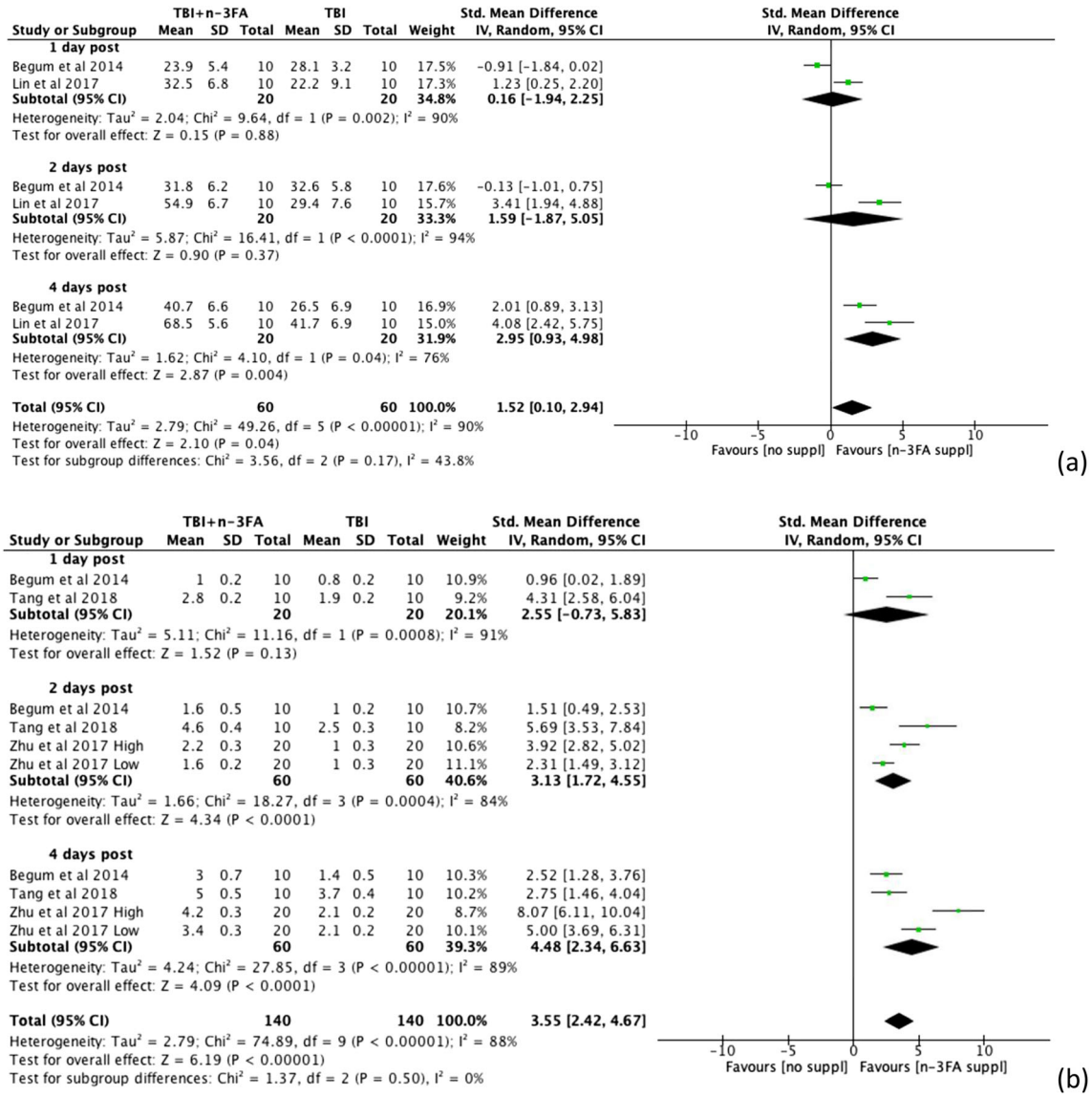


Fig 6 Beam walking–latency time (**a**) and Beam walking score (**b**)

(Syn-1), cAMP-responsive element-binding protein (CREB) and calcium/calmodulin-dependent kinase II (CaMKII) [44]. Each of these components is involved in neurological and cognitive processing. Thirdly, it has also been suggested that treatment with n-3FA DHA diets preceding brain injury can salvage levels of the antioxidants superoxide dismutase (SOD) and Silent Information Regulator 2 (the NAD+-dependent deacetylase; Sir2) and in doing so may provide resistance to oxidative stress [44]. Finally, n-3FA DHA supplementation can normalise levels of calcium-independent phospholipase A2 (iPLA2) and syntaxin-3, both of which have been suggested to contribute to maintaining membrane function following trauma injury in rodents [44]. The multiple mechanism of action could plausibly provide a unique therapeutic effect approach to the multifactorial problem of mTBI.

The studies included in this meta-analysis were moderate to good in quality, and the risk of bias assessment revealed elements of the studies that should be considered in future designs. Specifically, aspects of selection, performance and detection bias (Figure 2a) that were not described in the studies may open up interpretations of study bias. Further limitations observed include the different time point measures across the variables analysed. For example, neurological severity score was presented at 1, 7, 14 and 21 days, while the rotarod performance was presented at 3, 7, 14 and 21 days. Conversely, the water maze results were measured at 1, 2 and 4 days post-injury. Further, as molecular variables were presented as a one-off data points, it creates difficulty in interpreting the mechanistic molecular changes with the repeated neurological and cognitive measures presented for translation to human studies.

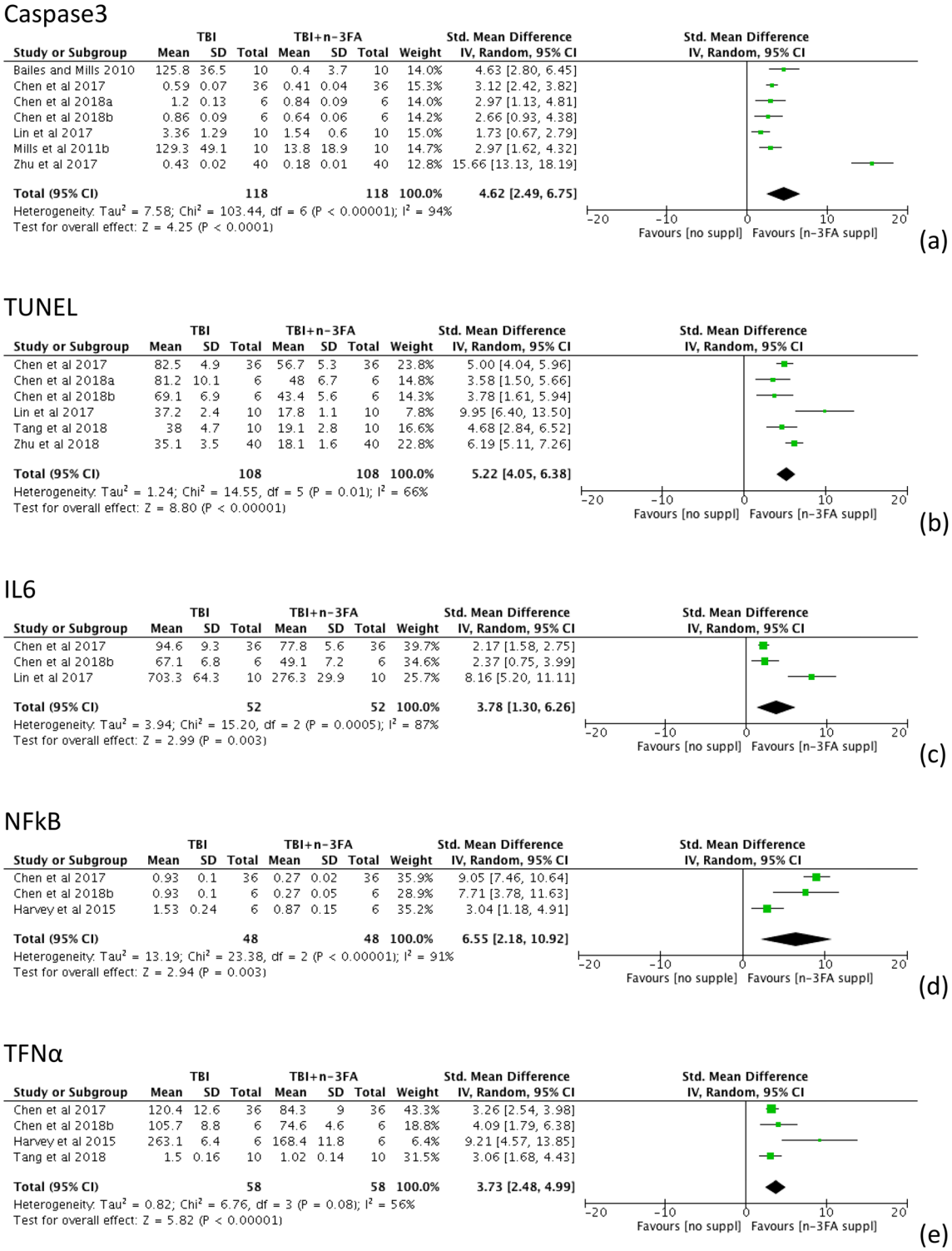


Fig 7 Molecular markers Caspase3 (**a**) and TUNEL (**b**) and inflammatory markers IL6 (**c**), NFKb (**d**) and TFNα (**e**)

In order to translate these preclinical findings to the clinical setting, determining the optimal therapeutic dose is paramount. Notably there have been a number of very informative clinical case studies reporting significant results in severe TBI patients given high-dose DHA (19,212 mg/day) via gastrointestinal delivery for more than a year with no adverse effects [23]. The studies presented in this analysis used DHA alone and DHA/EPA mixtures with varying dosage levels of omega-3 fatty acids in the range of 10–370 mg per day. Using the simple dose calculation to determine Human Equivalent Dose (HED) as outlined by Nair and Jacob [58] (HED (mg/kg)= Animal NOAEL (no observed adverse effect

levels) (mg/g) ×(Weight $_{\text{Animal}}$ Kg /Weight $_{\text{Human}}$ Kg)$^{(1\text{-body surface area rats }(0.67)}$, which when calculated equates to approximately HED 10, 000mg of DHA and EPA a day for at least 3-months. We would recommend this to be the minimal viable dose to be used in subsequent intervention trials for this indication. This dose is also supported in the 'real-world' setting with numerous American University sports programs including the US National Collegiate Athletic Association (NCAA) recommending 9000 mg/day on n-3FA. Given the favourable safety profile of n-3FA, high-dose interventions provide an exciting and potentially cost-effective therapeutic approach to the devastating public health concern of mTBI; it is long overdue to test this in humans under a placebo-controlled randomised control trial setting [22••].

Conclusions

The findings from this systematic review and meta-analysis demonstrate that despite variations in terms of study designs, variability of dose, risk of bias and disparity between mechanistic and functional data presented, the overall robust findings and strong effects suggest benefits in n-3FA supplementation following mild traumatic brain injury. Given the lack of data in human studies [23, 25••], the findings from this review in preclinical studies should provide suitable evidence to test in clinical studies. Consequently, the observed improvements across all neurological and cognitive variables warrant immediate consideration progression of n-3FA as a therapeutic candidate for mTBI intervention in humans.

Author Contribution CSP, EYH and AJP conceived the study. AJP completed the data analysis. CSP, EYH and AJP contributed to the writing of the manuscript. All authors contributed to the review and editorial suggestions for the writing of the manuscript.

Data Availability Data is available upon reasonable request.

Declarations

Ethics Approval No ethics approval was required to undertake this systematic review.

Consent to Participate N/A

Consent for Publication N/A

Conflict of Interest AJP currently receives partial research salary funding from Sports Health Check charity (Australia) and Erasmus+ strategic partnerships program (2019-1-IE01-KA202-051555). AJP has previously received partial research funding from the Australian Football League, Impact Technologies Inc., and Samsung Corporation, and has provided expert reports in concussion legal proceedings. CSP is Director of Feedback Nutrition Pty Ltd which sells and markets a DHA supplement is a shareholder and Non-Executive Director of Sea Dragon Ltd which sells and markets DHA tuna oil ingredients for the food and infant formula market. ML is a medical advisor to Nordic Naturals, Inc. No other author has any declaration of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

References

Papers of particular interest, published recently, have been highlighted as:
- • Of importance
- •• Of major importance

1. McInnes K, Friesen CL, MacKenzie DE, Westwood DA, Boe SG. Mild traumatic brain injury (mTBI) and chronic cognitive impairment: a scoping review. PLoS One. 2017;12(4):e0174847.
2. Alexander MP. Mild traumatic brain injury. Pathophysiology, natural history, and clinical management. Neurology. 1995;45(7):1253–60.
3. Yue JK, Upadhyayula PS, Avalos LN, Phelps RRL, Suen CG, Cage TA. Concussion and mild-traumatic brain injury in rural settings: epidemiology and specific health care considerations. J Neurosci Rural Pract. 2020;11(1):23–33.
4. Langlois JA, Rutland-Brown W, Wald MM. The epidemiology and impact of traumatic brain injury: a brief overview. J Head Trauma Rehabil. 2006;21(5):375–8.
5. Finch CF, Clapperton AJ, McCrory P. Increasing incidence of hospitalisation for sport-related concussion in Victoria, Australia. Med J Aust. 2013;198(8):427–30.
6. Faul M, Xu L, Wald MM, Coronado VG. Traumatic brain injury in the United States: emergency department visits, hospitalizations, and deaths 2002–2006. Atlanta, GA: US Department of Health and Human Services, CDC; 2010.
7. Wang K, Cui D, Gao L. Traumatic brain injury: a review of characteristics, molecular basis and management. Front Biosci. 2016;21:890–9.
8. McCrory P, Meeuwisse W, Dvorak J, Aubry M, Bailes J, Broglio S, et al. Consensus statement on concussion in sport—the 5th international conference on concussion in sport held in Berlin, October 2016. Brit J Sport Med. 2017;51:838–47.
9. Dimou S, Lagopoulos J. Toward objective markers of concussion in sport: a review of white matter and neurometabolic changes in the brain after sports-related concussion. J Neurotrauma. 2014;31(5):413–24.
10. Giza CC, Hovda DA. The neurometabolic cascade of concussion. J Athl Train. 2001;36(3):228–35.
11. Giza CC, Hovda DA. The new neurometabolic cascade of concussion. Neurosurgery. 2014;75(suppl_4):S24–33.
12. Romeu-Mejia R, Giza CC, Goldman JT. Concussion pathophysiology and injury biomechanics. Curr Rev Musculoskelet Med. 2019;12(2):105–16.
13. Kara S, Crosswell H, Forch K, Cavadino A, McGeown J, Fulcher M. Less than half of patients recover within 2 weeks of injury after a sports-related mild traumatic brain injury: a 2-year prospective study. Clin J Sport Med. 2020;30(2):96–101.
14. Alosco ML, Tripodis Y, Baucom ZH, Mez J, Stein TD, Martin B, et al. The late contributions of repetitive head impacts and tbi to depression symptoms and cognition. Neurology. 2020;95(7):e793–804.

15. Livingston G, Huntley J, Sommerlad A, Ames D, Ballard C, Banerjee S, et al. Dementia prevention, intervention, and care: 2020 report of the Lancet Commission. Lancet. 2020;396(10248): 413–46.
16. Barnes DE, Kaup A, Kirby KA, Byers AL, Diaz-Arrastia R, Yaffe K. Traumatic brain injury and risk of dementia in older veterans. Neurology. 2014;83(4):312–9.
17. Gardner RC, Yaffe K. Epidemiology of mild traumatic brain injury and neurodegenerative disease. Mol Cell Neurosci. 2015;66:75–80.
18. Rabinowitz AR, Li X, McCauley SR, Wilde EA, Barnes A, Hanten G, et al. Prevalence and predictors of poor recovery from mild traumatic brain injury. J Neurotrauma. 2015;32(19):1488–96.
19. Guskiewicz KM, Marshall SW, Bailes J, McCrea M, Cantu RC, Randolph C, et al. Association between recurrent concussion and late-life cognitive impairment in retired professional football players. Neurosurgery. 2005;57(4):719–26.
20. Lehman EJ, Hein MJ, Baron SL, Gersic CM. Neurodegenerative causes of death among retired National Football League players. Neurology. 2012;79(19):1970–4.
21. Mackay DF, Russell ER, Stewart K, MacLean JA, Pell JP, Stewart W. Neurodegenerative disease mortality among former professional soccer players. N Engl J Med. 2019;381:1801–8.
22.•• Mcgeown JP, Hume PA, Theadom A, Quarrie KL, Borotkanics R. Nutritional interventions to improve neurophysiological impairments following traumatic brain injury: a systematic review. J Neurosci Res. 2021;99(2):573–603 **The authors systematically reviewed the literature and show that n-3 FAs have shown the most promising pre-clinical results for altering neurophysiological outcomes following traumatic brain injury. Our study confirms that no clinical studies have yet been conducted in not only cognitive and neurological outcomes but also neurophysiological.**
23. Lewis M, Ghassemi P, Hibbeln J. Therapeutic use of omega-3 fatty acids in severe head trauma. Am J Emerg Med. 2013;31(1):273. e5–8.
24. Gomez-Pinilla F. The combined effects of exercise and foods in preventing neurological and cognitive disorders. Prev Med. 2011;52:S75–80.
25.•• Patch C, Hill-Yardin E, Ryan L, Daly E, Pearce AJ. Long chain omega-3 fatty acid intervention in ageing adults at risk of dementia following repeated head trauma. Low-level support or an opportunity for an unanswered question? J Prev Alzheimers Dis. 2021;8(1): 29–32 **This systematic review demonstrated that there are currently no studies that have employed n-3 FAs in humans with post-concussion syndrome. The study suggests that pre-clinical animal data is strong enough to take n-3 FA to trials in humans suffering from post-concussion syndrome.**
26. Moher D, Shamseer L, Clarke M, Ghersi D, Liberati A, Petticrew M, et al. Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015 statement. Syst Rev. 2015;4: 1.
27. Wright RW, Brand RA, Dunn W, Spindler KP. How to write a systematic review. Clin Orthop Relat Res. 2007;455:23–9.
28. Percie du Sert N, Ahluwalia A, Alam S, Avey MT, Baker M, Browne WJ, et al. Reporting animal research: explanation and elaboration for the ARRIVE guidelines 2.0. PLoS Biol. 2020;18(7): e3000411.
29. Percie du Sert N, Hurst V, Ahluwalia A, Alam S, Avey MT, Baker M, et al. The ARRIVE guidelines 2.0: updated guidelines for reporting animal research. J Cereb Blood Flow Metab. 2020;40(9):1769–77.
30. Higgins JP, Altman DG, Gøtzsche PC, Jüni P, Moher D, Oxman AD, et al. The Cochrane Collaboration's tool for assessing risk of bias in randomised trials. Brit Med J. 2011;343:d5928.
31. Bailes JE, Mills JD. Docosahexaenoic acid reduces traumatic axonal injury in a rodent head injury model. J Neurotrauma. 2010;27(9):1617–24.
32. Begum G, Yan HQ, Li L, Singh A, Dixon CE, Sun D. Docosahexaenoic acid reduces ER stress and abnormal protein accumulation and improves neuronal function following traumatic brain injury. J Neurosci Off J Soc Neurosci. 2014;34(10):3743–55.
33. Chen X, Wu S, Chen C, Xie B, Fang Z, Hu W, et al. Omega-3 polyunsaturated fatty acid supplementation attenuates microglial-induced inflammation by inhibiting the HMGB1/TLR4/NF-κB pathway following experimental traumatic brain injury. J Neuroinflammation. 2017;14(1):1–12.
34. Chen X, Chen C, Fan S, Wu S, Yang F, Fang Z, et al. Omega-3 polyunsaturated fatty acid attenuates the inflammatory response by modulating microglia polarization through SIRT1-mediated deacetylation of the HMGB1/NF-κB pathway following experimental traumatic brain injury. J Neuroinflammation. 2018;15(1): 1–15.
35. Chen X, Pan Z, Fang Z, Lin W, Wu S, Yang F, et al. Omega-3 polyunsaturated fatty acid attenuates traumatic brain injury-induced neuronal apoptosis by inducing autophagy through the upregulation of SIRT1-mediated deacetylation of Beclin-1. J Neuroinflammation. 2018;15(1):1–15.
36. Ghazale H, Ramadan N, Mantash S, Zibara K, El-Sitt S, Darwish H, et al. Docosahexaenoic acid (DHA) enhances the therapeutic potential of neonatal neural stem cell transplantation post—Traumatic brain injury. Behav Brain Res. 2018;340:1–13.
37. Harvey LD, Yin Y, Attarwala IY, Begum G, Deng J, Yan HQ, et al. Administration of DHA reduces endoplasmic reticulum stress-associated inflammation and alters microglial or macrophage activation in traumatic brain injury. ASN Neuro. 2015;7(6): 1759091415618969.
38. Lin C, Chao H, Li Z, Xu X, Liu Y, Bao Z, et al. Omega-3 fatty acids regulate NLRP3 inflammasome activation and prevent behavior deficits after traumatic brain injury. Exp Neurol. 2017;290:115–22.
39. Lucke-Wold BP, Turner RC, Logsdon AF, Nguyen L, Bailes JE, Lee JM, et al. Endoplasmic reticulum stress implicated in chronic traumatic encephalopathy. J Neurosurg. 2016;124(3):687–702.
40. Mills JD, Bailes JE, Sedney CL, Hutchins H, Sears B. Omega-3 fatty acid supplementation and reduction of traumatic axonal injury in a rodent head injury model. J Neurosurg. 2011;114(1):77–84.
41. Pu H, Jiang X, Wei Z, Hong D, Hassan S, Zhang W, et al. Repetitive and prolonged omega-3 fatty acid treatment after traumatic brain injury enhances long-term tissue restoration and cognitive recovery. Cell Transplant. 2017;26(4):555–69.
42. Tang R, Lin YM, Liu HX, Wang ES. Neuroprotective effect of docosahexaenoic acid in rat traumatic brain injury model via regulation of TLR4/NF-Kappa B signaling pathway. Int J Biochem Cell Biol. 2018;99:64–71.
43.• Thau-Zuchman O, Gomes RN, Dyall SC, Davies M, Priestley JV, Groenendijk M, et al. Brain phospholipid precursors administered post-injury reduce tissue damage and improve neurological outcome in experimental traumatic brain injury. J Neurotrauma. 2019;36(1):25–42 **This study in pre-clinical models demonstrated that n−3 FA treatment led to a significantly improved sensorimotor outcome and cognition and reduced lesion size and oligodendrocyte loss, and it restored myelin. It decreased microglia activation and the rise in ß-amyloid precursor protein and restored depressed neurogenesis resulting from TBI.**
44. 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. 2011;28: 2113–22.
45. Wu A, Ying Z, Gomez-Pinilla F. Dietary strategy to repair plasma membrane after brain trauma: implications for plasticity and cognition. Neurorehabil Neural Repair. 2014;28(1):75–84.
46. Yin Y, Li E, Sun G, Yan HQ, Foley LM, Andrzejczuk LA, et al. Effects of DHA on hippocampal autophagy and lysosome function after traumatic brain injury. Mol Neurobiol. 2018;55(3):2454–70.

47. Zhu W, Chi N, Zou P, Chen H, Tang G, Zhao W. Effect of docosahexaenoic acid on traumatic brain injury in rats. Exp Ther Med. 2017;14(5):4411–6.
48. Zhu W, Ding Y, Kong W, Li T, Chen H. Docosahexaenoic acid (DHA) provides neuroprotection in traumatic brain injury models via activating Nrf2-ARE signaling. Inflammation. 2018;41(4):1182–93.
49. Rohatgi A. WebPlotDigitizer: Version 4.2. https://automeris.io/WebPlotDigitizer/citation.html. 2019.
50. Borenstein M, Hedges LV, Higgins JP, Rothstein HR. A basic introduction to fixed-effect and random-effects models for meta-analysis. Res Synth Methods. 2010;1(2):97–111.
51. Cohen J. Statistical Power Analysis for the Behavioral Sciences. Hillsdale: Erlbaum; 1988.
52. Higgins JP, Thompson SG, Deeks JJ, Altman DG. Measuring inconsistency in meta-analyses. Brit Med J. 2003;327(7414):557–60.
53. Germano AF, Dixon CE, d ' Avella D, Hayes RL, Tomasello F. Behavioral deficits following experimental subarachnoid hemorrhage in the rat. J Neurotrauma. 1994;11(3):345–53.
54. Morris RG. Spatial localization does not require the presence of local cues. Learn Motiv. 1981;12(2):239–60.
55. Crawley JN. Behavioral phenotyping of rodents. Comp Med. 2003;53(2):140–6.
56. Goldstein LB, Davis JN. Beam-walking in rats: studies towards developing an animal model of functional recovery after brain injury. J Neurosci Methods. 1990;31(2):101–7.
57. Bailes JE, Patel V. The potential for DHA to mitigate mild traumatic brain injury. Mil Med. 2014;179(11):112–6.
58. Nair A, Morsy MA, Jacob S. Dose translation between laboratory animals and human in preclinical and clinical phases of drug development. Drug Dev Res. 2018;79(8):373–82.