Interaction of erythrocyte eicosapentaenoic acid and physical activity predicts reduced risk of mild cognitive impairment.

Steven J. Street, Natalie Parletta, Catherine M. Milte, Karen A. Sullivan, Andrew P. Hills, Jonathan D. Buckley, and Peter R. C. Howe.

Author Note

Steven J. Street, and Karen A. Sullivan, School of Psychology and Counselling, and Institute of Health and Biomedical Innovation, Queensland University of Technology; Andrew P Hills, Institute of Health and Biomedical Innovation, Queensland University of Technology; Natalie Parletta, Catherine M. Milte, Jonathan D Buckley, and Peter Howe, School of Health Sciences, Nutritional Physiology Research Centre, University of South Australia;

Steven J Street is now at the Mater Medical Research Institute, Centre for Nutrition and Exercise; Andrew P Hills is now at Mater Medical Research Institute, Centre for Nutrition and Exercise, and Griffith Health Institute, Griffith University; Peter R C Howe is now at the Clinical Nutrition Research Centre, University of Newcastle.

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38 Interaction of erythrocyte eicosapentaenoic acid and physical activity predicts reduced
39 risk of mild cognitive impairment.

40 Objectives: To evaluate relationships between self-reported physical activity,
41 proportions of long chain omega-3 polyunsaturated fatty acids (LCn3) in erythrocyte
42 content (% total fatty acids) and risk of Mild Cognitive Impairment (MCI) in older
43 adults.

44 Method: Cross-sectional study. Community dwelling male and female ($n = 84$)
45 participants over the age of 65 years with and without MCI were tested for
46 erythrocyte proportions of the LCn3s eicosapentaenoic acid (EPA) and
47 docosahexaenoic acid (DHA). Physical activity was measured using a validated
48 questionnaire.

49 Results: The interaction between erythrocyte EPA, but not DHA, and increased
50 physical activity was associated with increased odds of a non-MCI classification.

51 Conclusion: An interaction between physical activity and erythrocyte EPA content (%
52 of fatty acids) significantly predicted MCI status in older adults. Randomised control
53 trials are needed to examine the potential for supplementation with EPA in
54 combination with increased physical activity to mitigate the risk of MCI in aging
55 adults.

56 *Keywords:* aging, mild cognitive impairment, EPA, DHA, physical activity

57

58 Introduction

59 Mild cognitive impairment (MCI) is thought to be a transitional state between normal
60 and pathological cognitive aging (Petersen, 2004; Petersen et al., 2014). According to
61 the criteria developed by Petersen et al. (2001), MCI is diagnosed in a person who
62 reports a subjective memory complaint, has evidence of an objective memory
63 impairment without evidence of dementia, but has preserved general cognitive
64 function, and intact activities of daily living. Although several variations of MCI have
65 been identified, the most common presentation is amnesic MCI (aMCI) in which
66 diminished memory is the predominant symptom. With an estimated 5-10% of MCI
67 cases converting to dementia per year (Mitchell & Shiri-Feshki, 2009), finding ways
68 to reduce MCI may also help to reduce the rate of dementia onset.

69 The aetiology of MCI includes genetic predispositions and modifiable
70 environmental and lifestyle factors (Sachdev et al., 2012). Despite the immutability of
71 genetics, a growing body of research links adaptive and healthy lifestyle choices with
72 reductions in the risk of MCI. In particular, increased levels of physical activity
73 (Gonzalez-Palau et al., 2014; Tanigawa et al., 2014) and consumption of the
74 Mediterranean diet (Scarmeas et al., 2009) are inversely related to MCI risk. Studies
75 examining the combined effect of both increased physical activity and diets such as
76 the Mediterranean diet (Scarmeas et al., 2009), and high and low saturated fat diets
77 (Baker et al., 2012) have also shown an inverse relationship with dementia. Taken
78 together, these results suggest that consumption of certain nutrients independently or
79 in combination with a physically active lifestyle, may safeguard against the onset of
80 MCI.

81 A staple of the Mediterranean diet is fish that is high in long chain omega-3
82 polyunsaturated fatty acids (LCn3). Epidemiological research suggests a link between

cognitive function and LCn3, particularly the marine-based eicosapentaenoic (EPA) and docosahexaenoic (DHA). However, a negative relationship observed in epidemiological research between EPA and DHA intake and global cognitive decline (Gao, Niti, Feng, Yap, & Ng, 2011) has not been replicated in randomised control trials (Sydenham, Dangour, & Lim, 2012). One reason for these mixed findings may be that interactions between lifestyle factors have not been taken into account.

Examining the inter-relationship between LCn3 and other environmental factors such as physical activity may uncover important additive or synergistic effects with implications for cognition. Studies in rats provide support for an interaction between physical activity and DHA in terms of spatial memory improvements (Wu, Ying, & Gomez-Pinilla, 2008), leading to calls for closer examination of the link between diet and exercise for cognitive health in humans (Gomez-Pinilla, 2011). To our knowledge human research on LCn3 and physical interactions is yet to be conducted. The aim of the current study was to examine whether levels of DHA/EPA and physical activity interact to predict MCI in a sample of older adults. We report a significant but small increase in the odds of a non-MCI classification predicted by an interaction between EPA and an estimate of physical activity induced energy expenditure. However, in light of recent animal based research demonstrating significant interactions between LCn3 and physical activity, the findings reported here are of interest and warrant additional research. The current study draws on data from a larger research effort, including a randomised control trial, to examine relationships between mood, cognition and LCn3 in MCI (Milte et al., 2011; Natalie Sinn et al., 2011).

106 **Method**

107 **Participants**

108 Human Research Ethics Committees at the Queensland University of
109 Technology and University of South Australia approved this study. All participants
110 provided written informed consent prior to recruitment. Community dwelling adults
111 aged 65 years or older ($M = 72.48$ years, $SD = 6.12$) were recruited through
112 newspaper and shopping centre advertisements and interviews and radio and
113 television appeals. The screening procedures for MCI have been reported elsewhere
114 (Milte et al., 2011; Natalie Sinn et al., 2011). Briefly, MCI was identified according to
115 criteria developed by Petersen et al. (2001), viz. 1) the presence of a subjective
116 memory complaint assessed on the basis of a pre-screening telephone interview; 2)
117 normal activities of daily living (ADLs) inferred from independent living status; 3)
118 absence of dementia; 4) impairment in one or more cognitive domains. The MCI
119 group contained 50 participants (males = 34). A non-impaired comparison group ($n =$
120 34; males = 14) was also recruited.

121 **Control Measures**

122 Social networks are related to cognitive decline and dementia (Crooks, Lubben,
123 Petitti, Little, & Chiu, 2008) and may explain the cognitive enhancing effects of
124 physical activity when undertaken with others (Podewils et al., 2005). The Lubben
125 Social Network Scale Revised (LSNS-R) (Lubben et al., 2006) was included to
126 control for this potential confound. Depression is also associated with diminished
127 cognition; an association that may be related to physical activity (Hendrie et al., 2006)
128 and LCn3 levels (Butters et al., 2008; Sinn, Milte, & Howe, 2010). As EPA and DHA
129 proportions in erythrocytes were associated with Geriatric Depression Scale (GDS)

short form scores (Sheikh & Yesavage, 1986) in the current sample (Milte et al., 2011; Natalie Sinn et al., 2011), GDS scores were included in the current analysis.

Blood chemistry

A pre-prandial venous blood sample was drawn to measure relative proportions of the LCn3 eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) and the LCn6 arachidonic acid (AA) in erythrocytes. EPA, DHA and AA erythrocyte content (% of total fatty acids) was quantified using gas chromatography as previously described (Milte et al., 2011).

Physical Activity

Frequency and duration of several modes of physical activity over the preceding fortnight was determined with the Longitudinal Aging Study of Amsterdam Physical Activity Questionnaire (LAPAQ) (Stel et al., 2004). An index of daily physical activity (PAI) was calculated using the following formula where F is the frequency of reported activity i , T is reported time spent engaged in activity i , METS (Ainsworth et al., 2000) are metabolic equivalents, and 14 is the number of days for which physical activity behaviours are reported on:

$$PAI = \frac{\sum(F_i * T_i * METS)}{14}$$

This equation, adapted from Cairney, Faulkner, Veldhuizen, and Wade (2009), provides an estimate of active energy expenditure.

Procedure

On initial contact, participants were screened for LCn3 supplement consumption, recent subjective memory complaints and willingness to conform to project protocols (see Sinn et al., 2011). Following an appointment to determine MCI status, suitable participants were invited to attend a second appointment during which

a pre-prandial blood sample was drawn and processed before breakfast was provided. During breakfast, participants completed two questionnaire packages including the LSNS-R, LAPAQ, and GDS. Demographic and educational data were also obtained. A second group of unimpaired participants was recruited at the Adelaide site only. No non-impaired participants were recruited at the Brisbane site, given the recruitment of a sufficient sample of non-impaired participants in Adelaide.

Statistical analyses. PASW (SPSS version 18; SPSS, Inc., Chicago, IL, USA) was used for all statistical analyses with an alpha set at .05. Missing values were assessed using binary logistic regressions with missing values regressed on variables without missing values. Linear interpolation was used to impute values for variables with data deemed to be missing at random. Mann-Whitney U tests were conducted to examine differences in participant characteristics between the MCI and non-MCI groups. The primary analysis used binary logistic regressions for EPA and DHA separately using MCI/non-MCI as the outcome and three predictor variables: EPA or DHA; PAI; and an interaction between EPA or DHA, and PAI. The direct effects for both EPA and DHA are reported here for context; however, the reader is referred to Milte et al. (2011) and Sinn et al. (2011) for a complete discussion of the relationship between EPA, DHA and cognition in this sample. MCI status was coded as follows: MCI = 1, non-MCI = 2. Thus, negative β coefficients indicate an increased likelihood of a participant being classified with MCI while positive coefficients indicate an increased likelihood of being classified as non-MCI. Variables included in interaction terms were centred prior to analysis to reduce multicollinearity (Tabachnick & Fidell, 2007).

Results

Participant characteristics

An analysis of missing data indicated that data was missing at random (data not shown). Table 1 provides a summary of the sample characteristics broken down by MCI group, including average erythrocyte AA, EPA and DHA content and the physical activity intensity index (PAI). Participants identified with MCI were significantly older, had worse cognition and reported a lower mood as measured by the GDS. There were no significant differences between MCI and non-MCI participants on social networks, PAI, or erythrocyte proportion of DHA. However, there were significant group differences in EPA and AA erythrocyte content, with non-MCI participants recording higher EPA and lower AA than MCI participants (see also Milte et al., 2011).

INSERT TABLE 1 APPROXIMATELY HERE

Preliminary analysis

In a preliminary binary logistic regression analysis that excluded PAI, EPA and/or DHA, age and AA were the only variables contributing significantly to the prediction of MCI (χ^2 33.633 (4), $p = .001$). Model fit was good (Hosmer and Lemshow, χ^2 8.383 (8) $p > .05$), explaining between 34% (Cox and Snell R^2) and 47% (Nagelkerke R^2) of variance with an overall correct classification of 78.8%. Both age and AA were negatively related to MCI indicating an increased likelihood of participants being classified as MCI with every unit increase in age as measured by years, or AA as measured by erythrocyte content.

Primary analysis: individual and interactive effects of EPA or DHA, and PAI on MCI status

EPA, PAI and EPAxPAI. The first logistic regression included the following variables entered in sequential order using the forced entry method: block 1 EPA, block 2 PAI, block 3 an interaction term using the cross product of EPA and PAI (EPAxPAI), and block 4 age and AA. Compared to the constant only model, a test of the full model was significant (χ^2 39.468 (5), $p = .001$); MCI was reliably predicted by the combination of EPA, PAI, EPAxPAI, age and AA. The five predictors demonstrated acceptable fit (Hosmer and Lemshow, $\chi^2 = 15.467$ (8), $p = .051$), and explained between 38.9% (Cox and Snell R^2) and 53.1% (Naglekerke R^2) of the variance in MCI status. Classification was good with 88% of participants identified with MCI and 76.7% of participants identified as non-MCI correctly classified. The overall correct classification rate was 83.8%.

The regression coefficients, Wald statistics, odds ratios, and 95% confidence intervals for the odds ratios are shown in Table 3. An examination of the Wald statistic for each predictor revealed that age, and the interaction between EPA and PAI were the only variables that made unique contributions to the model. The interaction between EPA and PAI was positively related to MCI status. Thus, for every unit increase in the interaction between EPA and PAI, participants were 1.008 times more likely to be classified as non-non-MCI. However, age was still a stronger predictor of MCI; a one-unit increase in age was associated with a 0.804 times reduced likelihood of a non-MCI classification.

INSERT TABLE 2 APPROXIMATELY HERE.

DHA, PAI, and DHAxPAI. Entry of the variable into the second model was the same as for the first model with the exception that DHA was the LCn3

variable of interest. Compared to the constant only model, a test of the full model was significant (χ^2 29.124 (5), $p = .001$); MCI status was reliably discriminated when DHA, PAI, DHAxPAI, age and AA were included in the model. The five predictors demonstrated good fit (Hosmer and Lemshow, $\chi^2 = 9.003$ (8), $p = .342$) and explained between 30.5% (Cox and Snell R^2) and 41.6% (Naglekerke R^2) of the variance in MCI status. Classification was good with 82% of participants identified with MCI and 73.3% of participants identified as non-MCI correctly classified. The overall classification rate was 78.8%. However, as shown in Table 3, PAI, DHA, and the PAIxDHA interaction did not significantly contribute any unique variance to the predictive validity of the model¹. Additional analyses conducted to examine a DHA+EPAxPAI interaction term failed to demonstrate a significant relationship with MCI status (data not shown).

INSERT TABLE 3 APPROXIMATELY HERE.

Discussion

In the current study we examined whether self-reported physical activity, measured as daily PAI, interacted with either erythrocyte EPA or DHA content to predict MCI status. A significant interaction between PAI and EPA as a predictor of MCI was found. This finding extends other research suggesting an interactive effect between diet and greater intensity physical activity to mitigate cognitive pathology (Baker et al., 2012; Scarmeas et al., 2009). These findings also extend work examining cognition in rats as a function of interactions between DHA and physical

¹ The model was a poor fit for three cases only. All three cases were outliers with studentised residuals greater than 2 and were misclassified as MCI from the non-MCI sample. Examination of the case summaries data also revealed that all data were within acceptable limits on measures of influence (Analogue of Cook's influence statistics, leverage statistics and DF Betas). One of the cases with a studentised residual of greater than 5 was removed and the analysis was run again. No changes to the full model were identified with the case removed. Therefore, findings are reported with all cases retained.

activity (Wu et al., 2008). The current study also adds to a growing view that EPA and DHA may each have unique effects on cognitive function (Chiu, et al., 2012), and that, in the case of EPA, these effects may be enhanced by physical activity. However, it is not clear that the relationship reported here between EPA and PAI in terms of predicting MCI risk is clinically meaningful given the low odds reported. Therefore, these results should be interpreted with caution. Higher-powered randomised control studies are warranted.

These results are also interesting in the context of the relative amounts of LCn3 and LCn6 in erythrocyte measurements for this sample. We found AA levels that were 11.6 times greater than EPA and 2.5 times that of DHA. The antithetical effect of AA reported elsewhere in the literature did not seem to affect the interaction between EPA and PAI in the current study. The nature of the study design makes causal attributions impossible. However, a reasonable speculation is that physical activity enhanced the neuro-protective effects of EPA, or vice versa, over and above the deleterious effects of AA. The AA:EPA:DHA ratio found in the current study is consistent with findings in previous reports (Murphy et al., 2007), is indicative of a typical Western diet, and has been associated with disease and cognitive dysfunction (Simopoulos, 2011). An intervention that simply increases physical activity may be sufficient to potentiate the effect of EPA in terms of reducing MCI risk.

The finding that DHA was unrelated to MCI status is inconsistent with research suggesting DHA supplementation is associated with reduced cognitive pathology risk (Yurko-Mauro et al., 2010). Previous research has demonstrated improvements in entorhinal cortex neuronal function in animal models when the AA:DHA ratio is approximately 1:2 (Arsenault et al., 2009). Higher plasma DHA and a lower LCn6:LCn3 ratio may also mediate cognition in older adults (Feart et al.,

2011). In the current study, AA was still a significant predictor of MCI after variance from DHA was accounted for. One explanation for the absence of a relationship between DHA and MCI may be the content of DHA in erythrocytes. Although DHA levels in the current sample are typical of Western dietary intakes, it is conceivable that the amount was insufficient to minimise MCI risk. For example, Chiu et al. (2008) supplemented older adults with 1.8 g/d of combined EPA (1080 mg) and DHA (720 mg) and demonstrated no improvement cognitive function scores with an increase from 3.7% DHA in plasma to 5.1%, although cognitive improvements were associated with increases in erythrocyte EPA which did not change significantly over 24-weeks. However, the same study did find that daily supplementation of 1.7 g/d of DHA and 0.6 g/d of EPA for 24 weeks had a small effect on cognition in very mild Alzheimer's disease (Chiu et al., 2008). Thus, higher levels of DHA may be required before cognitive benefits are realised.

Participants in the current study reported engaging in any type of physical activity for approximately 15% of waking hours or about 2-hours and 20-minutes. In previous research, Australian adult populations have been shown to devote approximately 90% of waking hours to either sedentary behaviours or light physical activity and only 4% in moderate to vigorous physical activity (Armstrong, Bauman, & Davies, 2000; Healy et al., 2008), findings that are generally consistent with this study. The current study used the method reported in Cairney et al. (2009) to create the PAI, an estimate of active energy expenditure. However, increases in either intensity or amount of activity can affect the energy expenditure estimate of the PAI making it impossible to determine which of volume or intensity most affects MCI risk. The only possible conclusion from the current data is that increasing intensity, volume, or both, of physical activity is associated with decreased MCI risk through an

297 interaction with EPA. Experimental manipulation of the volume and/or intensity of
298 physical activity in combination with EPA or placebo should be the focus of future
299 research.

300 There were several limitations in the current study. First, the use of a physical
301 activity questionnaire, particularly in this sample of participants with MCI, may have
302 yielded inaccurate assessments of actual physical activity levels. Second, analysis was
303 conducted on a very small sample. Third, the classification of MCI made on the basis
304 of scores on one of two different screening tests, the VPA1 or Demtect, and an
305 inference about capacity to independently perform ADLs. Fourth, MCI is a
306 heterogeneous disorder and in this study subtypes were not separated. Fifth, the
307 apolipoprotein epsilon 4 (ApoE4) status of individual participants was also not
308 controlled for. Finally, the study was cross sectional and observational in nature with
309 a small sample size, meaning statements on direction of causation cannot be made.

310 In summary, this study found that a higher PAI in combination with greater
311 erythrocyte content of EPA was significantly associated with a small increase in the
312 odds of a non-MCI classification in a small sample of older adults. These findings
313 were stable after controlling for erythrocyte levels of AA and age. Much of the
314 rationale for this study came from the animal literature and, although the effect was
315 small, this study has demonstrated an interesting trend in humans that warrants
316 additional research. Larger randomised control trials are needed to examine the effect
317 of combining EPA, and different modes and intensities of physical activity for
318 cognitive benefit and protection against MCI.

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454 Table 1

455 *Sample characteristics of participants in both MCI and non-MCI groups*

Measure	MCI			Non-MCI		
<i>N</i>	50			34		
Male/Female	34/16			14/16		
	Mean	SD	Range	Mean	SD	Range
Age (years)	74.08**	5.52	65-87	69	4.601	65-81
MMSE	27.16**	2.65	19-30	28.97	1.47	24-30
DemTect	13.06**	3.1	8-18	16.87	1.98	9-18
VPA	6.41**	2.5	4-16	12.03	2.86	9-19
GDS	3.1**	2.61	0-12	1.25	1.17	0-3
LSNS-R Friends	15.98	7.7	0-29	14.5	9.33	0-27
LSNS-R Family	15.04	6.35	0-25	13.97	8.61	0-25
EPA	.96**	.28	.49-2.0	1.3	.451	.44-2.51
DHA	4.61	.81	3.2-7.36	4.6	.71	2.95-6.58
EPA+DHA	5.57	1.01	3.95-9.36	5.86	1.0	3.96-8.86
AA	11.54**	1.18	9.87-15.02	10.7	1.06	7.8-13.3
PAI	642.5	601.3	27.32-3562.01	624.33	374.6	171.07-1633.61

456 *Note.* MMSE = Mini Mental State Exam; VPA = Verbal Paired Associates 1; GDS =
457 Geriatric Depression Scale; LSNS-R Friends = Friends subscale of the Lubben Social
458 Network Scale; LSNS-R Family = Family subscale of the Lubben Social Network Scale;
459 EPA = eicosapentaenoic acid; DHA = docosahexaenoic acid; AA = Arachidonic acid; PAI =
460 Physical Activity Intensity index. Bolded data are statistically significant between groups
461 according to the Mann-Whitney U test statistic. Four participants did not provide gender
462 information in the non-MCI group. Consequently, the *N* and male/female numbers do not
463 match in the non-MCI section.

464

Table 2

Summary of a sequential logistic regression predicting MCI status from the individual and interactive effect of daily PAI, EPA, after controlling for effect of AA and age (n=84).

Variables	<i>B (SE)</i>	Wald χ^2 - test	Odds Ratio	<i>p</i>	95% CI for OR	
					Lower	Upper
Constant	22.8 (6.75)	11.41	NA	.001	NA	NA
EPA	-1.836 (1.99)	.851	.159	.356	.003	7.88
PAI	-.008 (.004)	3.404	.992	.065	.984	1.0
EPAxPAI	.008 (.004)	4.18	1.008	.041	1.0	1.016
Age	-.218 (.069)	9.94	.804	.002	.703	.921
AA	-.548 (.326)	2.82	.578	.093	.305	1.096

Note. PAI = Physical Activity Intensity, EPA = Eicosapentaenoic acid, PAIxEPA= interaction term for eicosapentaenoic acid and physical activity intensity. NA = not applicable. Positive beta weights predict non-MCI status.

Table 3

Summary of a sequential logistic regression predicting MCI status from the individual and interactive effect of daily PAI, DHA, after controlling for effect of AA and age (n = 84)

Variables	B (SE)	Wald χ^2 - test	Odds Ratio	p	95% CI for OR	
					Lower	Upper
Constant	25.204	15.48	NA	.001	NA	NA
DHA	-.911 (.711)	1.645	.955	.200	.100	1.62
PAI	-.005 (.004)	1.587	.995	.208	.987	1.003
DHAxPAI	.001 (.001)	1.76	1.001	.185	.999	.1.003
Age	-.183 (.061)	8.975	.833	.003	.739	.939
AA	.791 (.292)	7.354	.454	.007	.256	.803

Note. PAI = Physical Activity Intensity, DHA = docosahexaenoic acid, PAIxDHA= interaction term for docosahexaenoic acid and physical activity intensity. NA = not applicable. Positive beta weights predict non-MCI status.