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Original research article

The potential effects of polyunsaturated ω-3 fatty acids on spinal cord injury: A systematic review & meta-analysis of preclinical evidence

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ABSTRACT

Polyunsaturated fatty acids (PUFAs) have received attention for their anti-inflammatory and antioxidant properties. Preclinical studies have investigated the efficacy of PUFAs in animal models of spinal cord injury (SCI) to determine if these properties can translate to neuroprotection and locomotor recovery. Findings from such studies have been promising, suggesting PUFAs as potential treatments against the neurological dysfunction induced by SCI. This systematic review and meta-analysis sought to investigate the efficacy of PUFAs for promoting locomotor recovery in animal models of SCI. PubMed, Web of Science and Embase (Ovid) were searched for relevant papers and those that examined the restorative effects of PUFAs on locomotor recovery in preclinical SCI models were included in our analysis. A random effects meta-analysis (restricted maximum likelihood estimator) was employed. A total of 28 studies were included and the results showed the claim that PUFAs have a beneficial therapeutic effect for promoting locomotor recovery (SMD = 1.037, 95% CI = 0.809–1.2644, p = <0.001) and cell survival (SMD = 1.101, 95% CI = 0.889–1.313, p = <0.001) in animal models of SCI. No significant differences for the secondary outcomes of neuropathic pain and lesion volume. Moderate asymmetry was observed in the funnel plots for locomotor recovery, cell survival and neuropathic pain measures, suggesting publication bias. Trim-and-fill analysis estimated 13, 3, 0 and 4 missing studies for locomotor recovery, cell survival, neuropathic pain, and lesion volume, respectively. A modified CAMARADES checklist was also used to assess risk of bias, showing that the median score for all included papers was 4 out of a possible 7.

Summary

In this systematic review and meta-analysis, we have investigated the potential of polyunsaturated omega-3 fatty acids (PUFA) for improving neurological dysfunction resulting from spinal cord injury (SCI). The primary outcome was locomotor recovery and the secondary outcomes included cell survival, neuropathic pain and lesion volume. Our findings demonstrated that PUFAs provide a significant improvement in locomotor recovery and cell survival in preclinical SCI models. Moderate asymmetry was calculated, indicating publication bias and missing studies with negative or neutral effects were predicted among outcome measures. The modified CAMARADES checklist used here showed a median score of 4 when tabulating study design methods that diminish bias in studies. Overall, the results here demonstrate a significant increase in outcome measures for preclinical SCI models treated with PUFAs

1. Introduction

Spinal cord injury (SCI) is a devastating neurological impairment affecting between 250,000 and 500,000 people worldwide each year [1]. Around 90% of SCI patients are left with permanent disabilities that include the loss of motor and sensory function below the level of injury. SCI patients often develop other secondary complications including autonomic/bladder/bowel dysfunctions, neuropathic pain, pressure ulcers and urinary tract infections [2,3]. While there is currently no effective treatment available for SCI, there are promising preclinical studies that have examined the efficacies of the long chain polyunsaturated ω−3 fatty acids (hereafter referred to as PUFAs) using animal models of SCI. These PUFAs include alpha linolenic acid (ALA) and

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its metabolites docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), which have been shown to confer neuroprotection and neuroregeneration [4–7].

Treatment with dietary supplementation of ALA has been shown to elicit anti-inflammatory effects by inhibiting inflammatory cytokines production, such as interleukin-1 β (IL-1β), interleukin-6 (IL-6) and tumor necrosis-α (TNF-α) [8,9]. Moreover, previous studies reported neuroprotective effects in rat SCI models produced by spinal cord ischemia, in which ALA dietary treatment improved neurological function [10]. The findings from these studies suggest that ALA can confer protection against ischemia following SCI including preventing necrosis and apoptosis of motor neurons [10–12].

In addition to ALA, its metabolite DHA is crucial for the development and functioning of the nervous system and has been shown to inhibit proinflammatory cytokine production in cultured primary rat microglia [5]. When given as an intravenous (IV) injection alone or an IV injection combined with continuous dietary supplementation or repeated systemic IV injections, DHA treatment conferred neuroprotection and improved locomotor function in various rodent models of SCI [13]. There is also evidence showing that EPA, another metabolite of ALA, also conferred neuroprotection in SCI animals [13].

It has also been shown that prophylactic dietary supplementation with DHA and EPA led to some recovery of bladder function (14). Moreover, they showed attenuation in sensory deficits, inflammation, oxidative stress, and the size of the lesion cavity in SCI rat models [14–16].

Overall, these findings strongly suggest that PUFAs have a high therapeutic potential in animal models of SCI. In order to further evaluate the efficacy of PUFAs as neuroprotective agents for SCI and assess their translational potential, we proposed a systematic review and meta-analysis of the existing preclinical literature. While a previous systematic review and meta-analysis conducted this same investigation with DHA [17], ours differs by including studies that used other PUFAs (*e.g.,* ALA and EPA) and by investigating additional outcomes that reflect neurorestorative effects after SCI. When considering this and the inclusion of studies using mouse and rat models, our investigation is a novel insight into the preclinical evidence of PUFA treatment in SCI. Here we report the effects of PUFAs either alone or in combination with other PUFAs on functional recovery in animal models.

2. Materials and methods

The Preferred Reporting Items for Systematic Reviews and Meta-Analyses statement (PRISMA) guidelines were used for this systematic review [18]. The protocol of this systematic review and meta-analysis was registered in the PROSPERO database (CRD42020193073).

2.1. Search strategy

Databases included in the search for relevant papers were PubMed, Embase (OVID) and Web of Science. Relevant published articles available in English were found using the keyword search strategy detailed in our protocol. Previously published filters for animal studies were used to acquire preclinical animal studies only [19,20]. Relevant review papers were also screened for additional published articles. The final search was performed on the 31st of May 2022.

2.2. Study selection

After duplicate studies were excluded from the compiled search strategy output, titles were screened for relevance, followed by a subsequent screening of study abstracts. Ineligible publications including review papers, clinical studies or papers covering a different disease model were excluded. A final screening took place, where the full texts of remaining papers were read to assess against the full inclusion criteria as outlined in our protocol. Studies investigating the therapeutic potential of PUFAs on preclinical animal models of SCI were included. Papers that investigated locomotor recovery, using the Basso-Beattie-Bresnahan (BBB) and Basso mouse scale (BMS) scores, with an appropriate control group (*e.g.*, SCI with saline/vehicle) were included. Studies with only sham or naive groups were excluded. Two independent reviewers (WMS and AA) separately screened papers, with any disagreements resolved through discussions.

2.3. Data extraction

Two independent reviewers (WMS and AA) extracted relevant data from the included publications. Study design information was also extracted to collect the following information from included publications: species and strain; SCI injury model; sex; age; total animals used; treatment; single or combined treatment; control; route of administration; dosage; intervention timing; repeated dosing; behavioral assay; and histological measures. The quality of included studies was determined by an adapted 7-score CAMARADES (Collaborative Approach to Meta-Analysis and Review of Animal Data in Experimental Studies) Risk of Bias Checklist [21]. Items included on this list were: peer reviewed; randomization; allocation concealment; blinding; sample size calculation; animal welfare regulations; and conflicts of interest.

Primary outcome measure (locomotor score) and secondary outcome measures (lesion volume, cell survival and pain) were extracted from the included studies. Mean values with SD or SEM were extracted. In the event that multiple measures were made, data from the final timepoint were extracted since this represented the maximum recovery measured within the scope of included studies. Where one control group was used for comparison against multiple treatment groups, this was corrected for by dividing the number of animals in the control group by the number of treatment groups. In the event that exact animal numbers in a cohort were not stated but a range was reported, the lowest value of that range was used. When outcome measures were only reported graphically, results were extracted using the online graphical tool WebPlotDigitizer (https://apps.automeris.io/wpd/). Estimated outputs from this were verified by the second reviewer and where distinctions between independent outputs were <10%, the mean was taken. Differences >10% were resolved through discussion. Instances where extraction of data through WebPlotDigitizer was not possible, or where *n* numbers were not reported, study authors were contacted via E-mail for clarification. If no response with accompanying data was received after the second attempt, the corresponding studies were excluded from the meta-analysis.

2.4. Meta-analysis

Statistical analysis and graphing was conducted using the *metafor* package [22] in RStudio (RStudio, USA) with R version 3.6.3. Standardised mean difference (SMD) effect sizes were calculated by using Hedge's *g*, with all positive SMD values favoring treatment. Random effects meta-analyses were conducted using the restricted maximum likelihood method. Funnel plots were used to illustrate publication bias, and asymmetry was confirmed by Egger's regression test. The number of potential missing studies was determined by using trim-and-fill analysis, which can account for unpublished studies and provide an adjusted effect's size considering publication bias. Heterogeneity was quantified using I^2 and tau^2 (between-study variance that does not account for sampling error) and Tau^2 (between-study variance). Subgroup analysis was conducted to explore sources of heterogeneity for PUFA treatment and SCI model in locomotor score (primary outcome), cell survival and neuropathic pain measures (secondary outcomes). Study characteristics utilised for comparisons included PUFA type, route of administration, SCI model, randomization, and blinding. Subgroup analysis was performed only for conditions that had at least 4 comparisons in a subgroup [23]. Statistical significance was defined as $p < 0.05$. Comparison of subgroups was conducted by fitting random effects models to each

subgroup and comparing with a Wald-type test.

3. Results

3.1. Identification of publications

Our search strategy identified a total of 2177 studies, amongst which there were 28 that met the inclusion criteria. The breakdown of screening and exclusion of articles are illustrated in Fig. 1. While 28 of these were included in the systematic review, only 25 were included in the meta-analysis due to missing information that precluded analysis of extracted data.

3.2. Outcome measures

Our primary outcome locomotor recovery was assessed using the BBB and BMS scores, which were employed in the vast majority of included studies (85%). Meanwhile, cell survival, lesion volume and neuropathic pain, which we defined as our secondary outcomes, were assessed using a more varied range of techniques and methods. Cell survival in particular comprised numerous histological measures, including but not limited to NeuN, APC and NG2 staining at the lesion epicenter, or proximal rostral or caudal localities from the lesion site. Similarly, neuropathic pain was assessed using an array of evoked pain measures (Hargreaves, mechanical Von Frey and hot plate) and operant pain measures (burrowing and thigmotaxis).

3.3. Interventions

The most prevalent treatment intervention was DHA, either alone or in combination with other PUFAs ($n = 21$). The majority of studies used intravenous (IV) injection as a route of administration ($n = 14$), with dietary consumption being the second most widely used ($n = 11$). Additionally, several studies used a combination of IV and dietary routes of intervention ($n = 3$), this being an additional treatment group to either IV or dietary intervention alone. Intervention timings varied considerably between studies, but the most common timing was 30 min after SCI was induced ($n = 11$) or immediately after ($n = 6$). Interventions either involved single event administration ($n = 11$) or had a regime of repeated dosing ($n = 17$) that also varied considerably between studies.

3.4. Synthesised data

From the locomotor meta-analysis results, the outcome showed an improvement in locomotor recovery, compared with controls (SMD = 1.037, 95% confidence interval [CI] = (0.810–1.264), $p < 0.001$, $Tau^2 = 0.126$, $I^2 = 25.71\%$). PUFA treatment on the cell survival was also shown to have a favorable effect (SMD = 1.101, 95% CI = (0.889–1.313), $p < 0.001$, $Tau^2 = 0.604$, $I^2 = 51.96\%$). While only 4 studies were included for this outcome, a positive effect size was calculated for improved neuropathic pain measures, but the result was not significant (SMD = 0.749, 95% CI = (0.071–1.568), $p = 0.073$, Tau^2

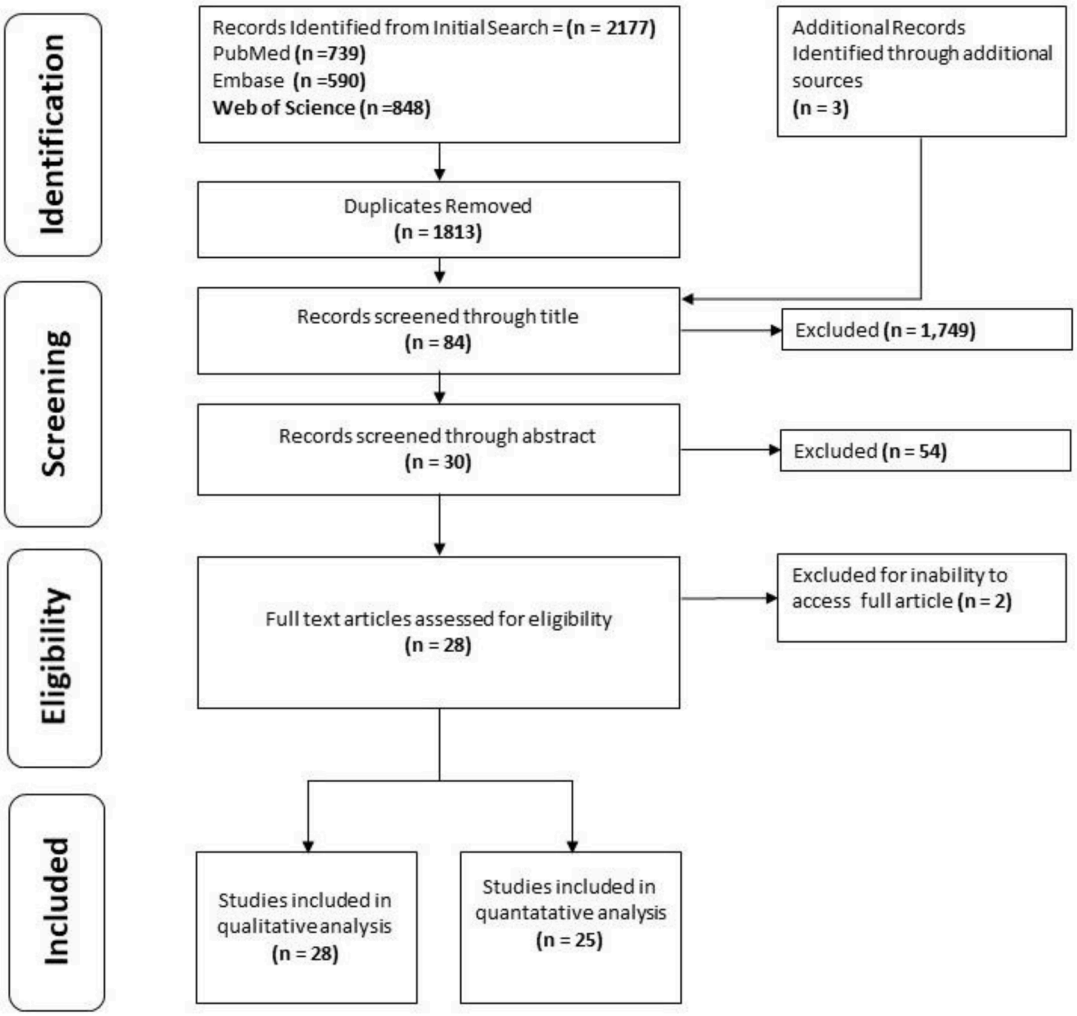

Fig. 1. PRISMA flowchart illustrating the process of study screening and showing the eventual number of included studies.

= 2.748, I^2 = 93.12%). The effect size of PUFA treatment on lesion volume was also calculated to have a positive effect size, but the result was also not significant (SMD = 0.875, 95% CI = (−0.168–1.1917), p = 0.1, Tau^2 = 2.033, I^2 = 78.96%). Forest plots for locomotor recovery (Fig. 2), cell survival (Fig. 3), and neuropathic pain and lesion volume (Fig. 4) are presented below.

3.5. Sources of heterogeneity

To identify sources of heterogeneity in our data, we conducted subgroup analysis for primary and secondary outcomes. PUFA treatment type (*i.e.,* DHA, ALA, or DHA + EPA) was not a source of any significant heterogeneity, within our primary outcome. While all three subgroups had a significant effect measure, none were statistically greater than alternative treatment subgroups. Meanwhile, significant heterogeneity for cell survival was calculated amongst DHA (I^2 = 46.29%, Tau^2 = 0.481, p = <0.001) and DHA + EPA (I^2 = 91.94%, Tau^2 = 3.26, p = <0.001) subgroups. Amongst the three treatment subgroups, only DHA provided a significant outcome measure (SMD = 0.9461, 95% CI = (0.715–1.176), p < 0.001). Assessing for heterogeneity with PUFA treatment was not possible for neuropathic pain or lesion volume secondary outcomes, due to the number of comparisons for ALA and DHA + EPA treatment falling below the minimum threshold (<4).

The route of administration was not a source of significant heterogeneity for locomotor recovery. For the primary outcome, IV + dietary administration accounted for the greatest proportion of between-study variance (I^2 = 48.53%, Tau^2 = 0.76, p = 0.102), despite this not being statistically significant. No subgroup was found to have a statistically greater effect measure than alternative routes of administration. For cell survival, IV injection alone accounted for significant between-study variance (I^2 = 45.19%, Tau^2 = 0.68, p = <0.001). The IV + dietary PUFA subgroup produced a significantly greater effect measure when compared to IV alone (SMD = 2.681, 95% CI = 1.987–3.375, p = <0.001). Assessing for heterogeneity via the route of administration was not possible for lesion volume or neuropathic pain, due to the low number of comparisons for dietary alone or in combination with IV injections.

Screening for heterogeneity amongst different SCI models revealed several sources of heterogeneity. Compression SCI models accounted for the greatest proportion of between-study variance for locomotor recovery (I^2 = 39.85%, Tau^2 = 0.355). Both contusion (p = 0.035) and compression (p = 0.008) models were significant sources of heterogeneity for the primary outcome. For locomotor recovery, all SCI models showed significant effect measures upon PUFA treatment. Subgroup comparison of contusion against compression models did not reveal any significant differences in effect measure. Injury model accounted for no

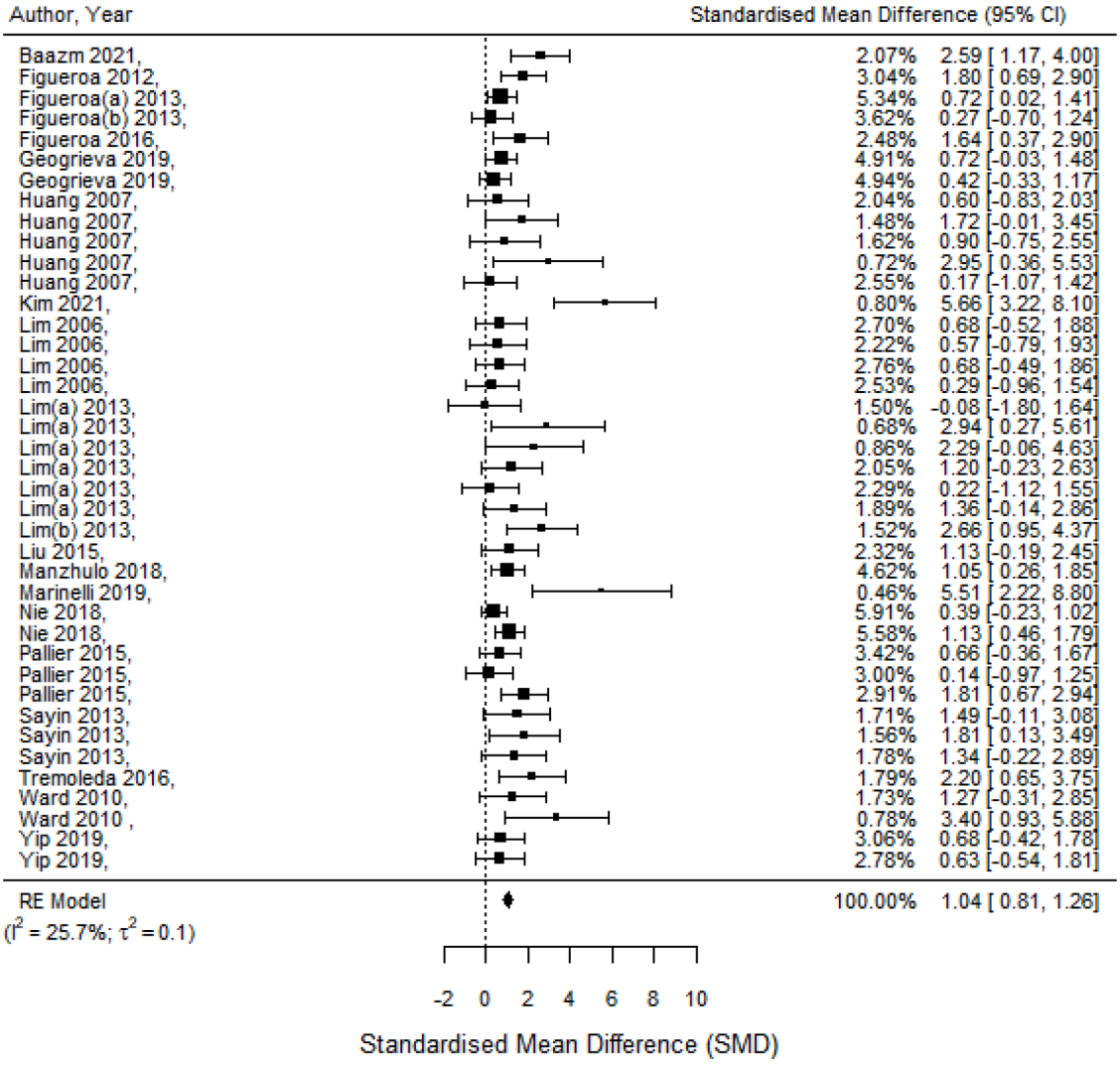

Fig. 2. Forest plot showing the effect size of PUFA treatments on locomotor recovery (primary outcome). Effect size is shown as the black dots right of study comparisons and the error bars indicate 95% confidence intervals. Estimate weight is indicated by the size of each individual dot and the diamond indicates the overall SMD (width represents 95% confidence intervals)[24].

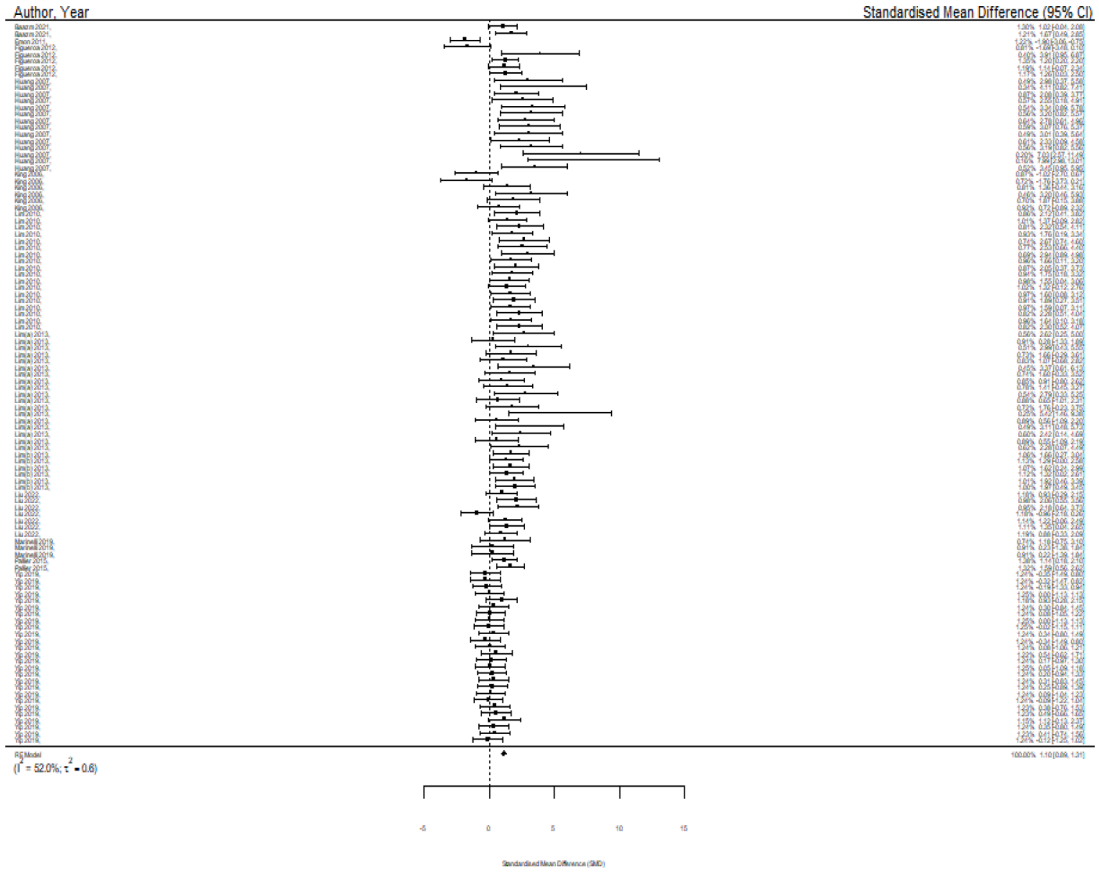

Fig. 3. Forest plot showing the effect size of PUFA treatments on cell survival (secondary outcome). Effect size is shown as the black dots right of study comparisons and the error bars indicate 95% confidence intervals. Estimate weight is indicated by the size of each individual dot and the diamond indicates the overall SMD (width represents 95% confidence intervals).

significant heterogeneity in cell survival. Contusion models accounted for significant between-study variance in lesion volume data (I^2 = 85.41%, Tau^2 = 10.21, p = 0.002), while being the only subgroup to have a significant effect measure (SMD = 3.965, 95% CI = 0.701–7.228, p < 0.017). Furthermore, when comparing the effect measures of contusion and compression subgroups in lesion volume, the latter had a significantly greater effect measure (p = 0.02). Heterogeneity could not be assessed for injury models in neuropathic pain measures due to too few comparisons.

Blinding and randomization were also included in the subgroup analysis for the primary outcome and all secondary outcomes. When comparing studies that reported blinding for locomotor recovery, there was a greater effect measure for studies that did not report blinding (SMD = 1.502, 95% CI = 0.836–2.169, p = <0.001) compared with those that did (SMD = 0.963, 95% CI = 0.732–1.193, p = <0.001), with the latter being a significant source of heterogeneity (p = 0.004). Despite this, there was no statistical difference between the effect measures of these subgroups. Subgroup analysis based on reported blinding for cell survival also illustrated an elevated effect measure for studies without blinding (SMD = 1.307, 95% CI = 0.518–2.095, p = <0.001) over those with (SMD = 1.1, 95% CI = 0.883–1.317, p = <0.0012). There was significant between-study variance for studies that reported blinding in cell survival (p = <0.001). This was also the case for studies reporting blinding in neuropathic pain measure (p = <0.001) and lesion volume (p = <0.001).

The effect size for locomotor recovery for studies that did not report randomization (SMD = 1.189, 95% CI = 0.832–1.546, p = <0.001) was greater than those that did (SMD = 0.844, 95% CI = 0.572–1.115, p = <0.001), but this was not calculated to be significant. Studies that did not report randomization were found to have significant between-study variance (p = 0.0012) for locomotor recovery. Amongst cell survival, studies that reported randomization (SMD = 1.422, 95% CI = 0.739–2.106, p = <0.001) had a greater effect measure compared with those that did not (SMD = 1.049, 95% CI = 0.834–1.266, p = <0.001), despite there being no statistical differences between their effect measures (p = 0.308). Both subgroups were significant sources of between-study variance (p = <0.001 for both subgroups). For the remaining secondary outcomes, there was significant between-study variance calculated for studies reporting randomization in neuropathic pain and lesion volume (p = <0.001 for both outcomes). In the case of neuropathic pain measures, the effect measure was greater studies that did not report randomization (SMD = 1.358, 95% CI = 0.716–1.999, p = 0.716) compared with those that did not, with the latter being a significant source of between-study variance (p = <0.001). Meanwhile, for lesion volume the effect measure was greatest amongst studies that reported randomization (SMD = 2.077, 95% CI = −0.147–4.169, p = 0.0516) compared with those that did not and was a significant source of heterogeneity (p = <0.001). Statistical comparisons of randomization subgroups for neuropathic pain and lesion volume was not possible due to a low quantity of comparisons that did not report randomization. Full results are provided in the supplementary data.

3.6. Risk of bias

We then investigated publication bias. As shown in Fig. 5a, there was a moderate degree of asymmetry present in the funnel plot for locomotor activity and this was confirmed with Egger's regression (p < 0.001). Trim-and-fill analysis showed that there were 13 predicted studies missing on the left side of the funnel that would show a neutral or negative effect (Fig. 5b). Incorporating these predicted missing studies

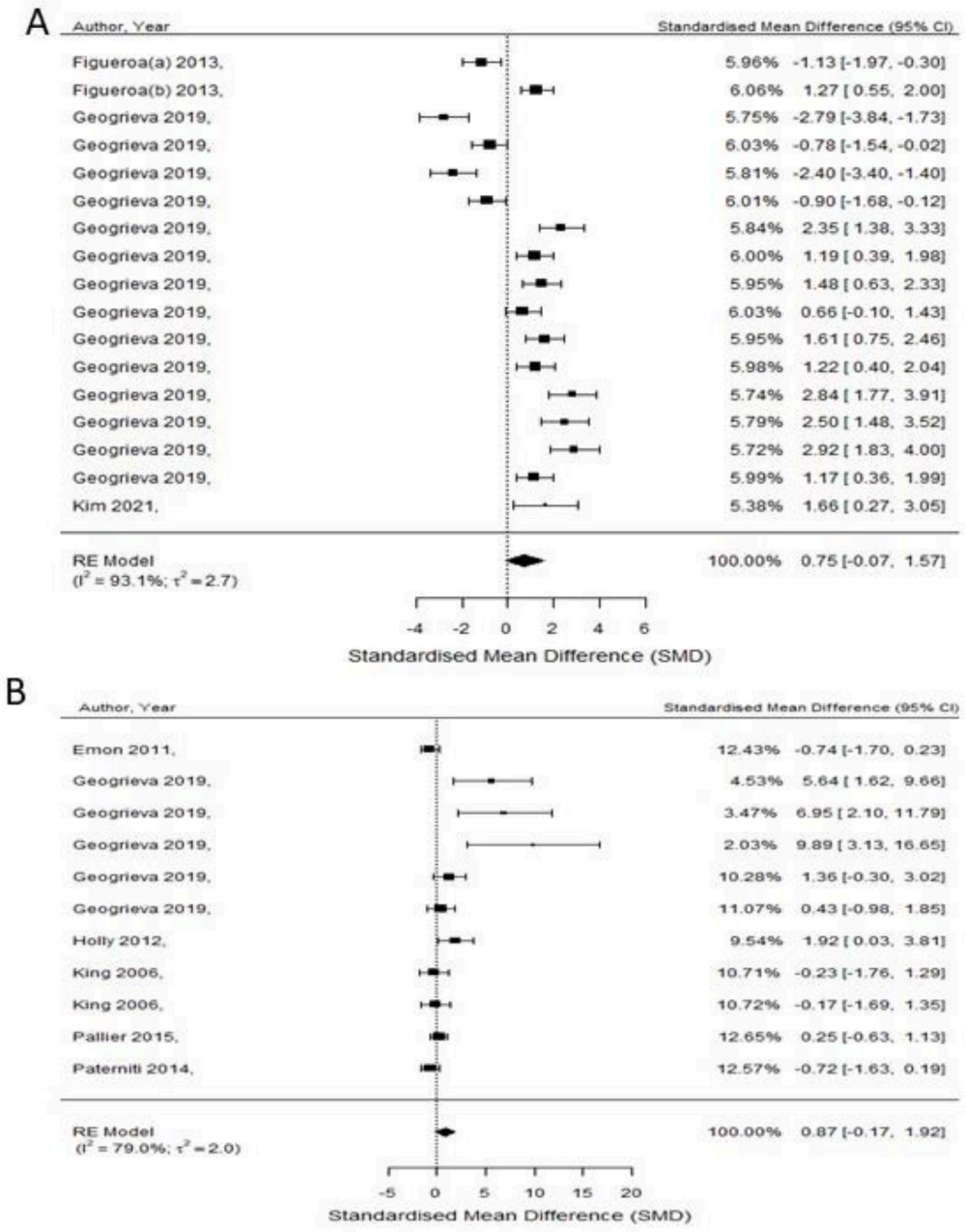

Fig. 4. Forest plot showing the effect size of PUFA treatments on (A) neuropathic pain and (B) lesion volume (secondary outcomes). Effect size is shown as the black dots right of study comparisons and the error bars indicate 95% confidence intervals. Estimate weight is indicated by the size of each individual dot and the diamond indicates the overall SMD (width represents 95% confidence intervals).

adjusted the effect size from 1.0371 (95% CI = 0.809–1.264, p = <0.001) to 0.726 (95% CI = 0.410–1.042, p = <0.001). Despite this, there was still a significantly positive effect towards locomotor recovery with this adjustment.

For the secondary outcome, cell survival, there was also considerable asymmetry (Fig. 5c) towards a positive outcome for treatment confirmed by Egger's regression (p < 0.001). Trim-and-fill analysis showed there were 3 predicted studies missing with neutral/negative effects resulting from treatment (Fig. 5d), and when adjusted for the effect size shifted from 1.097 (95% CI = 0.888–1.331, p = <0.001) to 1.076 (95% CI = 0.853–1.300 p = <0.001).

For neuropathic pain measures, there was very little asymmetry observable in the funnel plot (Fig. 5e), and trim-and-fill analysis (Fig. 5f) estimated that there were 0 missing studies. Egger's regression of this outcome did not show a significant output supporting bias towards treatment (p = 0.465).

Finally, lesion volume outcome measures showed considerable asymmetry favoring treatment in the funnel plot and trim-and-fill analysis calculated 4 missing studies with a negative or neutral effect, adjusting the effect measure from 0.875 (95% CI = −0.168–1.197, p = 0.1) to 0.749 (95% CI = −0.707–1.568, p = 0.963). Egger's regression also showed a significant result, indicating bias towards treatment (p = <0.001).

4. Discussion & conclusion

A recent systematic review and meta-analysis by Tian and colleagues (2020) also investigated the effects of DHA on preclinical SCI animal models [25], however, our analysis and results differ in several ways. Our search criteria incorporated studies that employed other PUFA

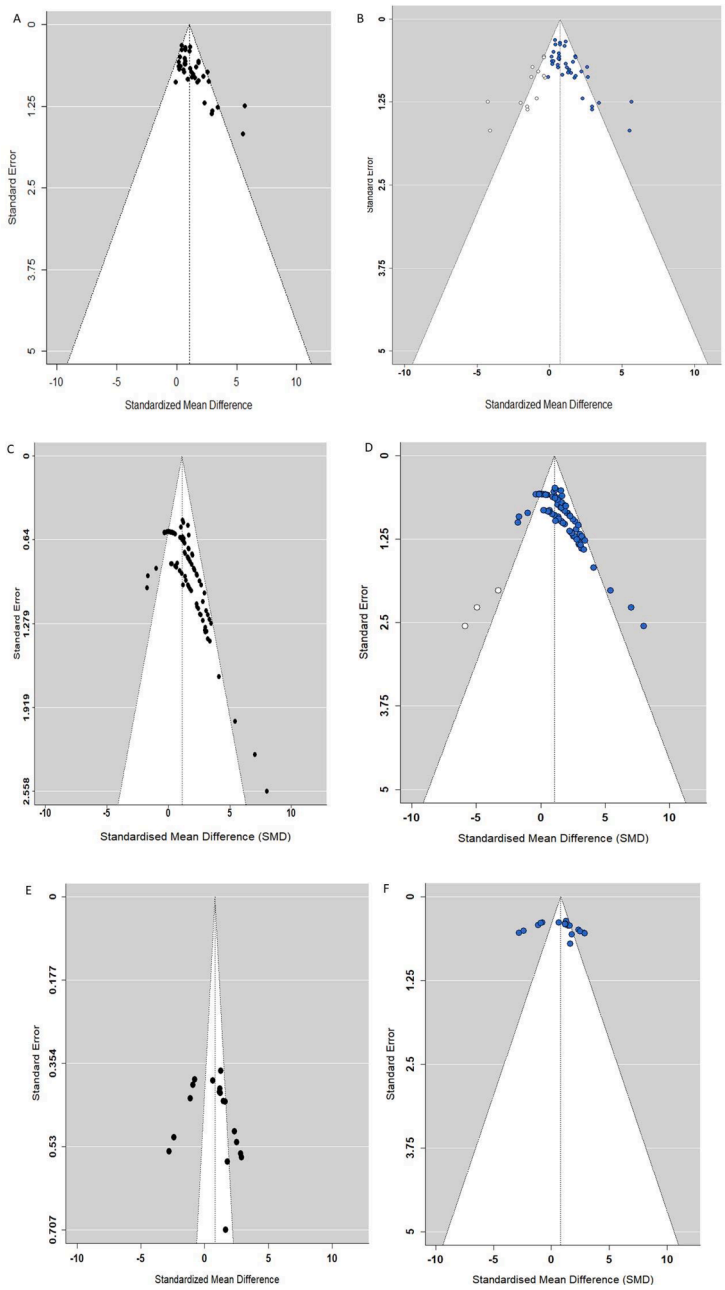

Fig. 5. Risk of publication bias and trim-and-fill analysis for locomotor recovery data (primary outcome), cell survival, neuropathic pain, and lesion volume (secondary outcomes). Funnel plots for locomotor recovery (A), cell survival (C) and neuropathic pain (E) are presented. Trim-and-fill analysis predicted 13 missing studies (white circles) for locomotor recovery data (B), 3 within cell survival dataset (D), 0 for the neuropathic pain dataset (F). White funnels for all figures depict 95% CIs.

molecules, and explored secondary outcomes (cell survival, neuropathic pain measures and lesion volume) that were not investigated in the other paper. Therefore, we believe our findings here are distinct enough to justify the synthesis of this systematic review and meta-analysis.

Our synthesized data has demonstrated that the systemic and dietary administration of ω−3 PUFAs can significantly reduce neuronal and glial cell death, and improve locomotor recovery after SCI. Our primary focus was on locomotor recovery in SCI animal models, utilizing studies employing the BBB or BMS scales. The secondary outcome cell survival was significantly improved in PUFA treated SCI animal models. Collectively, our findings present the neuroprotective effects of PUFA against SCI incurred damage.

Subgroup analysis for factors such as PUFA treatment, route of administration and SCI model revealed several interesting findings that highlighted where PUFA treatment is most effective in treating SCI. We calculated IV injection with dietary administration of PUFAs to have the greatest outcome for locomotion and cell survival, significantly so for the latter. However, there were no significant differences between the application of DHA, DHA + EPA or ALA among the included studies. Finally, PUFA treatment had the greatest effect on locomotor recovery and cell survival in compression models, significantly outperforming contusion SCI in cell survival. This outcome likely stems from the nature of these injury models, where compression results in more widespread secondary damage [26,27]. With PUFAs conferring anti-inflammatory effects and amelioration of oxidative stress, it is likely that the improved results from secondary damage played a larger role in the compression injury model. With this in mind, it highlights the need for a tailored approach to SCIs whereby treatment would be dependent on the

nature of the spinal cord trauma.

4.1. External validity of SCI animal models

The majority of included studies (89%) surgically induced SCI at the thoracic level of the spinal cord, with only three reporting injuries at the cervical level (11%). This does not reflect clinical SCI outcomes, where the most prevalent spinal injuries affect the cervical levels of the spinal cord (60%), with thoracic injuries coming in second (35%) [28,29]. This trend is seen in general SCI animal studies, and there are two primary reasons for this. Firstly, thoracic injury models are easier to reproduce and are less traumatic, leading to better injury consistency and much lower mortality rates compared with cervical models. Secondly, injury models that rely on an impactor device require firm clamping of the spinal processes, which is difficult to achieve at the cervical level [30–32]. With these factors in mind, it is understandable why SCI animal models at the thoracic level are favored in preclinical research. However, we advise that greater emphasis should be placed on cervical SCI animal models in order to more accurately represent clinical SCIs.

The sex of animal models amongst our included studies also does not reflect clinical representation of SCI in humans. Only 28% of included studies used male animal models for SCI, differing considerably from the reported 80% global SCI patients being male [33,34]. Female animal models of SCI are preferrable since bladder expression is easier to perform compared with males and they less frequently present with urinary infections [34]. Due to the overwhelming prevalence of male SCI patients, it would be advisable for preclinical studies to prioritize male animal models for SCI research.

The pathophysiology of SCI differs in mice and rats, where the latter are favorable due to pathophysiological events mimicking that of humans. This can be seen with the development of cystic cavities in both rats and humans but not mice, allowing for greater production and deposition of immunoprotective and pro-wound-healing factors [35–37]. Due to this, there is an overall favorability for rats in SCI research compared with mice [38]. Included studies reflected this trend, where 82% of studies employed rat models of SCI. This is promising as choosing animal models that better reflect the pathophysiology of SCI helps to improve the translatability of preclinical research to clinical trials.

4.2. Study quality and publication bias

Using a modified CAMARADES checklist (Table 1), we characterized study quality by looking at seven items of importance. From this, it was determined that there was a moderate degree of measures to reduce the risk of bias. Four of the seven criteria were found reported among greater than half of the extracted studies. Those three that fell below this threshold included randomization, allocation concealment and sample size calculations. These methods especially are reported to be underutilized in many studies [39], which is problematic given their reported efficacy in reducing study bias [40].

Only two of the included studies used power calculations, which potentially represents a collective issue of overestimation or underestimation among the included findings. Meanwhile allocation concealment and randomization were reported in five and twelve studies, respectively. The lack of papers reporting these measures suggests the presence of confounding factors like experimental bias and selection bias. This could impact the outcome measures such as baseline readings, thereby distorting a study's overall outcome to potentially favor treatment and exaggerate findings [41]. This highlights the importance of implementing these practices in preclinical experimental design.

Table 1
Modified CAMARADES risk of bias checklist, where the percentage of papers meeting each criterion are displayed.

Modified CAMARADES checklist CAMARADES criteria	Percentage of included studies
1. Peer reviewed	60.71%
2. Random allocation	42.86%
3. Allocation concealment	17.86%
4. Blinded assessment	78.57%
5. Sample size calculation	7.14%
6. Animal welfare	89.29%
7. Conflicts of interest	64.29%
Median study quality	4 (IQR: 3–5)

It has been estimated that studies in neuroscience may in fact report overestimated results in greater than 50% of published papers [42,43]. This suggests that our extracted data may be an overestimated value from the true effect. In this systematic review we found that in most outcome measures effect size was greater among studies that did not report blinding (locomotor recovery, cell survival, neuropathic pain, and lesion volume) and randomization (locomotor recovery and neuropathic pain). While these outcomes were not statistically significant from one another, this apparent exaggeration of effect size demonstrates the potential for publication bias among our included studies. Egger's regression confirmed bias towards favorable results in three of our outcome measures, while trim-and-fill analysis also showed missing studies with negative or neutral findings in these same three outcome measures. Based upon these results, it is evident that the global effect measures here are moderately overestimated, further indicating the presence of publication bias amongst our synthesized results.

4.3. Experimental design

Preclinical animal models of SCI have been largely standardized for the last few decades [33], which has been beneficial towards the experimental output of research in this area. This was also seen amongst included studies, where the three most common SCI animal models were employed (compression, contusion and hemisection [30]) in all but one paper. Using standardized SCI models helps to improve between-study comparability and reduce the influence of potential undescribed surgical factors that can influence experimental outcomes. This is likely reflected by low heterogeneity scores for locomotion and cell survival when comparing SCI models, suggesting that the use of standardized injury models are beneficial towards consistent and comparable experimental output.

The majority of studies utilised either the BBB or BMS scores when assessing locomotor recovery, either alone or in addition to other locomotor assays. These locomotor scoring schemes are widely used and have been a staple of assessing locomotor recovery for many years [44,45]. The widespread use of these locomotor scores also helps to standardize the measurement and assessment of our primary outcome measure, allowing for improved external validity.

Experimental design for treatment dosage and treatment timing/regimes was considerably varied between the included studies. A commonly extracted treatment time was 30 min after surgery (39%), but these were either the only reported treatment administered, or the first before additional treatments. The extent of variability here complicates comparison of treatment efficacy between studies, which limits the ability for comparison of treatment regimes. One of the proposed explorations for our subgroup analysis was treatment at different time points, but due to the extent of variability in this field we did not pursue analysis here. Another aspect of treatment that varied considerably was dosage. The most frequently reported dosage for PUFAs was 250 nM/kg (46%), either alone or in addition to other concentrations applied. The extent of variability here demonstrates a lack of consistency or consensus between studies making it difficult to ascertain an ideal concentration in the context of a systematic review.

4.4. Limitations

In conducting this systematic review and meta-analysis, we were

able to characterize the restorative effects of PUFA treatment in preclinical SCI models with several measures. However, the scope of our investigation did not seek to capture additional aspects of recovery characterizable in these models. For example, we did not seek to explore bladder and bowel function which is another neurological dysfunction associated with SCI [28,46]. Another consequence of SCI that clinically diminishes quality of life is the development of central neuropathic pain, known to occur in 40–50% of SCI patients [47–50]. While neuropathic pain measures were one of our secondary outcomes, only one study used assays assessing chronic pain via operant behaviors [5]. The majority of neuropathic pain measures incorporated into our data represent acute pain and given the chronic nature of CNP [51], this diminishes the clinical comparability of this outcome measure.

4.5. Conclusion

In this systematic review and meta-analysis, we report that PUFA treatment in preclinical models of SCI has a beneficial effect on locomotor recovery and cell survival. These outcomes agree with the literature, which has a growing body of evidence to demonstrate the restorative effects of PUFAs not only in SCI, but in other intractable conditions resulting from trauma as well. Our assessment on the risk of bias has shown there is detectable bias in the studies included, which may exaggerate their findings. We recommend that measures to reduce the risk of bias should be employed to improve the validity of future publications. Furthermore, means to improve the external validity of such studies, such as prioritizing male animals and rat models, are also advisable. The data and discussion here can be used to guide researchers towards robust experimental design for future preclinical SCI studies. The goal of regenerating neuronal damage caused by SCI remains an intractable challenge in medicine. Further research is necessary to characterize the underlying mechanisms behind this but determining what treatments can improve recovery outcomes for such an intractable condition will help pave the way for a restorative solution to address SCI induced dysfunction.

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CRediT authorship contribution statement

W.A.C. MacIntosh-Smith: Conceptualization, Methodology, Software, Formal analysis, Investigation, Writing – original draft. **A. Abdallah:** Conceptualization, Methodology, Investigation, Writing – original draft. **C.J. Cunningham:** Methodology, Software, Validation, Writing – review & editing, Supervision.

Declaration of Competing Interest

None.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.plefa.2023.102554.

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