AWARD NUMBER: W81XWH-16-1-0657

TITLE: Omega-3 Polyunsaturated Fatty Acid Status, Microglial Activation, Stress Resilience, and Cognitive Performance

PRINCIPAL INVESTIGATOR: Rajesh Narendran, MD

RECIPIENT: University of Pittsburgh
Pittsburgh, PA 15213

REPORT DATE: October 2017

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
October 2017	Annual	30 Sep 2016 - 29 Sep 2017

4. TITLE AND SUBTITLE
Omega-3 Polyunsaturated Fatty Acid Status, Microglial Activation, Stress Resilience, and Cognitive Performance

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-16-1-0657

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Rajesh Narendran, MD

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
.
University of Pittsburgh
Pittsburgh, PA 15213

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT
Background: Recent studies in healthy civilian and military populations indicate a strong relationship between red blood cell (RBC) n-3 polyunsaturated fatty acids (PUFA) levels and cognitive performance, mood, impulsivity and suicide. However, the precise brain mechanisms that underlie these behavioral impairments in n-3 PUFA deficient individuals are largely unknown. Here we seek to elucidate the underlying immunological mechanisms by which n-3 PUFA exerts its benefits on brain and behavior.

15. SUBJECT TERMS-
None listed

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
U	U	U	UU	12	19b. TELEPHONE NUMBER *(include area code)*

TABLE OF CONTENTS

1. INTRODUCTION:

Background: Recent studies in healthy civilian and military populations indicate a strong relationship between red blood cell (RBC) n-3 polyunsaturated fatty acids (PUFA) levels and cognitive performance, mood, impulsivity and suicide. However, the precise brain mechanisms that underlie these behavioral impairments in n-3 PUFA deficient individuals are largely unknown. Here we seek to elucidate the underlying immunological mechanisms by which n-3 PUFA exerts its benefits on brain and behavior.

Purpose (Aim 1)*: To determine with [^{11}C]PBR28 positron emission tomography (PET) whether there is greater microglial activation in humans with low compared to high RBC docosahexaenoic acid* (*DHA) levels.* [^{11}C]PBR28 PET will be used to measure binding to the 18 KDa translocator protein (TSPO) in young healthy males with constitutive low and high RBC DHA levels (n=42 subjects/group). Low and high RBC DHA levels will be defined as subjects with one standard deviation below and above the mean RBC DHA levels in the sample. The [^{11}C]PBR28 binding in the midbrain, striatal and cortical regions will provide an estimate of the in vivo status of TSPO, which is a marker of activated microglia. Subjects will also complete a comprehensive stress resilience and neurocognitive battery to correlate with [^{11}C]PBR28 binding. We hypothesize that [^{11}C]PBR28 binding will be greater in individuals with low RBC DHA levels compared to high levels (after correction for the TSPO Alanine147Threonine polymorphism, which has been shown to influence [^{11}C]PBR28 binding), and will be linked to impaired stress resilience and cognitive performance.

Scope: Linking RBC DHA status to TSPO levels in the brain would suggest that n-3 PUFAs modulate neuroinflammation. This has the potential to inform the clinical testing of oral and parenteral n-3 PUFA formulations as a treatment for conditions in which inflammation is a focus, such as traumatic brain injury, multiple sclerosis, etc.

2. KEYWORDS:

PET imaging; TSPO; omega-3 fatty acids,

3. ACCOMPLISHMENTS:

What were the major goals of the project?

Major goals of the project (aim 1, human study):

The major tasks listed in the approved SOW (6/2016) with listed milestones and target dates within the first 12 months are included below

1. Prepare regulatory documents and research protocol for the Human study

(a) Milestone/Target date: Local RDRC and IRB approval at University of Pittsburgh/ 4 months

The initial and annual renewal for RDRC and IRB approval for this protocol was completed by the target date of **4 months.** The date of initial approval by RDRC and IRB was 5/31/2016. The annual renewal for IRB approval for this protocol was completed on 3/29/2017

(b) Milestone and Target date: HRPO approval in place/6 months

The HRPO approval was in place by the target date of **6 months.** The date of initial approval by HRPO was complete on 7/13/2016. The annual renewal by HRPO was completed on 6/28/2017

2. Coordinate staff for human study

(a) Milestone/Target date: Research staff trained/6 months

Research staff were trained in the screening procedures by the target date of **6-months**. This was necessary to initiate the screening procedures. The training for experimental procedures is currently underway and will be initiated soon.

3. Participant Recruitment

(a) Milestone/Target date: 1st participant consented, screened and enrolled for TSPO genotype and RBC DHA analysis/6 months

The first participant was consented, screened and enrolled for TSPO genotype and RBC DHA analysis on 10/14/2016. This was within the first **6-month**s of initiation of the award.

To date we have successfully consented a total of 115 subjects, out of which RBC DHA and genotype analysis have been completed in 95 subjects (cohort 1). Based on this we have met the n=100 subjects projected to be enrolled by the end of Y1 (i.e., first four quarterly enrollments for screening)

(b) Milestone/Target date: First 28 subjects selected for imaging based on DHA and TSPO status/13 months

TSPO genotype and RBC DHA status were analyzed in= 95 subjects (cohort 1) as mentioned in the previous milestone. Based on these data, we have selected subjects to be included in the experimental phase. This milestone was met ahead of schedule at **11 months.**

4. Data analysis

No specific milestones were listed to be met within the first 12 months

What was accomplished under these goals?

The major activity during the first year was consenting and screening n=115 healthy males. Of these healthy males TSPO genotype and RBC DHA results are now available for n=95 subjects. These data are necessary to identify the first cohort (projected n=14 low DHA and 14 high DHA) to undergo the experimental procedures. The experimental procedures will involve [^{11}C]PBR28 PET imaging, cognitive and stress-resilience measurements in the subjects with low and high DHA levels.

Screening procedures that were performed in the n=95 subjects included:

(a) *Clinical questionnaires:* Demographic information and clinical assessments were collected as outlined in the PhenXToolkit for Mental Health Research (Core: Tier 1 and Tier 2). These clinical assessments include screening questions for broad psychopathology, substance abuse, medical conditions, perceived stress and life events.

(b) *Genotype analysis:* The proposed genotype analysis was performed by the lab of Dr. Vishwajit Nimgaonkar, Director, Program for Human Genetics and Psychoses, Department of Psychiatry, University of Pittsburgh, Pittsburgh, PA (a co-investigator on the proposal). The TSPO rs6971- genotype has been shown to predict PBR28 binding affinity in human platelets and brain V_T in a trimodal distribution (high C/C, low T/T and mixed affinity C/T binders) (Owen et al., 2010; Owen et al., 2012). Thus, it is necessary to correct the measured [^{11}C]PBR28

V_T for genotype to allow for the detection of differences in TSPO availability between low and high DHA groups. Genomic DNA isolation and analysis were conducted as reported previously in (Narendran et al., 2014). The rs6971 polymorphism has a reported minor allele (Thr147) and major allele (Ala147) frequency of 30% and 70% in Caucasians (the minor allele frequency is 25% African Americans) (Owen et al., 2010; Owen et al., 2012). This should roughly translate to ~ 50 % high affinity binders, 40% mixed affinity binders and 10% low affinity binders.

Consistent with this estimate our data in n=95 men, demonstrated 52, 34 & 9 high, mixed & low affinity binders respectively (54%, 35% and 10%). The low binders will not be included in the experimental procedures because it is not possible to reliably quantify [^{11}C]PBR28 binding (V_T) in these subjects (Fujita et al., 2008).

(c) RBC fatty acid composition analysis: The proposed RBC fatty acid composition analysis were conducted in the lab of CAPT. Joseph R Hibbeln, MD., USPHS, Acting Chief, Section on Nutritional Neurosciences, National Institute on Alcoholism and Alcohol Abuse (NIAAA). These samples were processed for the separation of RBC membranes using previously described methods and stored at -80 degree Celsius (Reddy et al., 2004). The frozen RBC samples were batch processed for fatty acid composition using gas chromatography (Sekikawa et al., 2008). Individual PUFA levels will be expressed as percentages of the total fatty acid pool (weight or mol %).

In this dataset of n=95 men, the mean age of subjects was (age 22.3 ± 1.9 years). The mean RBC DHA level of 2.91 ± 0.75 % (range 1.8 to 5.6%) mol of total fatty acid pool. Based on this, individuals with RBC DHA values one standard deviation below and above the mean can be stratified as low (≤ 2.2%) and high (≥ 3.6%) DHA groups. After excluding low affinity binders for [^{11}C]PBR28, the stratification of RBC DHA data allows for the selection of n=12 subjects /group (low DHA and high DHA) to move on to the experimental (imaging) procedures. The total number of subjects arrived at using this method (n=24, 12 low DHA and 12 high DHA individuals) is consistent with 32% of individuals falling outside of one standard deviation of the mean in a normally distributed curve (95- 9 low binders =86 subjects; 86*32% =25 subjects). However, this number is below the n=14 per group we had proposed in the grant to scan with [^{11}C]PBR28 and PET in each cohort in the experimental phase. In addition, it is likely that not all n=12 subjects/group will consent to the imaging, cognitive and resilience studies, and some may not tolerate the procedures (such as A-line failures etc.). Preliminary calls to these n=12 subjects/group suggest 50% of the sample is either unavailable or uninterested due to changes in their life situation since screening in 9-12 months ago (such as moved out of the area, have a job that prevents them to return for imaging etc.,). It is now clear to us that the criteria proposed in the grant for low and high DHA (i.e., one

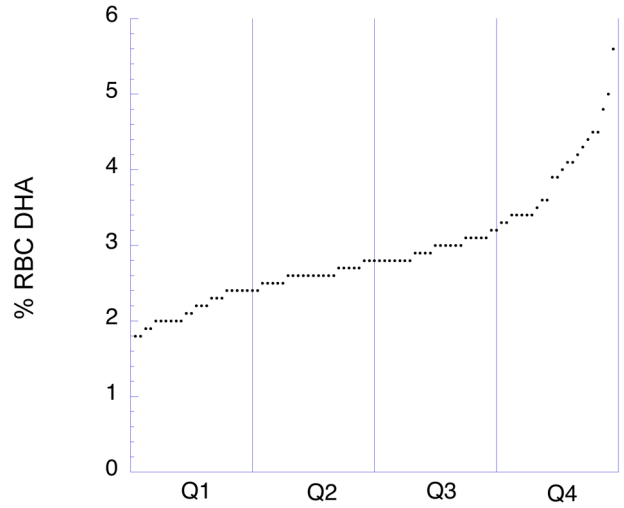

Figure 1 shows the cumulative distribution of %RBC DHA in n=95 subjects; the vertical blue lines parallel to the Y-axis denote the separation of the group by quartiles (Q1-4)

standard deviation above and below the mean) is too restrictive as it did not account for relatively high subject attrition. Thus, we suggest dividing the cohorts into quartiles and offering the imaging to individuals in the lowest and highest quartile groups. Based on this approach, individuals with RBC DHA values in the lowest and highest quartile have a DHA level of ≤ 2.4% and ≥ 3.2% respectively in cohort 1 (see Figure 1 in which the cumulative distribution of % RBC DHA in n=95 individuals is shown; vertical blue lines parallel to the Y-axis show the separation by quartiles, Q1-4). After excluding low affinity binders for [^{11}C]PBR28, the use of quartiles allows for us to select from a larger pool of eligible subjects, i.e., **n=24 high DHA and 22 low DHA individuals in cohort 1**. This should allow for us to successfully scan the proposed target of n=14 low and 14 high DHA individuals. We still intend to offer the imaging to individual subjects with the highest and lowest DHA values (as far as they are within the highest and lowest quartile) to maximize the separation amongst groups. The use of the quartile approach should allow for us to meet the specific objectives of this award as outlined.

What opportunities for training and professional development has the project provided?
Nothing to report

How were the results disseminated to communities of interest?
Nothing to report

What do you plan to do during the next reporting period to accomplish the goals?
1. To initiate the imaging procedures in subjects with low and high DHA
2. Complete imaging procedures in cohort 1 subject who meet inclusion/exclusion criteria
3. Continue consenting and screening procedures in cohort 2

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?
Nothing to report

What was the impact on other disciplines?
Nothing to report

What was the impact on technology transfer?
Nothing to report

What was the impact on society beyond science and technology?
Nothing to report

5. **CHANGES/PROBLEMS:**
Changes in approach and reasons for change
(1) Based on the analysis of cohort 1 data, it is clear that the previously proposed criteria to characterize low and high DHA groups (i.e., below and above one standard deviation of the cohort's mean DHA levels) is too restrictive (see section 3). Thus, we propose to separate the

group into quartiles and offer the imaging component to individuals in the lowest and highest quartiles. Based on this approach, individuals with RBC DHA values in the lowest and highest quartile will have a DHA level of ≤ 2.4% and ≥ 3.2% respectively (see Figure 1 data that shows a good spread of % RBC DHA data by quartiles). This is not meaningfully different from the DHA level of ≤ 2.2% and ≥ 3.6% for low and high DHA groups arrived by using the 1-standard deviation criteria originally proposed in the award. In addition, we first intend to offer the imaging to individuals with the highest and lowest DHA values (as far as they are in the highest and lowest quartile) to maximize the separation between groups.

(2) The other change we intend to make for cohort 2 is to analyze blood samples for TSPO genotype and %RBC DHA after screening 30-45 subjects as opposed to waiting to analyze them until we have enrolled all 90 subjects. This should allow for us to pool the newly acquired data with existing cohort 1 data to refine the quartiles, and offer the imaging to cohort 2 subjects sooner compared to cohort 1. The reduction in lag time between the screening and imaging components should improve subject retention in the study.

Actual or anticipated problems or delays and actions or plans to resolve them
Nothing to report

Changes that had a significant impact on expenditures
Nothing to report

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

Significant changes in use or care of human subjects
Nothing to report

Significant changes in use or care of vertebrate animals.
Not applicable

Significant changes in use of biohazards and/or select agents
Nothing to report

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**
  Nothing to report

  Journal publications.
  Nothing to report

  Books or other non-periodical, one-time publications.
  Nothing to report

Other publications, conference papers, and presentations.
Nothing to report

- **Website(s) or other Internet site(s)**
  Nothing to report
- **Technologies or techniques**
  Nothing to report
- **Inventions, patent applications, and/or licenses**
  Nothing to report
- **Other Products**
  Nothing to report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name:	*Rajesh Narendran, MD*
Project Role:	*PI*
Nearest person month worked:	*1*
Contribution to Project:	*Dr. Narendran has overseen the recruitment and subject selection*

Name:	*Brian Lopresti, MSNE*
Project Role	*Co-I*
Nearest person month worked	*1*
Contribution to the Project:	*Mr. Lopresti assisted the PI with recruitment and subject selection and assembling the imaging protocol*

Name:	*Matthew Muldoon, MD*
Project Role	*Co-I*
Nearest person month worked:	*1*
Contribution to the Project:	*Dr. Muldoon assisted the PI in subject selection and defining criteria for high and low DHA groups*

Name:	*Vishwajit Nimgaonkar, MD*
Project Role:	*Co-I*
Nearest person month worked	*1*
Contribution to the Project:	*Dr. Nimgaokar oversaw the TSPO Genetics analyses of subject blood samples*

Name:	*Kodavali Chowdari, PhD*
Project Role:	*Scientist*
Nearest person month worked	*1*
Contribution to the Project:	*Dr. Chowdari analyzed the blood samples for TSPO genotype, did the quality control and final reporting*
Name:	*Antonio Paris, BA*
Project Role	*Research coordinator*
Nearest person month worked	*5*
Contribution to the Project:	*Screened and enrolled subjects for cohort 1*
Name:	*Joshua Gertler, BS*
Project Role	*Research coordinator/data analyst*
Nearest person month worked	*1*
Contribution to the Project	*Worked to get MRI, cognitive testing and rating scales administration protocols together for experimental procedures*
Name:	*Savannah Tollefson, BS*
Project Role	*Research coordinator/data analyst/compliance coordinator*
Nearest person month worked	*1*
Contribution to Project	*Worked to standardize blood collection protocols for RBC DHA, genetics analyses and assisted the PI in overseeing compliance for the study*
Name:	*Rehima Jordan, BS*
Project Role:	*Research coordinator*
Nearest person month worked 1	*1*
Contribution to Project	*Being trained to conduct screening for cohort 2 subjects and assist with experimental procedures*
Name:	*Katherine Roach, BS*
Project Role:	*Research coordinator*
Nearest person month worked	*2*
Contribution to Project	*Screened and enrolled subjects for cohort 1; ensured data safety monitoring and medical monitor visits were documented*
Funding:	*National Institute of Health (NIH)*
Name:	*Michael Himes, BA*
Project Role:	*Image analyst/Data base administrator/lab supervisor*
Nearest person month worked	*1*
Contribution to Project	*Oversaw the work of lab personnel, assisted the PI in writing standard operating procedures for the*

	research protocol. Assisted the PI in formulating a PET data analysis pipeline to analyze experimental data that will be acquired as part of the study going forward
Funding:	*National Institute of Health (NIH)*
Name:	*Konasale Prasad, MD*
Project Role:	*Medical monitor*
Nearest person month worked:	*1*
Contribution to the Project	*Monitoring of conduct of the study and data acquisition*

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?
Nothing to report

What other organizations were involved as partners?
Organization Name: National Institute on Alcoholism and Alcohol abuse
Location of Organization: (if foreign location list country) USA
Partner's contribution to the project: CAPT. Joseph R Hibbeln, MD., USPHS, Acting Chief, Section on Nutritional Neurosciences, NIAAA
Supported the analysis of RBC DHA samples in his lab at NIAAA

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.
Partnering PI report will be filed separately for Aim 2 (animal study) by Dr. Bita Moghaddam, Chair of Neuroscience, OHSU, Portland, Oregon

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.
Nothing to report

REFERENCES

Costa B, Pini S, Gabelloni P, Da Pozzo E, Abelli M, Lari L, Preve M, Lucacchini A, Cassano GB, Martini C. 2009. The spontaneous Ala147Thr amino acid substitution within the translocator protein influences pregnenolone production in lymphomonocytes of healthy individuals. Endocrinology 150(12):5438-5445.

Fujita M, Imaizumi M, Zoghbi SS, Fujimura Y, Farris AG, Suhara T, Hong J, Pike VW, Innis RB. 2008. Kinetic analysis in healthy humans of a novel positron emission tomography radioligand to image the peripheral benzodiazepine receptor, a potential biomarker for inflammation. NeuroImage 40(1):43-52.

Narendran R, Lopresti BJ, Mason NS, Deuitch L, Paris J, Himes ML, Kodavali CV, Nimgaonkar VL. 2014. Cocaine abuse in humans is not associated with increased microglial activation: an 18-kDa translocator protein positron emission tomography imaging study with [11C]PBR28. J Neurosci 34(30):9945-9950.

Owen DR, Howell OW, Tang SP, Wells LA, Bennacef I, Bergstrom M, Gunn RN, Rabiner EA, Wilkins MR, Reynolds R, Matthews PM, Parker CA. 2010. Two binding sites for [3H]PBR28 in human brain: implications for TSPO PET imaging of neuroinflammation. Journal of cerebral blood flow and metabolism : official journal of the International Society of Cerebral Blood Flow and Metabolism 30(9):1608-1618.

Owen DR, Yeo AJ, Gunn RN, Song K, Wadsworth G, Lewis A, Rhodes C, Pulford DJ, Bennacef I, Parker CA, StJean PL, Cardon LR, Mooser VE, Matthews PM, Rabiner EA, Rubio JP. 2012. An 18-kDa Translocator Protein (TSPO) polymorphism explains differences in binding affinity of the PET radioligand PBR28. Journal of cerebral blood flow and metabolism : official journal of the International Society of Cerebral Blood Flow and Metabolism 32(1):1-5.

Reddy RD, Keshavan MS, Yao JK. 2004. Reduced red blood cell membrane essential polyunsaturated fatty acids in first episode schizophrenia at neuroleptic-naive baseline. Schizophrenia bulletin 30(4):901-911.

Sekikawa A, Curb JD, Ueshima H, El-Saed A, Kadowaki T, Abbott RD, Evans RW, Rodriguez BL, Okamura T, Sutton-Tyrrell K, Nakamura Y, Masaki K, Edmundowicz D, Kashiwagi A, Willcox BJ, Takamiya T, Mitsunami K, Seto TB, Murata K, White RL, Kuller LH. 2008. Marine-derived n-3 fatty acids and atherosclerosis in Japanese, Japanese-American, and white men: a cross-sectional study. J Am Coll Cardiol 52(6):417-424.