Dietary Omega-3 Polyunsaturated Fatty-Acid Supplementation reduces Painful Diabetic Neuropathy and Upregulates Neuroprotective Cellular Pathways in Latinos with Type 2 Diabetes

Alfonso M Durán[1], Lorena M Salto[1], Justin Câmara[1], Ivette Paquien[1], W Lawrence Beeson[1,2], Anthony Firek[3], Zaida Cordero-MacIntyre[1,2], Marino De León[1]

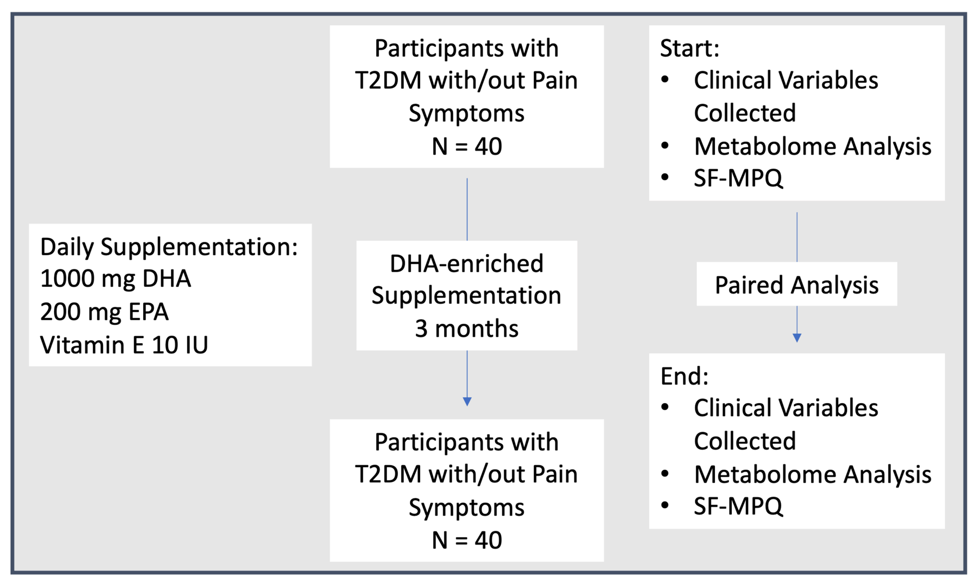
[1]Center for Health Disparities and Molecular Medicine, Department of Basic Sciences, Loma Linda University School of Medicine, Loma Linda, CA, USA; [2]Center for Nutrition, Healthy Lifestyle and Disease Prevention, School of Public Health, Loma Linda University, Loma Linda, CA, USA;
[3]Comparative Effectiveness and Clinical Outcomes Research Center, Riverside University Health System Medical Center, Moreno Valley, CA, USA

LOMA LINDA UNIVERSITY
School of Medicine
Center for Health Disparities and Molecular Medicine

Introduction

Omega-3 polyunsaturated fatty acids (PUFAs) are increasingly reported to improve chronic neuroinflammatory diseases in peripheral and central nervous systems. Specifically, docosahexaenoic acid (DHA) protects nerve cells from noxious stimuli in vitro and in vivo. In addition, recent reports in animal models link PUFA supplementation to improving painful diabetic neuropathy (pDN). However, the molecular mechanism behind omega-3 PUFAs ameliorating pDN symptoms is lacking with concordant human pain evaluation for pDN symptoms. Therefore, we sought to determine if dietary omega-3 supplementation improved pDN symptoms and define the distinct cellular pathways impacted by our dietary supplementation in patients with type 2 diabetes mellitus.

Study Description



Scheme 1. Study Description. DHA, Docosahexaenoic acid; EPA, Eicosapentaenoic acid; T2DM, type 2 diabetes mellitus; SF-MPQ, Short-form McGill Pain Questionnaire

Characteristics of all En Balance-Plus study participants pre and post DHA-rich dietary intervention (n=35)

Clinical Parameter	Baseline	3 Months	Difference 3Mo-BL	Significance, *P*
Age (y)	55.5 ± 11.8	-	-	-
Male %, Female %	43%, 57%	-	-	-
% Hispanic	100	-	-	-
BMI, kg/m²	29.7 ± 5.5	29.8 ± 5.7	0.1	0.706‖
Cholesterol, mg/dL	176.3 ± 35.7	179.0 ± 34.3	2.7	0.294
LDL, mg/dL	117.1 ± 32.5	111.6 ± 27.9	-5.5	0.083
HDL, mg/dL	47.2 ± 13	48.2 ± 14	1	0.600
Cholesterol:HDL	3.9 ± 1.0	3.9 ± 1.1	0.0	0.561
Triglycerides, mg/dL	169.3 ± 83.9	163.7 ± 123.0	-5.6	0.347
Fasting Glucose, mg/dL	154.1 ± 71.9	142.7 ± 65.5	-11.4	0.095
HbA1c %	7.6 ± 2.3	7.4 ± 2.1	0.2	0.014

Table 1. Percentage of individuals or mean ± SD shown for each variable and each time point (Baseline vs 3-months). ‖Normally distributed (other distributions were non-normally distributed). *P*-values based on match pairs *t*-test for BMI and Mann–Whitney *U* test for LDL, HDL, cholesterol:HDL, triglycerides, HbA_{1c}, and fasting glucose.

Total of 124 Biochemicals significantly change from baseline after 3-months of DHA-rich dietary supplementation

Total Biochemicals Identified	695
Matched Paired *t*-Test	3Mo / BL
Total Biochemicals p ≤ 0.05	124
Biochemicals (↑↓)	75 | 49

Table 2. Metabolomic compound identification and statistical comparison, matched pairs *t*-test

En Balance-Plus participants reported dietary and GC-MS relative omega-3 PUFAs plasma levels at baseline and 3 months (n = 40)

	Baseline (BL)	3 Months (3Mo)	Difference 3Mo-BL	Significance, *P*
GC-MS				
DHA	.71 ± .4	1.85 ± 1.1	1.14	< 0.001
EPA	1.03 ± .68	1.62 ± 1.1	0.59	0.001
Dietary Intake				
DHA mg	57.6 ± 98.9	1035.2 ± 30.1	977.6	0.0001
EPA mg	26.8 ± 65.5	211.7 ± 11.3	184.9	0.0001

Table 3. Percentage of individuals or mean ± SD shown for each variable and each time point (Baseline vs 3-months). *P*-values based on match pairs *t*-test.

Metabolic maps showing major biochemicals alteration after DHA-rich supplementation

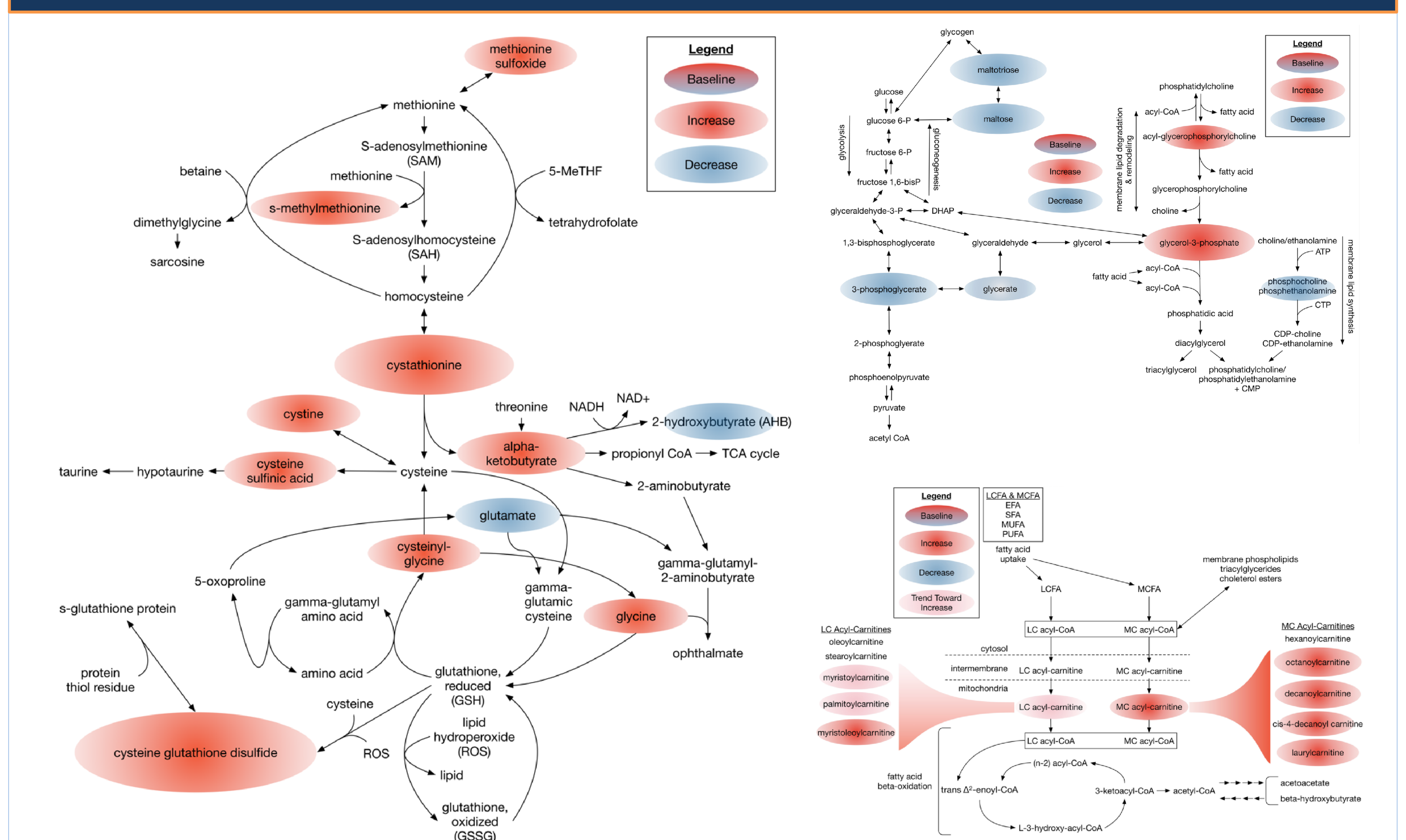


Figure 1. Changes in cysteine, methionine, and glutathione metabolism overlaid on a metabolic map. The size of each colored bubble is proportional to the fold change (increase, red; decrease, blue). The baseline bubble in the legend corresponds to a fold change of 1.

Random Forest classification of plasma metabolites identifies lipid metabolites as important features for group separation

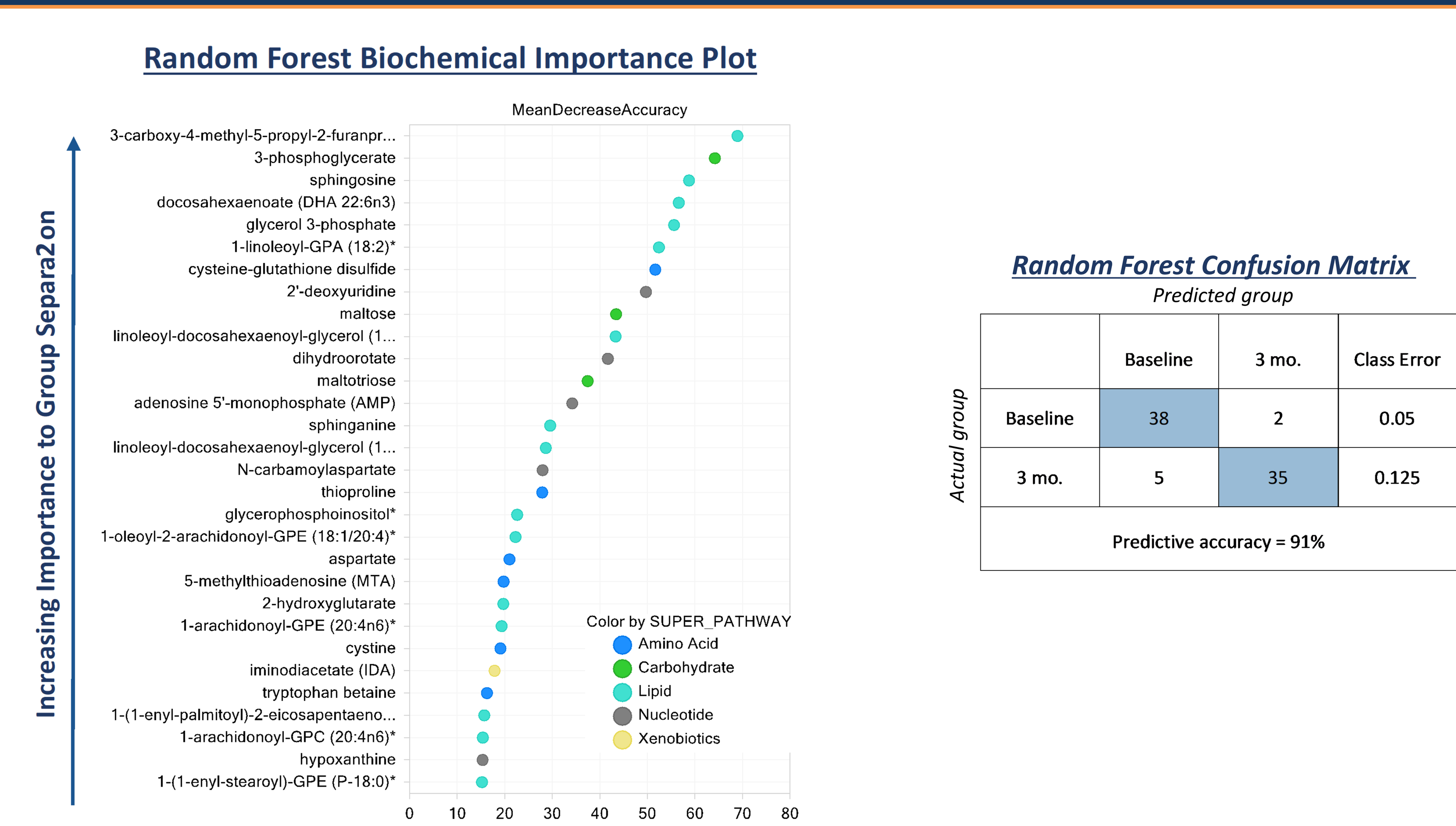


Figure 2. Classification was ~91% accurate for samples when a value of 50% would be expected by random chance. Top factors contributing to group separation shown in the biochemical importance plot.

SF-MPQ sensory score correlate with pain metabolites

Metabolite	Correlation	p-value
cysteine-glutathione disulfide	-0.460	<0.001
linoleoyl-docosahexaenoyl-glycerol (18:2/22:6) [1]	-0.266	0.059
Sphingosine	0.489	<0.001
3-phosphoglycerate	0.572	<0.001
Glycerol 3-phosphate	-0.163	0.252
1-linoleoyl-GPA (18:2)	0.406	0.003
2′-deoxyuridine	-0.492	<0.001
Maltose	0.360	0.009

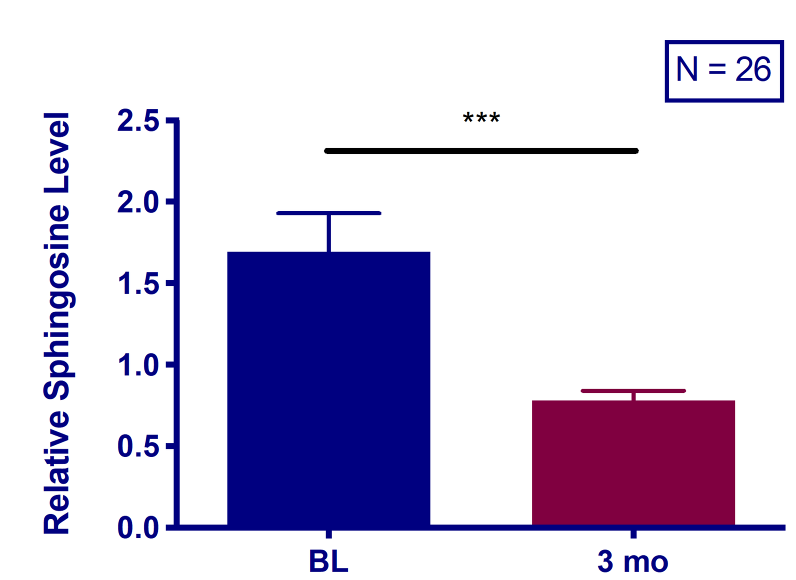


Table 4. Partial spearman correlation between SF-MPQ sensory score and top metabolites contributing to group separation per RF, adjusted for ID. **Figure 3.** Paired *t*-test comparison of baseline relative plasma concentration of sphingosine metabolite. Mean ± SD shown. *** p ≤ 0.001

IPA demonstrates increase in neurorestorative bio-functions post intervention

Bio Function Categories	Diseases or Functions Annotation	*P*-Value	Activation Z-Score	Number of Biochemicals
Free Radical Scavenging	Synthesis of reactive oxygen species	7.21 E-07	-1.69	13
	Formation of reactive oxygen species	4.19E-04	-1.96	4
	Biosynthesis of hydrogen peroxide	4.95E-05	-1.95	5
Cellular Compromise, Lipid Metabolism, Small Molecule Biochemistry	Peroxidation of Lipids	2.25E-05	-1.94	6
Cell-to-Cell Signaling and Interaction, Hematological System Development and Function, Inflammatory Response	Aggregation of blood platelets	5.7E-05	-1.78	7
Carbohydrate Metabolism, Molecular Transport, Small Molecule Biochemistry	Uptake of D-glucose	1.19E-04	-1.72	6
Cell Signaling, Molecular Transport, Vitamin and Mineral Metabolism	Quantity of Ca2+	1.52E-04	-1.83	9
	Entrance of Ca2+	2.15E-04	-1.97	4
Drug Metabolism, Molecular Transport, Small Molecule Biochemistry	Concentration of glutathione	3.43E-04	1.97	5

Significant Bio Functions, increases and decreases are highlighted in red and blue, respectively

Table 4. Top 10 Bio functions reported by IPA analysis of significantly changed metabolites post omega-3 PUFA supplementation

Participants Report dramatic decrease in sensory pain scores

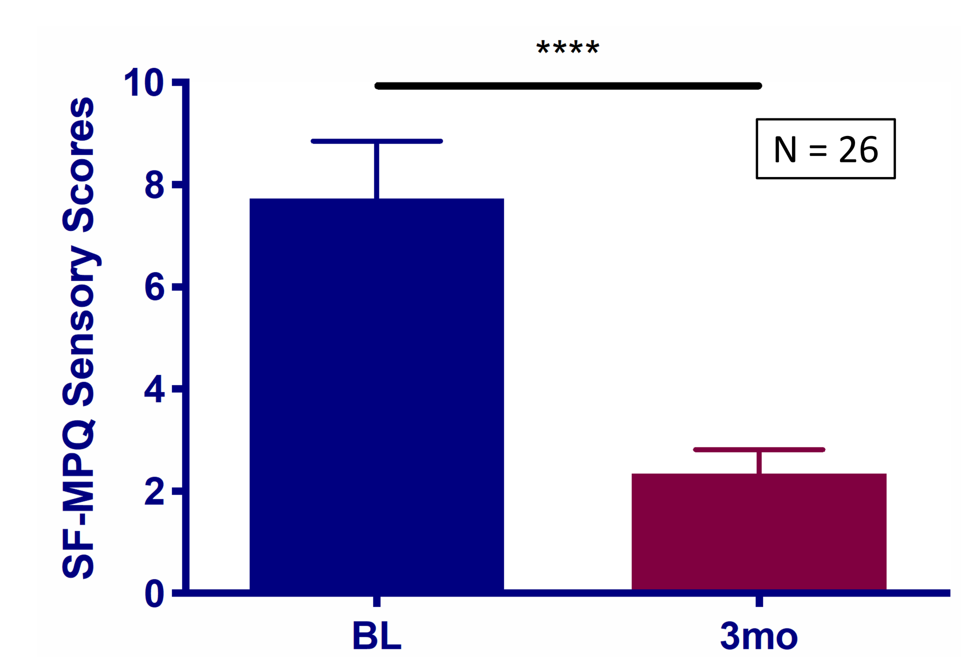


Figure 4. Paired *t*-test comparison of baseline and 3-month sensory pain scores. mean ± SD shown. **** p ≤ 0.0001

Conclusions

- DHA-rich supplementation preferentially targets pathways associated with cellular homeostasis and neuronal health
- DHA-rich supplementation improved metabolic profiles regarding omega-3 PUFA metabolism, glycerolipid metabolism, cysteine, methionine, and glutathione metabolism, and glucose and fatty acid homeostasis **without major changes** clinical parameters.
- Circulating plasma metabolites showed a significant shift toward a decrease in reactive oxygen species biosynthesis, lipid peroxidation, improved Ca2+ homeostasis, and increased glutathione activity
- Participants report dramatic **decrease in sensory pain scores** after omega-3 PUFA dietary supplementation
- These findings support the promising use of omega-3 fatty acids to reduce adverse symptoms associated with neuroinflammatory diseases and painful neuropathy.

Recent Publications

- Durán, A. M., Beeson, W. L., Firek, A., Cordero-MacIntyre, Z. & De León, M. Dietary Omega-3 Polyunsaturated Fatty-Acid Supplementation Upregulates Protective Cellular Pathways in Patients with Type 2 Diabetes Exhibiting Improvement in Painful Diabetic Neuropathy. *Nutrients* **14**, doi:10.3390/nu14040761 (2022).
- Durán, A. M. *et al.* Effects of omega-3 polyunsaturated fatty-acid supplementation on neuropathic pain symptoms and sphingosine levels in Mexican-Americans with type 2 diabetes. *Diabetes Metab Syndr Obes* **12**, 109-120, doi:10.2147/dmso.S187268 (2019).
- Montero, M. L., Liu, J. W., Orozco, J., Casiano, C. A. & De Leon, M. Docosahexaenoic acid protection against palmitic acid-induced lipotoxicity in NGF-differentiated PC12 cells involves enhancement of autophagy and inhibition of apoptosis and necroptosis. *J Neurochem* **155**, 559-576, doi:10.1111/jnc.15038 (2020).
- Figueroa, J. D. *et al.* Metabolomics uncovers dietary omega-3 fatty acid-derived metabolites implicated in anti-nociceptive responses after experimental spinal cord injury. *Neuroscience* **255**, 1-18, doi:10.1016/j.neuroscience.2013.09.012 (2013).

Acknowledgements

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