The Role of Vitamin D and Omega-3 Long Chain Polyunsaturated Fatty Acids in Children with Autism Spectrum Disorder

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A Dedication to All the Kids with Autism and Their Families Who Participated in This Study

A story of hope…

"And the best thing is that she now says "Mommy" when she wants me. She never says this unless being prompted by me but now she does it on her own. I have been waiting for this for 5 years and I was so ecstatic and soooo happy when she did!"

Abstract

Background: The efficacy of vitamin D and omega-3 long chain polyunsaturated fatty acid (omega-3 LCPUFA), each individually, in Autism Spectrum Disorder (ASD) has been tested in a few trials and the results are inconclusive. Furthermore, several observational studies have observed low vitamin D and omega-3 LCPUFA status in populations with ASD. Children with ASD are susceptible to nutritional issues and poor diet quality due to sensory, behavioural and gastrointestinal issues associated with the condition, though no information regarding these children's nutritional status is available in New Zealand. Also, no validated nutritional quality assessment tools are available for this population.

Aim and Objectives: The overall aim of this study was to investigate the role of vitamin D (VID), omega-3 LCPUFA (OM), or both (VIDOM) in ASD in children through systematically reviewing literature and conducting an intervention trial with these nutrients. The primary objective was to investigate the efficacy of vitamin D, omega-3 LCPUFA, both on core symptoms and sensory issues after correcting major nutritional deficiencies and secondary objectives were to investigate the efficacy of intervention on irritability and hyperactivity, to study dietary adequacy/nutritional status of children with ASD, and to validate a Dietary Index of Children's Eating (DICE) questionnaire against 4-day estimated food record (4DFR).

Methods/Design: New Zealand children with ASD (age 2.5-8.0 years) participated in a 12-month randomised, double-blind, placebo-controlled, 2x2 factorial trial. Prior to trial entry, children's dietary adequacy and nutritional status were assessed by 4DFR, DICE questionnaire (designed based on New Zealand Ministry of Health Food and Nutrition guidelines), and nutritional biomarkers (25(OH)D, red blood cell fatty acids, iron, calcium, albumin, vitamin B_{12}, and folate). Data regarding dietary supplement use and special/exclusion diet, demographics and anthropometrics (height and weight) were also collected. Children then were randomly assigned to one of four treatment groups; daily 2000IU vitamin D_3, 722 mg docosahexaenoic acid (DHA), both supplements, or placebo, and behaviours were assessed. Core symptoms were assessed using Social Responsiveness Scale (SRS), sensory issues using Sensory Processing Measure (SPM), problem behaviours including irritability and hyperactivity using Aberrant Behaviour Checklist (ABC). Outcome measures were analysed pre- and post-intervention. Pair-wise mixed effects longitudinal models were used for data analysis.

Results: 309 families registered their interest in the study, of whom 190 families were either excluded or not enrolled. The children of remaining families (n=119) were screened for nutritional deficiencies and high serum 25(OH)D concentrations, of whom two children were excluded. Overall, 62% (73/117) of children completed the trial (placebo 16, VID 19, OM 23, VIDOM 15). The mean serum 25(OH)D concentrations (nmol/L) increased in the VID (27±14, P<0.001) and VIDOM (36±17, P<0.001) groups and changed slightly in the OM (1.1±14, P>0.05) and placebo (8.9±23, P>0.05) groups. The median omega-3 index (%) increased in the OM [4.4 (3.3, 5.9), P<0.001] and VIDOM [4.0 (2.0, 6.0), P<0.001] groups and decreased in the VID [-0.2 (-1.0, 0.1)] and placebo [-0.5 (-0.9, -0.1), P>0.05] groups. Compared to placebo, a greater improvement in multiple outcomes in the intervention groups was observed: SRS-social awareness for OM (0.4±2.9 *vs.* -1.4±2.3, P=0.03) and VIDOM (0.4±2.9 *vs.* -1.7±3.5, P=0.03); SRS-social communicative functioning for VIDOM (-5.6±10 *vs.* -16±24, P=0.07); SRS-total for OM (-5.8±12 *vs.* -17±18, P=0.08); SPM-taste and smell for VIDOM (-0.3±1.7 *vs.* -2.5±4.3, P=0.06), SPM-balance and motion for OM (-0.1±4.7 *vs.* -2.6±4.3, P=0.09), ABC-irritability for VID (0.8±6.1 *vs.* -4.0±4.9 P=0.01) and OM (0.8±6.1 *vs.* -5.0±5.0, P=0.001); and ABC-hyperactivity for VID (-0.8±5.6 *vs.* -5.2±6.3, P=0.047).

Out of 86 children whose food records were available, approximately 50% (39/86) reported taking dietary supplements and 15% (13/86) were on a special/exclusion diet. A large proportion of children had dietary intake for vitamin D below the Adequate Intake (AI, 96%), protein below the Average Macronutrient Distribution Range (AMDR, 65%), and iodine below the Estimated Average Requirement (EAR, 54%). Dietary intake of fibre (43%) and vitamin E (37%) was also below the AI by at least one third of children. All or most children exceeded the recommendations for sodium (100%), total saturated fat (80%) and sugar (52%). There was a significant and positive correlation (r=0.7; P<0.001) and good agreement (κ=0.6) between total scores from DICE (64±16) and 4DFR (58±11). Participants in the highest tertile of DICE had higher intakes of magnesium (P=0.02), vitamin A (P=0.03) and fibre (P=0.06).

Conclusions: Vitamin D and omega-3 LCPUFA, each individually or together, improved some behavioural symptoms of ASD. However, large attrition rates and resultant loss of statistical power preclude definitive conclusion and warrant further trials.

Also, the baseline assessment of nutrition confirms nutritional issues and poor diet quality in children with ASD. Given the importance of nutrition in growth and development and in the management of ASD, screening of the nutritional status of children with ASD for nutrient adequacy to reduce under- or over-consumption of nutrients is recommended. DICE is a valid tool for the assessment of diet quality in children with ASD living in New Zealand.

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Abbreviations

ABC	Aberrant Behaviour Checklist
AD	Autism Disorder
ADHD	Attention Deficit/Hyperactivity Disorder
ADI-R	Autism Diagnostic Interview-Revised
ADOS	Autism Diagnostic Observation Schedule
AI	Adequate Intake
ALA	Alpha-linolenic acid
AMDR	Acceptable Macronutrient Distribution Range
ANCOVA	Analysis of Covariance
ANOVA	Analysis of Variance
ARA	Arachidonic Acid
Anti-MAG	Anti-Myelin-Associated Glycoprotein
ASC	Autism Screening Questionnaire
ASD	Autism Spectrum Disorder
ATEC	Autism Treatment Evaluation Checklist
BASC	Behaviour Assessment System for Children
BDNF	Brain-Derived Neurotrophic Factors
CARS	Childhood Autism Rating Scale
CGI-I	Clinical Global Impression-Improvement
CGI-S	Clinical Global Impression-Severity
CI	Confidence Interval
D_2	Ergocalciferol
D_3	Cholecalciferol
DCS	Dendritic Cells
DHA	Docosahexaenoic Acid
DICE	Dietary Index of Children's Eating
DISCO	Diagnostic Interview for Social and Communication Disorders
DPA	Docosapentaenoic Acid
DSM	Diagnostic and Statistical Manual of Mental Disorder
EAR	Estimated Average Requirement
EPA	Eicosapentaenoic Acid
FABP	Fatty Acid Binding Protein
FADS	Fatty Acid Desaturase
FFQ	Food Frequency Questionnaire
HNRU	Human Nutrition Research Unit
ICD	International Classification of Diseases and Related Health Problems
IFN-γ	Interferon Gamma
IL	Interleukin
IQ	Intelligence Quotient
IQR	Interquartile Range
IU	International Unit
LA	Linoleic Acid
MIP2	Macrophage Inflammatory Protein-2
MOH	Ministry of Health
MUFA	Monounsaturated Fatty Acids

NGF	Nerve Growth Factor
NRV	Nutrient Reference Values
NZ	New Zealand
OM	Omega-3 Long Chain Polyunsaturated Fatty Acids
Omega-3 LCPUFA	Omega-3 Long Chain Polyunsaturated Fatty Acids
Omega-6 LCPUFA	Omega-6 Long Chain Polyunsaturated Fatty Acids
PDD	Pervasive Developmental Disorder
PDD-BI	Pervasive Developmental Disorder-Behaviour Inventory
PDD-NOS	Pervasive Developmental Disorder-Not Otherwise Specified
PGE2	Prostaglandin E2
PUFA	Polyunsaturated Fatty Acids
RBC	Red Blood Cell
RCT	Randomised Controlled Trial
RDI	Recommended Dietary Intake
RRB	Repetitive and Restricted Interests and Behaviour
SCQ	Social Communication Questionnaire
SD	Standard Deviation
SFA	Saturated Fatty Acids
SNP	Single Nucleotide Polymorphism
SPM	Sensory Processing Measure
SRS	Social Responsiveness Scale
TNF–α	Tumour Necrosis Factor Alpha
UL	Tolerable Upper Intake Level
US	United States
UVB	Ultra-Violate B
VABS	Vineland Adaptive Behaviour Scales
VDR	Vitamin D Receptor
VID	Vitamin D
VIDOM	Vitamin D + Omega-3 Long Chain Polyunsaturated Fatty Acids
WDHB	Waitemata District Health Board
1-OHase	25-hydroxyvitamin D-1α-hydroxylase
25(OH)D	25-hydroxyvitamin D
$1,25(OH)_2D$	1,25-dihydroxyvitamin D
3di	Developmental, Dimensional, and Diagnostic Interview
4DFR	4-Day Estimated Food Record

List of Papers and Conference Presentations

Papers (Published, Submitted or to be submitted)

The following publications have been included in this thesis and incorporated as different chapters and sections in manuscript format. Therefore, in some cases, there may be replication.

Paper I Mazahery, H., C.A. Camargo, Jr., C. Conlon, K.L. Beck, M.C. Kruger, and P.R. von Hurst, Vitamin D and Autism Spectrum Disorder: A literature review. Nutrients, 2016. **8**(4): p. 236.

Incorporated as Chapter 2: Section 2.

Paper II Mazahery, H., W. Stonehouse, M. Delshad, M.C. Kruger, C.A. Conlon, K.L. Beck, and P.R. von Hurst, relationship between long chain n-3 polyunsaturated fatty acids and Autism Spectrum Disorder: systematic review and meta-analysis of case-control and randomised controlled trials. Nutrients, 2017. **9**(2).

Incorporated as Chapter 2: Section 3.

Paper III Mazahery, H., C. Conlon, K.L. Beck, M.C. Kruger, W. Stonehouse, C.A. Camargo, Jr., B.J. Meyer, B. Tsang, O. Mugridge, and P.R. von Hurst, Vitamin D and omega-3 fatty acid supplements in children with Autism Spectrum Disorder: A study protocol for a factorial randomised, double-blind, placebo-controlled trial. Trials, 2016. **17**(1): p. 295.

Incorporated as Chapter 3

Paper IV Mazahery, H., M. Delshad, C. Conlon, K.L. Beck, M.C. Kruger, and P.R. von Hurst, Dietary adequacy and nutritional status of New Zealand children with Autism Spectrum Disorder and validation of a Dietary Index of Children's Eating (DICE) (Not submitted yet)

Incorporated as Chapter 4.

Paper V Mazahery, H., C. Conlon, K.L. Beck, O. Mugridge, M.C. Kruger, W. Stonehouse, C.A. Camargo, Jr., B.J. Meyer, B. Tsang, B. Jones, and P.R. von Hurst, A randomised controlled trial of vitamin D and omega-3 long chain polyunsaturated fatty acids in the treatment of core symptoms of Autism Spectrum Disorder in children. J. Autism Dev. Disord., 2019 (online)

Incorporated as Chapter 5.

Paper VI Mazahery, H., C. Conlon, K.L. Beck, O. Mugridge, M.C. Kruger, W. Stonehouse, C.A. Camargo, Jr., B.J. Meyer, B. Jones, and P.R. von Hurst, A randomised controlled trial of vitamin D and omega-3 long chain polyunsaturated fatty acids in the treatment of irritability and

hyperactivity among children with Autism Spectrum Disorder. The Journal of Steroid Biochemistry and Molecular Biology, 2018 (online).

Incorporated as Chapter 6.

Conference Presentations

I Mazahery, H. and O. Mugridge, Adaptations to standard nutrition research protocol for a nutritional supplement study in ASD children. South Pacific Congress, 2015.
Oral presentation

II Mazahery, H., C. Conlon, K.L. Beck, M.C. Kruger, O. Mugridge, C.A. Camargo, Jr., and P.R. von Hurst, Vitamin D status and its predictors in children with Autism Spectrum Disorder. The 20th workshop on Vitamin D, Orlando, 2017. Poster presentation

III Mazahery, H., C. Conlon, K.L. Beck, M.C. Kruger, W. Stonehouse, C.A. Camargo, Jr., O. Mugridge, and P.R. von Hurst, A randomised, double-blind, placebo-controlled trial of vitamin D and n-3 long chain polyunsaturated fatty acids in the treatment of irritability and hyperactivity in children with Autism Spectrum Disorder. The 21st Workshop on Vitamin D, Barcelona, 2018.
Oral presentation. Recipient of "Claude McCarthy Fellowship Award" and "Trainee Travel Award".

IV Mazahery, H., C. Conlon, K.L. Beck, M.C. Kruger, W. Stonehouse, C.A. Camargo, Jr., O. Mugridge, and P.R. von Hurst, Omega-3 long chain polyunsaturated fatty acids may modulate the effect of vitamin D supplementation on vitamin D status in children with Autism Spectrum Disorder. The 21st Workshop on Vitamin D, Barcelona, 2018.
Poster presentation.

V Mazahery, H., C. Conlon, K.L. Beck, O. Mugridge, M.C. Kruger, W. Stonehouse, C.A. Camargo, Jr., Meyer, B.J., Jones, B., Tsang, B., and P.R. von Hurst, Vitamin D and omega-3 long chain polyunsaturated fatty acids improve behavioural symptoms in children with Autism Spectrum Disorder. Nutrition Society of New Zealand: Annual Scientific Meeting, Auckland, 2018

Oral presentation.