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AWARD NUMBER: W81XWH-16-1-0753

TITLE: Prenatal Polyunsaturated Fatty Acid Levels and Risk of Autism Spectrum Disorders

PRINCIPAL INVESTIGATOR: Kristen Lyall

RECIPIENT: Drexel University
PHILADELPHIA, PA 19104

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PHILADELPHIA, PA 19104

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14. ABSTRACT

Polyunsaturated fatty acids (PUFAs) are dietary factors that play a critical role in fetal neurodevelopmental processes that have been implicated in the etiology of autism spectrum disorder (ASD). It is not known whether prenatal PUFA levels influence risk of ASD.

15. SUBJECT TERMS

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
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TABLE OF CONTENTS

1. INTRODUCTION: Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.

The purpose of this project is to determine whether prenatal levels of polyunsaturated fatty acids (PUFAs), as classes (omega 3, omega 6, and total PUFA) as well as individual fatty acids, are associated with offspring autism spectrum disorder (ASD). These fats are critical in neurodevelopmental processes with evidence for disruption in ASD, and thus we hypothesize that altered levels of them during critical windows of neurodevelopment may influence risk. To address this hypothesis, we are conducting a population-based case control study, including 500 cases with ASD identified through the California Department of Developmental Services (DDS) and 500 general population controls identified through state birth certificates and matched by birth year (2011-2013), birth month, and sex, after excluding DDS clients. Using banked prenatal serum specimens collected through routine prenatal screening in California, levels of PUFAs are measured using liquid chromatography-mass spectrometry/high resolution mass spectrometry (LC-MS/MHMS). In a subset of participants (n=400), we will also examine measured levels of PUFAs in neonatal blood spots. Results from this work will provide novel information about the relationship between PUFAs and ASD, in the first study with measured levels of PUFAs during pregnancy.

2. KEYWORDS: Provide a brief list of keywords (limit to 20 words).

Autism, etiology, epidemiology, polyunsaturated fatty acids, prenatal risk factors, nutrition

3. ACCOMPLISHMENTS: The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency Grants Officer whenever there are significant changes in the project or its direction.

What were the major goals of the project?
List the major goals of the project as stated in the approved SOW. If the application listed milestones/target dates for important activities or phases of the project, identify these dates and show actual completion dates or the percentage of completion.

Major goals included in the SOW, and information on target and actual dates and percent completion, are listed below:

1. **Procurement of Maternal and Neonatal Stored Blood Samples (relevant to Aims 1, 2, and 3)**

   Target completion: Year 1, quarter 3-4

   Actual completion/% complete: Year 1, quarter 4- all maternal and neonatal samples have been obtained; thus, this task is 100% complete.

   Description: We have obtained all maternal prenatal serum samples (n=1002; an additional 2 were obtained due to unexpected differences in availability of samples and the need to balance case-control birth years) neonatal blood spots (n=400). This major goal included the following sub-tasks: completing and submitting IRB and vital record use applications (completed by the second quarter of our first year, slightly later than expected due to California review board meeting dates); selecting cases and controls from California databases (projected for quarter 1; completed slightly later than anticipated, due to waiting for approvals); obtaining approval

from the Genetic Disease Screening Program (GDSP) for use of samples (completed as projected); requesting and obtaining samples from GDSP and sample shipment to the Snyder laboratory (obtaining of samples completed approximately one quarter later than expected, due to delays in the California Biobank queue process.)
Major milestones sought and achieved: Local and CPHS IRB approvals; HRPO approval; procurement of samples.

2. **Measurement of PUFAs in maternal serum and newborn blood spots (relevant to Aims 1 and 3)**
   Target completion: Year 1, quarter 4- year 2, quarter 2
   Actual completion/% complete: We have completed laboratory analyses of all samples (100% complete).
   Description: Measurement of PUFAs in maternal samples was completed in the Spring of 2018. Measurement of PUFAs in newborn spots was just recently completed. There had been some delay in shipment of newborn blood spots due to California Biobank administrative delays.
   Major milestones sought and achieved: Completion of biosample assays for PUFAs.

3. **Data analysis of PUFAs in association with ASD (relevant to Aims 1, 2, and 3)**
   Target completion: (Year 2, quarters 2-4.)
   Actual completion/% complete: 100% complete; we have completed analyses of maternal PUFA levels and ASD, as well as analyses of newborn PUFAs and ASD.
   Description: Subtasks 1 (analyses of maternal levels) and subtasks 2 (examination of modifers and subgroups) were completed in the previous grant year. Subtask 3 (analyses of newborn levels) was completed in this grant year, with preliminary statistical analyses completed between November 2018 and January 2019, and analyses finalized between February and May 2019. Results relevant to Aim 3 are presented in Appendix 1.
   Milestone achieved: Completion of statistical analyses.

4. **Presentation of findings (relevant to Aims 1, 2, and 3)**
   Target completion: (Year 3, quarters 1-4)
   Actual completion/% complete: 60%
   Description: We have presented results based on newborn levels at the 2019 INSAR conference and 2019 Society for Pediatric and Perinatal Epidemiologic Research (SPER). We drafted a manuscript describing maternal serum results; the draft must be reviewed by CDPH prior to submission in a peer-reviewed journal. A draft of the manuscript describing newborn results is in progress. Thus, subtask 2 of this major task will be completed shortly.
   Milestone achieved: Presentation of findings.

What was accomplished under these goals?
For this reporting period describe: 1) major activities; 2) specific objectives; 3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative); and/or 4) other achievements. Include a discussion of stated goals not met. Description shall include pertinent data and graphs in sufficient detail to explain any significant results achieved. A succinct description of the methodology used shall be provided. As the project progresses to completion, the emphasis in reporting in this section should shift from reporting activities to reporting accomplishments.

Major accomplishments under these goals (since the last report) include: completion of the aims of this study with the finalization of data analyses of newborn blood spot levels. In addition, drafting of the manuscript of maternal analyses was completed and drafting of the manuscript based on newborn analyses is in progress. An additional major accomplishment is the presentation of newborn blood spot findings at the 2019 INSAR and SPER meetings. We conducted logistic regression to examine crude and adjusted odds ratios, by percentiles of PUFA levels, in 200 cases and controls for these newborn analyses. These results are summarized in Appendix 1. Characteristics of individuals included in our neonatal bloodspot group are shown in Table 1 (note, these are comparable to our maternal sample, as reported in prior Annual Reports, demonstrating effectiveness of random selection). We observed strong correlations between neonatal PUFAs measured in newborn bloodspots, as well as between maternal PUFAs measured in serum, but notably, not across these time points (Appendix Figure 1). This in itself is an interesting finding, and will be a novel contribution to the literature, suggesting the importance of measuring these fats at different timepoints to examine potential critical windows. Overall, when examining associations between maternal PUFAs and ASD (as presented in our 2018 annual report), we observed mostly null associations (though with some suggested associations in certain subgroups or sub-analyses). Here, we also observed similar neonatal levels between cases and controls, and several null associations based on neonatal PUFA levels (Appendix Tables 2 and Table 3), but we did find that there may be increases or decreases in risk of ASD at extremes of the distribution of PUFA levels (Appendix Table 4). Our results also suggested some non-linear associations (Appendix Figure 2). Specifically, results suggested non-linear associations between neonatal levels of the n-6 PUFA linoleic acid and alpha-linoleic/gamma-linolenic acid. Increases in risk of ASD were observed for those with very lowest levels of dihomo-γ-linolenic acid (DGLA) and total PUFAs. Decreases in risk were observed for those with the highest levels of linoleic acid, alpha-linoleic/gamma-linolenic acid, and total PUFAs. Overall, our findings from this project suggest that maternal PUFAs, particularly as measured mid-pregnancy, do not have a strong association with ASD overall, but that high levels of PUFAs in late pregnancy (as represented by our neonatal analyses) may be associated with a reduction in risk of ASD. These findings suggest the need for continued work focused on the role of PUFAs in ASD and neurodevelopment, in large samples with the ability to examine late pregnancy levels and consider phenotypic subgroups.

What opportunities for training and professional development has the project provided?
If the project was not intended to provide training and professional development opportunities or there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe opportunities for training and professional development provided to anyone who worked on the project or anyone who was involved in the activities supported by the project. "Training" activities are those in which individuals with advanced professional skills and experience assist others in attaining greater proficiency. Training activities may include, for example, courses or

one-on-one work with a mentor. "Professional development" activities result in increased knowledge or skill in one's area of expertise and may include workshops, conferences, seminars, study groups, and individual study. Include participation in conferences, workshops, and seminars not listed under major activities.

Under funding from this project, the PI attended the 2019 meeting of the International Society for Autism Research (INSAR), where she presented findings from this study. The PI also presented findings on newborn blood spots at the Society for Pediatric and Perinatal Epidemiologic Research (SPER) conference.

In addition, this year, work on newborn blood spot analyses providing a training opportunity for a masters-level student who assisted in these analyses. Dr. Lyall provided mentoring to this student with an interest in how biomarkers during pregnancy may predict autism.

Describe how the results were disseminated to communities of interest. Include any outreach activities that were undertaken to reach members of communities who are not usually aware of these project activities, for the purpose of enhancing public understanding and increasing interest in learning and careers in science, technology, and the humanities.

Results from newborn blood spot analyses from this work were presented at the INSAR and SPER meetings as described above. As premiere autism and epidemiology conferences respectively, findings were disseminated to professionals in the field. We also plan to disseminate findings through publishing in peer reviewed journals; the manuscript based on associations with maternal PUFA levels is drafted and the manuscript with newborn blood spot levels is currently being prepared.

What do you plan to do during the next reporting period to accomplish the goals?
If this is the final report, state "Nothing to Report."

Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.

All major goals of our project have been accomplished. We have been approved for a no-cost extension, during which we will finalize manuscript submissions. In addition, during the NCE period, we plan to finalize supplemental analyses examining interactions with cotinine levels.

4. IMPACT: Describe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:

What was the impact on the development of the principal discipline(s) of the project?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how findings, results, techniques that were developed or extended, or other products from the project made an impact or are likely to make an impact on the base of knowledge, theory, and research in the principal disciplinary field(s) of the project. Summarize using language that an intelligent lay audience can understand (Scientific American style).

Our study provides the first results on the association between measured levels of maternal and newborn PUFA levels in association with ASD diagnosis. Because there was some suggestion of increased risk for having a child with ASD with comorbid intellectual disability among mothers with low levels of certain PUFAs, our work suggests the need for continued investigation into the potential relationship between PUFAs and phenotypic subgroups within ASD. The majority of pregnant women do not eat recommended levels of fish, which are a key source of these PUFAs. If our findings are further supported in work seeking to replicate these results specific to ASD with comorbid intellectual disability, there is the potential for risk reduction with dietary modifications for certain subgroups. Furthermore, because few studies have measured levels of these fatty acids in newborn blood spot samples, our study has provided novel information on methods to conduct such measurements, which could be applied in other studies.

What was the impact on other disciplines?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the findings, results, or techniques that were developed or improved, or other products from the project made an impact or are likely to make an impact on other disciplines.

The suggestion of potentially increased risk with certain PUFA levels for ASD with comorbid intellectual disability from our maternal PUFA analyses suggests the need to further examine these associations in study populations with detailed information on broader neurodevelopmental outcomes in order to tease apart specificity of associations and better understand how risk may differ for different neurodevelopmental conditions and for individuals with comorbid conditions. Thus, our findings could have an impact on other fields of specialty focused on other, non-ASD neurodevelopmental disorders. In addition, as stated above, the measurement of PUFAs in newborn blood spots may have an impact on other disciplines seeking to measure these fatty acids in novel and more readily available matrices.

What was the impact on technology transfer?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe ways in which the project made an impact, or is likely to make an impact, on commercial technology or public use, including:

- *transfer of results to entities in government or industry;*
- *instances where the research has led to the initiation of a start-up company; or*
- *adoption of new practices.*

Nothing to Report

What was the impact on society beyond science and technology?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how results from the project made an impact, or are likely to make an impact, beyond the bounds of science, engineering, and the academic world on areas such as:

- *improving public knowledge, attitudes, skills, and abilities;*
- *changing behavior, practices, decision making, policies (including regulatory policies), or social actions; or*
- *improving social, economic, civic, or environmental conditions.*

Findings here should be replicated and further studied prior to making wide-scale public health recommendations. However, there is evidence that PUFA intake is below recommended levels for pregnant women in the US; thus, if there is further support for increased risk to certain subgroups with levels outside of average ranges, risk reduction strategies based on dietary recommendations could ultimately be created, and this may have an impact on risk of ASD.

5. CHANGES/PROBLEMS: The Project Director/Principal Investigator (PD/PI) is reminded that the recipient organization is required to obtain prior written approval from the awarding agency Grants Officer whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, "Nothing to Report," if applicable:

Changes in approach and reasons for change
Describe any changes in approach during the reporting period and reasons for these changes. Remember that significant changes in objectives and scope require prior approval of the agency.

No significant changes in approach have been made during the reporting period.

Actual or anticipated problems or delays and actions or plans to resolve them
Describe problems or delays encountered during the reporting period and actions or plans to resolve them.

We have not experienced any major problems during this reporting period. Within the newborn bloodspot samples, we were able to measure and quantify the majority of PUFAs of interest, but two fatty acids of the same chain length, ALA and GLA, could not be further resolved and were reported together (see also Appendix Figure 1 legend). This did not affect our study aims or conclusions, but results for these two fatty acids will be presented as combined levels rather than individually.

Changes that had a significant impact on expenditures

Describe changes during the reporting period that may have had a significant impact on expenditures, for example, delays in hiring staff or favorable developments that enable meeting objectives at less cost than anticipated.

None

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Describe significant deviations, unexpected outcomes, or changes in approved protocols for the use or care of human subjects, vertebrate animals, biohazards, and/or select agents during the reporting period. If required, were these changes approved by the applicable institution committee (or equivalent) and reported to the agency? Also specify the applicable Institutional Review Board/Institutional Animal Care and Use Committee approval dates.

Significant changes in use or care of human subjects

None

Significant changes in use or care of vertebrate animals.

Not applicable

Significant changes in use of biohazards and/or select agents

Not applicable

6. PRODUCTS: List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."

- **Publications, conference papers, and presentations**
  Report only the major publication(s) resulting from the work under this award.

  Journal publications. *List peer-reviewed articles or papers appearing in scientific, technical, or professional journals. Identify for each publication: Author(s); title; journal; volume: year; page numbers; status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

Nothing to report

  Books or other non-periodical, one-time publications. *Report any book, monograph, dissertation, abstract, or the like published as or in a separate publication, rather than a periodical or series. Include any significant publication in the proceedings of a one-time conference or in the report of a one-time study, commission, or the like. Identify for each one-time publication: Author(s); title; editor; title of collection, if applicable; bibliographic information; year; type of publication (e.g., book, thesis or dissertation); status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

None

  Other publications, conference papers, and presentations. *Identify any other publications, conference papers and/or presentations not reported above. Specify the status of the publication as noted above. List presentations made during the last year (international, national, local societies, military meetings, etc.). Use an asterisk (*) if presentation produced a manuscript.*

Conference presentation: INSAR, Montreal, CA May 2019. Lyall K, Windham GC, Whitman C, Snyder N, Kuskovsky R, Robinson L, Newschaffer C. "Neonatal polyunsaturated fatty acid levels in association with autism spectrum disorder: Results from a California population-based case-control study." Poster presentation given on May 4th, 2019. Abstract presentation: SPER, Minneapolis, MN, June 2019. Lyall K, Windham GC, Whitman C, Snyder N, Kuskovsky R, Robinson L, Newschaffer C. "Neonatal polyunsaturated fatty acid levels and autism spectrum disorder." Acknowledgement of federal support was given in both presentations.

- **Website(s) or other Internet site(s)**

List the URL for any Internet site(s) that disseminates the results of the research activities. A short description of each site should be provided. It is not necessary to include the publications already specified above in this section.

Nothing to report

- **Technologies or techniques**
  Identify technologies or techniques that resulted from the research activities. In addition to a description of the technologies or techniques, describe how they will be shared.

Nothing to report

- **Inventions, patent applications, and/or licenses**
  Identify inventions, patent applications with date, and/or licenses that have resulted from the research. State whether an application is provisional or non-provisional and indicate the application number. Submission of this information as part of an interim research performance progress report is not a substitute for any other invention reporting required under the terms and conditions of an award.

- **Other Products**
  Identify any other reportable outcomes that were developed under this project. Reportable

Nothing to report

outcomes are defined as a research result that is or relates to a product, scientific advance, or research tool that makes a meaningful contribution toward the understanding, prevention, diagnosis, prognosis, treatment, and/or rehabilitation of a disease, injury or condition, or to improve the quality of life. Examples include:

- *data or databases;*
- *biospecimen collections;*
- *audio or video products;*
- *software;*
- *models;*
- *educational aids or curricula;*
- *instruments or equipment;*
- *research material (e.g., Germplasm; cell lines, DNA probes, animal models);*
- *clinical interventions;*
- *new business creation; and*
- *other.*

Nothing to report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?
Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate "no change."

Name: Dr. Kristen Lyall
Project Role: Principle Investigator
Researcher Identifier
Nearest person month worked: 2
Contribution to Project: Oversaw all project activities, obtained appropriate approvals, and conducted data analyses.

Name: Dr. Nathaniel Snyder
Project Role: Co-Investigator
Researcher Identifier
Nearest person month worked: 1
Contribution to Project: Performed laboratory analysis of PUFAs in maternal serum samples and newborn blood spots.

Name: Dr. Gayle Windham
Project Role: Co-Investigator
Researcher Identifier
Nearest person month worked:1
Contribution to Project: Oversaw data linkage and aided in preparation of data files; helped to coordinate case control selection process.

Name: Casey Whitman
Project Role: Data Analyst
Researcher Identifier
Nearest person month worked: 1
Contribution to Project: Under supervision of PI, Kristen Lyall, performed data analyses.

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

If the active support has changed for the PD/PI(s) or senior/key personnel, then describe what the change has been. Changes may occur, for example, if a previously active grant has closed and/or if a previously pending grant is now active. Annotate this information so it is clear what has changed from the previous submission. Submission of other support information is not

necessary for pending changes or for changes in the level of effort for active support reported previously. The awarding agency may require prior written approval if a change in active other support significantly impacts the effort on the project that is the subject of the project report.

Please see attached appendix. Active support has changed for some investigators, but there is no overlap and support changes do not impact effort on the current project.

What other organizations were involved as partners?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe partner organizations – academic institutions, other nonprofits, industrial or commercial firms, state or local governments, schools or school systems, or other organizations (foreign or domestic) – that were involved with the project. Partner organizations may have provided financial or in-kind support, supplied facilities or equipment, collaborated in the research, exchanged personnel, or otherwise contributed.
Provide the following information for each partnership:
Organization Name:
Location of Organization: (if foreign location list country)
Partner's contribution to the project (identify one or more)

- *Financial support;*
- *In-kind support (e.g., partner makes software, computers, equipment, etc., available to project staff);*
- *Facilities (e.g., project staff use the partner's facilities for project activities);*
- *Collaboration (e.g., partner's staff work with project staff on the project);*
- *Personnel exchanges (e.g., project staff and/or partner's staff use each other's facilities, work at each other's site); and*
- *Other.*

(NO CHANGE)
Organization name: California Department of Public Health (CDPH)
Location: Richmond, CA
Contribution: Collaboration- Co-Investigator Dr. Gayle Windham and her study staff at CDPH collaborated with the study PI to ensure data linkage and study sample selection necessary for this project. As noted in the previous annual report for this project, Dr. Windham and her team have extensive experience with California birth certificate and DDS data, and conducting data linkages for similar projects. Dr. Windham and her staff have maintained close communication with the PI of this project, Dr. Lyall, including through attending project meetings via conference calls. Facilities have not been exchanged.

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: *For collaborative awards, independent reports are required from BOTH the Initiating PI and the Collaborating/Partnering PI. A duplicative report is acceptable; however, tasks shall be clearly marked with the responsible PI and research site. A report shall be submitted to* ***https://ers.amedd.army.mil*** *for each unique award.*

QUAD CHARTS: *If applicable, the Quad Chart (available on https://www.usamraa.army.mil) should be updated and submitted with attachments.*

N/A

9. APPENDICES: *Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.*

Please see attached. Appendix 1: Results Summary. Appendix 2: Other support documentation.

ADDITIONAL NOTES:

Appendices provided include other support documents for all key investigators (PI and Co-Investigators with support from this project) as well as results summaries.

MARKING OF PROPRIETARY INFORMATION: Data that was developed partially or exclusively at private expense shall be marked as "Proprietary Data" and Distribution Statement B included on the cover page of the report. Federal government approval is required before including Distribution Statement B. The recipient/PI shall coordinate with the COR/GOR to obtain approval. **REPORTS NOT PROPERLY MARKED FOR LIMITATION WILL BE DISTRIBUTED AS APPROVED FOR PUBLIC RELEASE.** It is the responsibility of the Principal Investigator to advise the COR/GOR when restricted limitation assigned to a document can be downgraded to "Approved for Public Release." **DO NOT USE THE WORD "CONFIDENTIAL" WHEN MARKING DOCUMENTS. DO NOT USE WATERMARKS WHEN MARKING DOCUMENTS.**

APPENDIX 1: NEONATAL PUFA ANALYSIS RESULTS

Figure 1: Correlation between maternal and neonatal PUFA levels

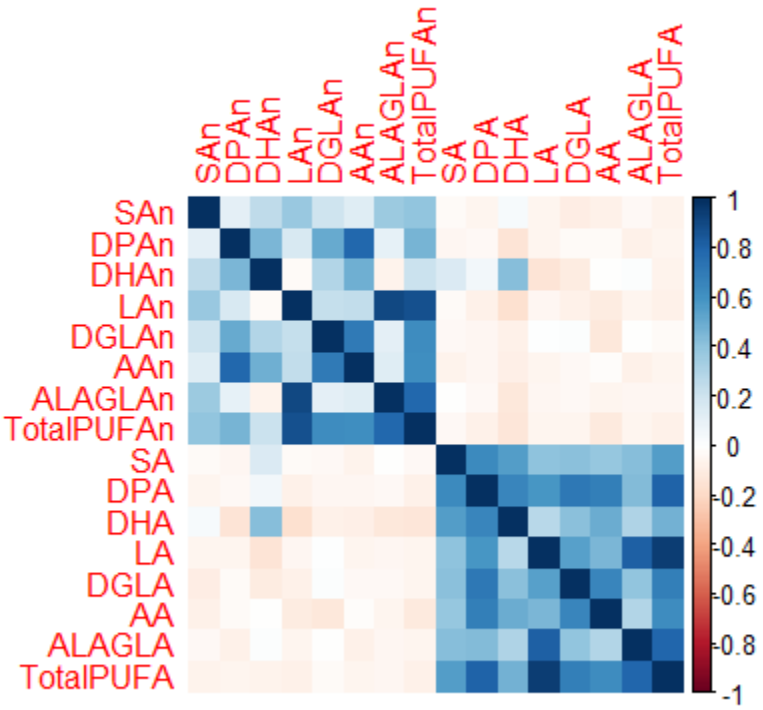


Figure legend: PUFAs with 'n' appended indicate neonatal measurements. ALAGLA reported together due to inability to separate these molecules in newborn bloodspots.

Abbreviation	PUFA name	Lipid name/type
ALA	Alpha-linolenic acid	18:3 n-3
SA	Stearidonic acid (SDA)	18:4 n-3
EPA	Eicosapentaenoic acid	20:5 n-3
DPA	Docosapentaenoic acid	22:5 n-3
DHA	Docosahexaenoic acid	22:6 n-3
LA	Linoleic acid	18:2 n-6
GLA	Gamma-linolenic acid	18:3 n-6
ECA	Eicosadienoic acid	20:2 n-6
DGLA	Di-homo-gamma linolenic acid	20:3 n-6
AA	Arachidonic acid	20:4 n-6

Table 1:
Basic characteristics of the NBS subgroup by case status:

	ASD Cases (n=200)	Controls (n=200)	P-value
Continuous variables (mean, SD)			
Maternal age (yrs)	29.6 (6.0)	27.92 (5.7)	0.005
Paternal age (yrs)	33.2 (7.3)	30.7 (6.5)	0.001
Gestational age (days)	274.0 (13.9)	273.8 (14.1)	0.90
Birth weight (g)	3393.3 (487.7)	3370.1 (492.9)	0.64
Pre-pregnancy BMI (kg/m2)	27.3 (6.8)	25.5 (5.7)	0.006
Categorical variables (n, %)			
Offspring sex Male Female	 164 (82%) 36 (18%)	 164 (82%) 36 (18%)	
Maternal education 1-<HS (no diploma) 2-HS diploma 3-Some college or 2yr 4-College degree 5-Graduate degree 6-Missing	 36 (18%) 37 (19%) 68 (34%) 35 (18%) 19 (10%) 5 (3%)	 50 (25%) 37 (19%) 43 (22%) 40 (20%) 20 (10%) 10 (5%)	0.08
Maternal race/ethnicity 1-Non-Hispanic White 2-Asian 3-Black 4-Hispanic 5-Other	 46 (23%) 35 (18%) 9 (5%) 102 (51%) 8 (4%)	 46 (23%) 29 (15%) 7 (4%) 110 (55%) 8 (4%)	0.89
Maternal birthplace outside the US	86 (43%)	93 (47%)	0.48
Insurance status at delivery 1-Private 2-Government program 3-Other	 86 (43%) 111 (56%) 3 (2%)	 89 (45%) 110 (55%) 1 (1%)	0.59
Smoking[1]	5 (3%)	2 (1%)	0.25
Weight change for BMI [2] 1-Appropriate 2-Less than recommended 3-Greater than recommended	 103 (52%) 23 (12%) 68 (34%)	 107 (54%) 27 (14%) 60 (30%)	0.83
IPI groupings (different vars)	15 (8%)	9 (5%)	0.21

Within 1 year Within 2 years Within 1 year or ≥10yrs	37 (19%) 28 (14%)	29 (15%) 15 (8%)	0.28 0.04
Metabolic Conditions [3]	71 (36%)	49 (25%)	0.02
Any pregnancy complication	50 (25%)	35 (18%)	0.06
Any delivery complication	153 (77%)	148 (74%)	0.56
Labor medications *(+sep.var)* Induction Oxytocin Epidural	133 (67%) 24 (12%) 35 (18%) 125 (63%)	134 (67%) 32 (16%) 34 (17%) 124 (62%)	0.92 0.25 0.89 0.92
Mode of delivery 1-Spontaneous vaginal 2-Assisted vaginal 3-Cesarean section (all types)	 122 (61%) 8 (4%) 70 (35%)	 129 (65%) 11 (6%) 60 (30%)	 0.49
Preterm birth	16 (8%)	17 (9%)	0.86
Low birth weight	10 (5%)	7 (4%)	0.46

[1]Defined as any smoking 3 months prior to conception through pregnancy
[2]Weight gain appropriate for BMI according to ACOG recommendations, with 15% of recommendation.
[3] indicator for any of GDB, pre-pregnancy obesity according to a BMI>=30, and/or pregnancy-related hypertension, according to Vital Statistics data

Table 2: Neonatal PUFA levels by case status

PUFA Name	Geometric Mean		Range		p-value
	Case	Control	Case	Control	
ALA/GLA	57.19	57.73	(14.22, 385.6)	(8.07, 194.1)	.8559
AA	71.05	71.09	(27.92, 234.9)	(12.42, 170.9)	.9893
DGLA	106.5	106.4	(40.96, 377.1)	(26.81, 373.2)	.9787
DHA	15.87	15.32	(5.08, 59.14)	(2.47, 61.14)	.5055
DPA	6.13	5.76	(0.16, 20.05)	(0.24, 25.22)	.3075
LA	45.89	56.09	(0.96, 868.5)	(1.92, 517.1)	.0839
SA	0.082	0.083	(0.052, 0.202)	(0.058, 0.209)	.4627
Total PUFA	338.3	343.4	(155.1, 1458.1)	(129.6, 1091.9)	.7134
Total n-6	319.6	325.6	(142.7, 1450.9)	(123.0, 1078.0)	.6501
Total n-3	76.63	76.10	(22.34, 392.9)	(18.29, 208.0)	.8682

Table 3: Association (odds ratios and 95% confidence intervals) between quartiles of neonatal PUFA levels measured in newborn bloodspots and offspring autism spectrum disorder (ASD)

PUFA	Quartile Median	Case/Control n	Crude OR (95%CI)	Adjusted OR (95% CI)[1]
AA (20:4)				
Q1	49.67	59/50	1.0	1.0
Q2	64.36	34/50	0.59 (0.33, 1.04)	0.55 (0.30, 1.02)
Q3	76.72	43/50	0.73 (0.42, 1.28)	0.65 (0.36, 1.18)
Q4	106.71	64/50	1.09 (0.63, 1.86)	1.07 (0.60, 1.91)
ALAGLA				
Q1	32.44	44/50	1.0	1.0
Q2	47.51	50/50	1.15 (0.65, 2.01)	1.13 (0.62, 2.05)
Q3	67.50	57/50	1.32 (0.74, 2.34)	1.27 (0.69, 2.33)
Q4	100.58	49/50	1.23 (0.64, 1.99)	0.95 (0.52, 1.74)
DGLA (20:3)				
Q1	68.70	56/50	1.0	1.0
Q2	90.90	32/50	0.57 (0.32, 1.04)	0.62 (0.33, 1.16)
Q3	116.20	55/50	0.97 (0.55, 1.69)	1.08 (0.60, 1.95)
Q4	171.15	57/50	1.03 (0.59, 1.81)	1.18 (0.65, 2.13)
DPA				
Q1	3.30	58/50	1.0	1.0
Q2	5.69	52/50	0.90 (0.53, 1.53)	1.08 (0.61, 1.92)
Q3	7.77	41/50	0.70 (0.40, 1.23)	0.81 (0.44, 1.51)
Q4	10.60	49/50	0.84 (0.48, 1.46)	0.95 (0.53, 1.73)
DHA (22:6)				
Q1	8.50	58/50	1.0	1.0
Q2	13.68	51/50	0.88 (0.52, 1.50)	0.85 (0.47, 1.52)
Q3	18.99	35/50	0.59 (0.33, 1.06)	0.53 (0.27, 1.03)
Q4	27.46	56/50	0.97 (0.58, 1.64)	0.96 (0.48, 1.91)
LA (18:2)				
Q1	10.90	31/50	1.0	1.0
Q2	31.88	46/50	1.52 (0.84, 2.77)	1.57 (0.82, 3.01)
Q3	76.10	74/50	2.45 (1.37, 4.40)	**2.49 (1.31, 4.70)**
Q4	180.98	49/50	1.62 (0.89, 2.98)	1.34 (0.70, 2.57)
SA (18:4)				
Q1	0.067	40/50	1.0	1.0
Q2	0.077	61/50	1.54 (0.87, 2.71)	1.66 (0.91, 3.03)
Q3	0.086	50/50	1.26 (0.70, 2.25)	1.42 (0.77, 2.64)
Q4	0.099	49/50	1.24 (0.69, 2.21)	1.16 (0.62, 2.15)
Total PUFA				
Q1	216.41	42/50	1.0	1.0
Q2	280.83	46/50	1.09 (0.62, 1.93)	0.92 (0.50, 1.71)
Q3	372.89	59/50	1.41 (0.81, 2.47)	1.29 (0.71, 2.35)
Q4	544.48	43/50	1.25 (0.72, 2.19)	1.09 (0.60, 1.97)
Total n6				
Q1	202.20	41/50	1.0	1.0
Q2	264.97	49/50	1.19 (0.67, 2.11)	1.04 (0.57, 1.91)
Q3	357.38	56/50	1.37 (0.78, 2.41)	1.23 (0.67, 2.26)
Q4	518.87	54/50	1.31 (0.75, 2.28)	1.13 (0.63, 2.05)
Total n3				
Q1	48.27	53/50	1.0	1.0

Q2	66.40	38/50	0.72 (0.41, 1.28)	0.70 (0.38, 1.28)
Q3	86.19	62/50	1.20 (0.69, 2.09)	1.23 (0.67, 2.24)
Q4	123.36	47/50	0.89 (0.51, 1.55)	0.75 (0.41, 1.35)

[1]Adjusted for: sex, child's age, mother's education, mother's age, BMI, race, insurance status at delivery, and short inter-pregnancy interval.

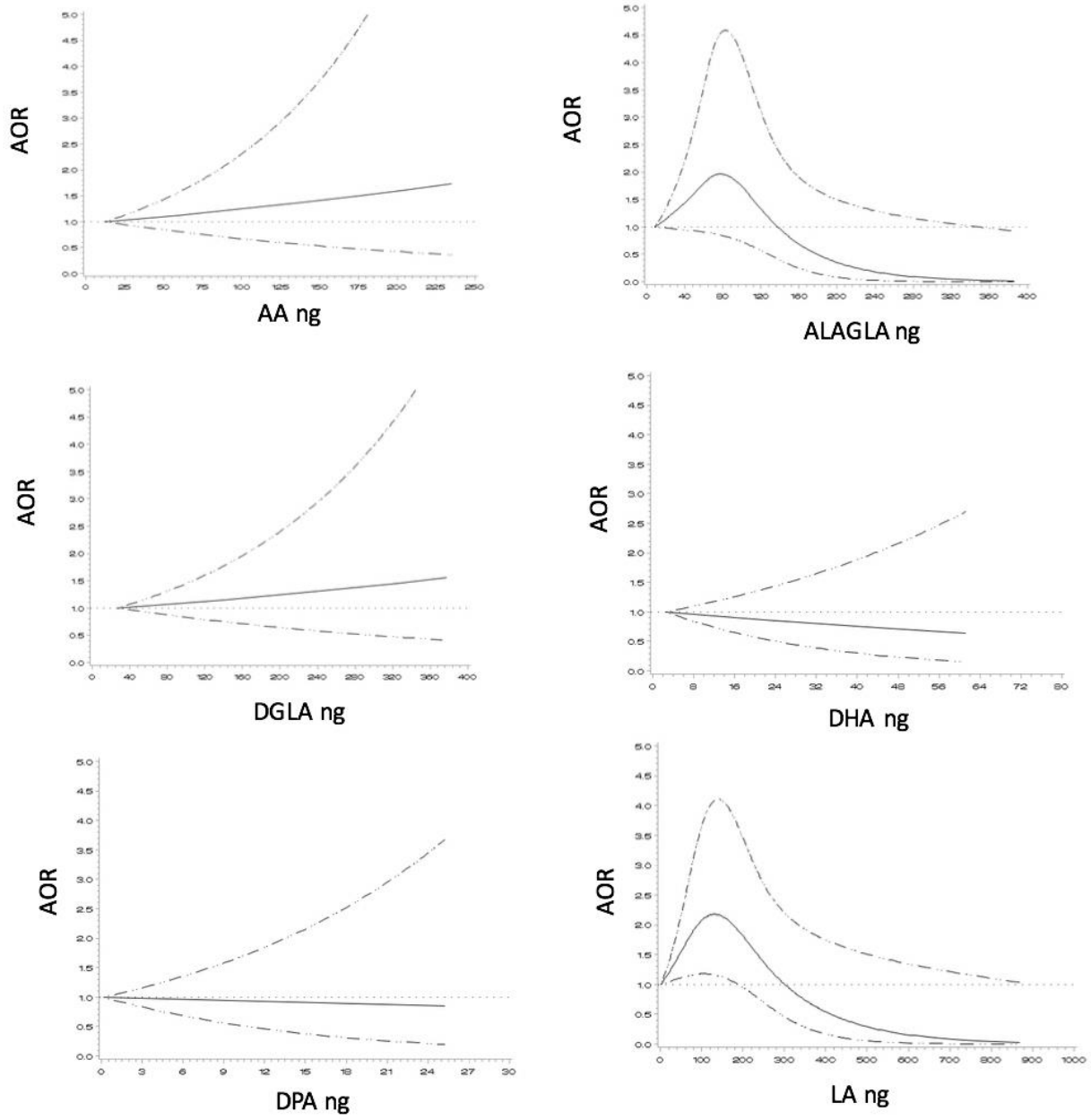
Table 4: Association (odds ratios and 95% confidence intervals) between distributional extremes of neonatal PUFA levels measured in newborn bloodspots and offspring autism spectrum disorder (ASD)

PUFA	Median value (ng) of the category	Case/Control n	Crude OR (95% CI)	Adjusted OR (95% CI)
AA				
Lowest 5th percentile	34.74	16/10	2.04 (0.87, 4.77)	1.98 (0.81, 4.86)
6th-<25th percentile	51.39	43/40	1.38 (0.82, 2.33)	1.56 (0.89, 2.73)
IQR (referent)	70.30	77/100	1.0	1.0
>75th percentile-<95th percentile	99.46	44/40	1.42 (0.85, 2.38)	1.54 (0.89, 2.66)
Top 5th percentile	136.69	20/10	2.62 (1.15, 5.97)	**2.89 (1.20, 6.95)**
ALA-GLA				
Lowest 5th percentile	19.52	5/10	0.48 (0.16, 1.44)	0.49 (0.16, 1.55)
6th-<25th percentile	33.46	39/40	0.88 (0.52, 1.49)	0.91 (0.52, 1.59)
IQR (referent)	57.67	107/100	1.0	1.0
>75th percentile-<95th percentile	97.98	46/40	1.09 (0.66, 1.80)	0.91 (0.53, 1.57)
Top 5th percentile	166.04	3/10	0.29 (0.08, 1.07)	0.28 (0.07, 1.12)
DGLA				
Lowest 5th percentile	54.29	24/10	2.71 (1.22, 6.01)	**2.56 (1.11, 5.90)**
6th-<25th percentile	73.31	32/40	0.93 (0.53, 1.64)	0.84 (0.46, 1.52)
IQR (referent)	103.20	87/100	1.0	1.0
>75th percentile-<95th percentile	154.86	41/40	1.18 (0.68, 2.04)	1.21 (0.68, 2.15)
Top 5th percentile	259.71	16/10	1.80 (0.78, 4.15)	1.97 (0.82, 4.70)
DHA				
Lowest 5th percentile	5.84	9/10	1.06 (0.41, 2.71)	1.11 (0.41, 3.01)
6th-<25th percentile	8.92	49/40	1.41 (0.86, 2.33)	1.49 (0.84, 2.63)
IQR (referent)	15.87	86/100	1.0	1.0
>75th percentile-<95th percentile	26.34	49/100	1.41 (0.85, 2.32)	1.59 (0.90, 2.80)
Top 5th percentile	47.49	7/10	0.82 (0.30, 2.28)	0.68 (0.22, 2.16)
DPA				
Lowest 5th percentile	1.55	15/10	1.63 (0.69, 3.87)	1.55 (0.63, 3.85)
6th-<25th percentile	3.67	43/40	1.15 (0.69, 1.91)	0.93 (0.54, 1.61)
IQR (referent)	6.51	93/100	1.0	1.0
>75th percentile-<95th percentile	10.24	37/40	0.99 (0.58, 1.69)	0.97 (0.55, 1.71)
Top 5th percentile	14.67	12/10	1.26 (0.52, 3.04)	1.12 (0.44, 2.84)
LA				
Lowest 5th percentile	3.03	3/10	0.24 (0.06, 0.89)	**0.23 (0.06, 0.91)**
6th-<25th percentile	12.49	28/40	0.57 (0.33, 0.99)	0.56 (0.30, 1.02)
IQR (referent)	52.34	120/100	1.0	1.0
>75th percentile-<95th percentile	166.10	43/40	0.90 (0.54, 1.49)	0.74 (0.43, 1.29)
Top 5th percentile	340.57	6/10	0.49 (0.17, 1.39)	0.38 (0.13, 1.13)
SA				
Lowest 5th percentile	0.060	9/10	0.81 (0.32, 2.08)	0.78 (0.29, 2.11)
6th-<25th percentile	0.069	31/40	0.69 (0.40, 1.20)	0.63 (0.35, 1.11)
IQR (referent)	0.080	111/100	1.0	1.0
>75th percentile-<95th percentile	0.096	31/40	0.71 (0.42, 1.21)	0.63 (0.35, 1.11)
Top 5th percentile	0.118	18/10	1.64 (0.72, 3.74)	1.23 (0.51, 2.97)
Total n-3 PUFA				
Lowest 5th percentile	31.49	4/10	0.34 (0.10, 1.16)	0.30 (0.08, 1.15)
6th-<25th percentile	50.40	49/40	1.23 (0.74, 2.05)	1.25 (0.72, 2.15)
IQR (referent)	77.92	100/100	1.0	1.0
>75th percentile-<95th percentile	120.67	45/40	1.14 (0.68, 1.90)	0.96 (0.55, 1.65)

Top 5th percentile	182.26	2/10	0.18 (0.04, 0.87)	**0.17 (0.03, 0.82)**
Total n-6 PUFA				
Lowest 5th percentile	161.76	16/10	1.54 (0.67, 3.54)	1.83 (0.75, 4.46)
6th-<25th percentile	208.81	25/40	0.62 (0.35, 1.09)	0.69 (0.37, 1.26)
IQR (referent)	302.43	105/100	1.0	1.0
>75th percentile-<95th percentile	514.70	51/40	1.21 (0.73, 2.01)	1.23 (0.73, 2.08)
Top 5th percentile	890.33	3/10	0.30 (0.08, 1.11)	**0.24 (0.06, 0.93)**
Total PUFA				
Lowest 5th percentile	178.71	20/10	1.93 (0.86, 4.32)	2.03 (0.88, 4.70)
6th-<25th percentile	225.65	22/40	0.54 (0.30, 0.97)	0.63 (0.34, 1.19)
IQR (referent)	322.19	105/100	1.0	1.0
>75th percentile-<95th percentile	532.42	50/40	1.19 (0.72, 1.97)	1.22 (0.72, 2.06)
Top 5th percentile	903.96	3/10	0.31 (0.08, 1.14)	**0.24 (0.06, 0.96)**

[1]Adjusted for: sex, child's age, mother's education, mother's age, BMI, race, insurance status at delivery, and short inter-pregnancy interval.

Figure 2: Cubic spline analyses of neonatal PUFA levels in association with offspring ASD



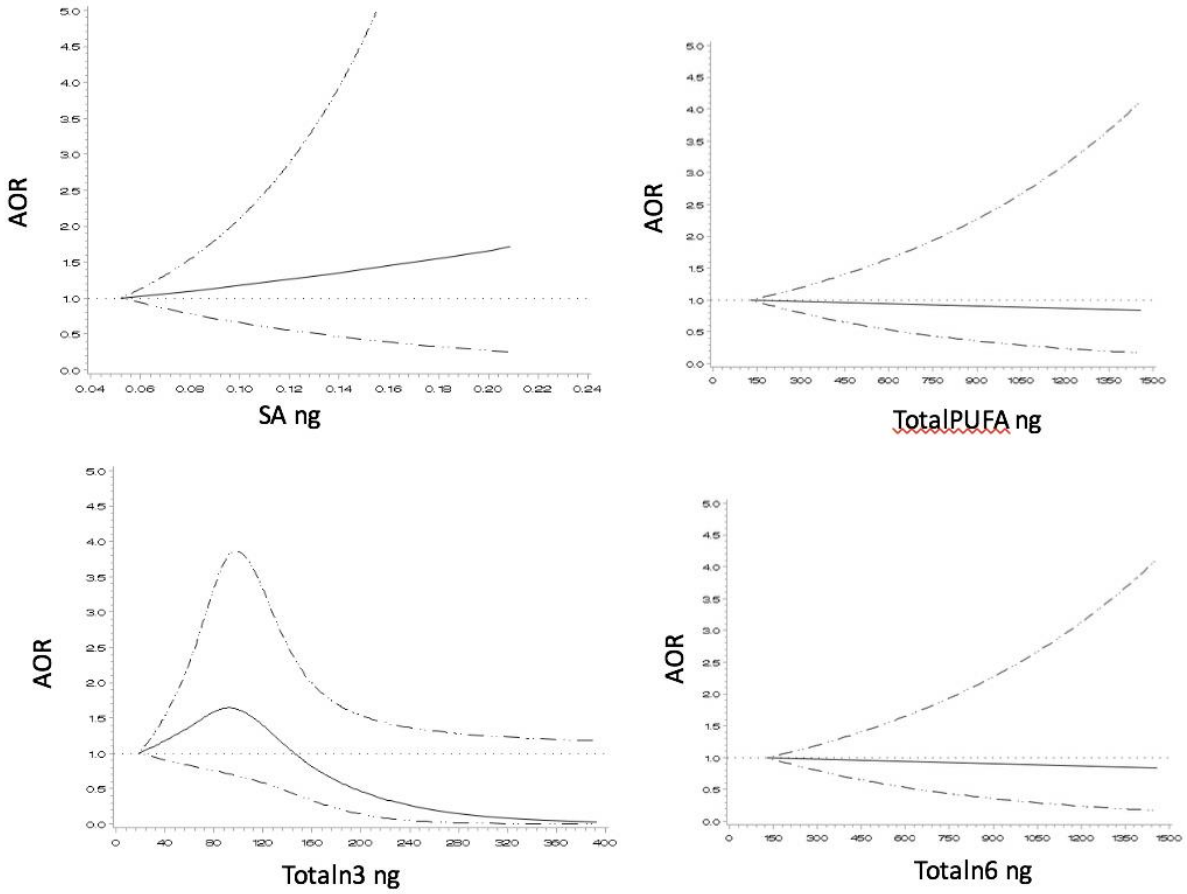


Figure legend: Spline models adjusted as in Tables 3 and 4.