Title: No association between the Omega-3 Index and depressive symptoms in patients with heart disease who are low fish consumers

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Abstract

Background: Long chain omega-3 polyunsaturated fatty acids (LCn3PUFAs) may improve cardiovascular health and depression. This study investigated the relationships between erythrocyte membrane LCn3PUFA status, depression and angina symptoms in patients with heart disease.

Methods: We recruited 91 patients (65 males and 26 females, mean age 59.2 ± 10.3 years) with heart disease and depressive symptoms (Center for Epidemiological Studies Depression Scale, CES-D ≥ 16) and low fish/fish oil intakes. The Omega-3 Index (EPA+DHA) of erythrocyte membranes (as a percentage of total fatty acids) was assessed by gas chromatography. Depression status was measured by both self -report and clinician-report scales; CES-D and the Hamilton depression scale (HAM-D). Angina symptoms were measured using the Seattle Angina Questionnaire and the Canadian Cardiovascular Society Classification for Angina Pectoris.

Results: The mean Omega-3 Index was 4.8 ± 1.0% (±SD). Depression scores measured by CES-D and HAM-D were 29.2 ± 8.8 (moderate to severe) and 11.0 ± 5.7 (mild) (arbitrary units) respectively reflecting a different perception of depressive symptoms between patients and clinicians. Angina status was inversely associated with depressions scores ($r > -0.26$, $P < 0.03$). There were no significant relationships between individual LCn3PUFA or the Omega-3 Index and either the depression scores or the angina symptoms.

Conclusion: Worse angina status was associated with worse depression, but the Omega-3 Index was not associated with symptoms of depression or angina in patients with heart disease.

Keywords

Omega-3 Index, Depression, Heart disease

Introduction

In patients with cardiovascular disease (CVD), depression is common and associated with high mortality rates [1]. Depression is also an independent risk factor for the development of coronary heart disease [2]. A number of pathophysiological factors including endothelial dysfunction, altered platelet activity and aggregation, inflammation and autonomic nervous system dysfunction have been suggested to link depression with adverse cardiac outcomes [3]. Also, as a major manifestation of coronary heart disease (CHD), angina may be one of the possible mediators in the relationship between CHD and depression. Depression can augment perceived pain [4] thus the sensation of angina may be increased in patients with depression [5].

The long chain omega-3 polyunsaturated fatty acids (LCn3PUFAs) eicosapentaenoic acid (EPA) and docosahexaenoic acid) (DHA) may improve cardiovascular health and depression via a number of mechanisms. Cardiovascular benefits of LCn3PUFAs include lowering blood pressure and resting heart rate, improving heart rate variability and vascular endothelial function, decreasing serum triglycerides levels, inhibiting inflammation, platelet aggregation and new plaque development [6, 7]. The exact mechanism by which LCn3PUFAs may influence depression remains unclear. However, DHA is highly concentrated in the retina, brain and nervous system [8], and plays important roles in the structure (neuronal membranes) and function (neurotransmission and receptor function) of neurons, the growth of neural cells, and the gene expression in the brain [9].

The role of LCn3PUFAs in improving endothelial function in the brain might optimise the auto-regulation of cerebral perfusion and blood brain barrier integrity, thus enhancing mental health by adequate oxygen and nutrition supply to brain regions [10]. LCn3PUFAs may also increase levels of monoaminergic neurotransmitters in the brain [11], and inhibit inflammation that is associated with depression [9]. The mechanisms underlying depression are complex and, while there is some evidence linking change to cerebrovascular structure and function with depression [12], other evidence suggests that inflammatory changes are central [13]. Changes in inflammatory markers following supplementation with LCn3PUFAs have been proposed to be central to improvements seen in vascular function in adults with metabolic syndrome [14]. Furthermore, low levels of omega 3s have been

associated with cerebral small vessel disease in acute ischemic stroke patients [15]. In a meta-analysis of prospective cohort studies and randomised controlled trials (RCT), Chowdhury et al [16] found moderate inverse associations between fish consumption and incidence of cerebrovascular disease. Interestingly, LCn3PUFAs measured as circulating biomarkers in observational studies or given as supplements in primary and secondary prevention trials were not associated with cerebrovascular disease, suggesting that the positive effects may be due to the wide variety of nutrients in fish or may reflect a healthier dietary pattern or higher socioeconomic status.

Despite evidence of mechanisms for how LCn3PUFAs may reduce the risk of developing CHD and depression, whether supplementation is beneficial for either condition remains controversial. Epidemiological studies have reported low LCn3PUFA status in patients with CHD compared with healthy age and gender matched controls [17]. Furthermore, The PREDIMED study reported [18] protection from cardiac mortality with LCn3PUFAs. Hazard ratios for meeting the recommended intake of LCn3PUAFS (500mg/day) (n=5452, 75.7%) were, 0.61 (95% CI 0.39-0.96) for fatal cardiovascular disease, and 0.54 (95% CI 0.29-0.99) for fatal coronary heart disease. Evidence from large RCTs have shown beneficial effects of LCn3PUFAs on CHD mortality. Supplementation with LCn3PUFAs as part of cardiovascular disease management have been found to reduce the risk of non-fatal coronary events in Japanese hypercholesterolaemic patients (JELIS study), reduce the risk of death, non-fatal myocardial infarction and stroke in Italian patients with a history of myocardial infarctions (original GISSI-P) [19] and improve survival for patients with chronic heart failure (GISSI-HF) [20]. However, recent meta-analyses have generated conflicting results, with one concluding that supplementation with LCn3PUFA may not reduce the incidence of coronary events but may improve the odds of survival [21] while another concluded that there was no significant reduction in mortality from supplementation with LCn3PUFAs.[22]. Differences may be due to inclusion or exclusion of individual studies; however, it is currently suggested that there is neither a beneficial nor adverse effect of LCn3PUFA supplementation for primary or secondary prevention of coronary heart disease [23].

A major manifestation of CHD is the presence of angina symptoms. There is limited research investigating angina symptoms and LCn3PUFA and what few studies there are have provided mixed results. A small RCT of 20 patients with stable angina reported benefits associated with fish oil supplementation [24] but two other studies found no benefits [25, 26]. Understanding relationships between angina symptoms, depression and omega 3 status may help to explain these mixed findings.

Epidemiological studies have also reported low levels of LCn3PUFAs in people with depression [27]. Cross-sectional studies have also reported that depressed patients with heart disease have significantly lower blood levels of DHA compared with non-depressed patients [28-30]. There are mixed conclusions from meta-analyses as to whether omega 3 supplements are beneficial for reducing the risk of depression [31, 32]. A meta-analysis of clinical trials of treatment with LCn3PUFAs for depression concluded that the use of these supplements is effective in patients with a diagnosis of Major Depressive Disorders (MDD) and in depressive patients without a diagnosis of MDD.[33]

This study investigated associations of LCn3PUFA status, with (a) depression severity and (b) angina symptoms in patients with cardiac disease and depression who were low fish consumers. It was hypothesised that in patients with heart disease, LCn3PUFA status would be inversely associated with depression severity and angina symptoms.

Methods

Study design

The present study represents a secondary analysis of baseline data collected in a randomized placebo-controlled trial that evaluated the effects of supplementation with long chain omega-3 polyunsaturated fatty acids on depression scores in patients with heart disease. The RCT was approved by the Human Research Ethics Committees at the Queen Elizabeth Hospital (Ethics Reference Number: 2008104) and the University of South Australia (Ethics Reference Number: P223/08) and registered on the Australian New Zealand Clinical Trials Registry (ACTRN12608000598381). Each participant provided written informed consent before enrolment.

Patients

Patient inclusion criteria comprised: (1) angiographically-documented coronary artery disease (defined as >50% stenosis in an epicardial coronary artery on selective coronary angiography [34]) or systolic heart failure (heart failure admission and an ejection fraction < 40%), (2) co-morbid clinically relevant depression (determined by a score of greater than or equal to 16 on the Center for Epidemiological Studies Depression Scale (CES-D) [35]), and (3) able to complete questionnaires in written English. Exclusion criteria were the consumption of more than one fish meal per week or the regular use of fish oil supplements (containing more than 300mg EPA + DHA per day).

Procedure

Participants were recruited in 2009-2013 during hospital admission or outpatient visits and by mail-out invitations from two public hospitals that service the North-Western Adelaide suburbs (population approximately 550,000) and by newspaper advertisements.

Demographic data, cardiovascular risk factors, current diagnoses, and medication history were obtained by patient interview and case note extraction. If eligible, participants completed a self-reported depression assessment and a clinician completed an assessment of depression. Height and

body mass were used to calculate body mass index (BMI) and fasting venous blood samples were collected from which LCn3PUFA status was measured in erythrocyte membranes.

Quantification of Angina Symptoms

Angina status was measured by the Seattle Angina Questionnaire (SAQ) [36] and the Canadian Cardiovascular Society Classification for Angina Pectoris (CCSA) [37]. The SAQ assessed four domains of interest for this study (angina frequency, physical limitation, quality of life and treatment satisfaction). Scores of each subscale ranges from 0 (lowest level) to 100 (highest level). The CCSA measured severity of angina symptoms across 4 grades from best (I) to worst (IV), (i.e. the reverse order to the SAQ instrument). Both have good reliability and validity [36, 38].

Quantification of Depressive Symptoms

Self-reported depressive symptoms were assessed using the CES-D, a 20 item scale designed to measure the level of depressive symptoms during the past week [39]. Standard threshold scores were not depressed (CES-D < 16), mild depression (CES-D = 16-26) and moderate to severe depression (CES-D > 26).

Clinician-rated depression was assessed using HAM-D, a 21 item rating scale assessing a patient's current level of depression [40]. Levels of depression were classified as normal (0 – 7), mild depression (8 –16), moderate depression (17 – 23), and severe depression (≥ 24) [41].

Determination of fatty acids

Fasting venous blood samples were collected in 10ml tubes containing ethylenediaminetetraacetic acid. Erythrocytes were separated from plasma via centrifugation at 1780 *g* for ten minutes at 4℃, washed with isotonic saline and stored at -80℃. After thawing, fatty acid contents of erythrocytes were determined using previous published modifications of Lepage and Roy's technique [42]. Samples were analyzed by a gas chromatography (GC2010, Shimadzu) and fatty acids were identified and quantified against a reference standard (GLC-463, Nu-Chek Prep Inc. Elysian, MN, USA). Individual fatty acid contents were reported as percentages of total fatty acids in erythrocytes. The Omega-3 index was calculated as the sum of erythrocyte membrane eicosapenatenoic acid (EPA) and docosahexaenoic acid (DHA).

Statistical Analysis

Statistical analysis was performed using SPSS Statistics for windows, version 21.0 (IBM Corp, Armonk, NY). Data were checked for normality and variables presented as mean and standard deviation (mean ± SD). Student's t-tests and 1-way ANOVAs were performed to test for differences between groups based on depression severity and Spearman Rank and Pearson correlations were used where relevant to determine the relationships between LCn3PUFA status, angina status and depression scores. $P < 0.05$ was considered statistically significant.

Results

Patients

3817 patients were invited to participate and 340 patients were screened. 110 patients agreed to participate, but 18 withdrew before the baseline interview. Thus 92 patients took part in the study and 91 had complete baseline data (Figure 1).

Patient characteristics are provided in Table 1. The majority of patients (78%) had coronary heart disease, with 51% having stable angina. Most had very mild angina status (reflected by levels equal or greater than 50 (arbitrary units) on the five subscales of the SAQ) (data not shown). This was consistent with the measurement of angina severity by the CCSA with the majority of patients (88.8%) being classified into grade I and grade II indicating either no or slight limitation of ordinary activity related to angina (data not shown). Of note, 43% of patients were taking at least one antidepressant medication.

Depression status

Depression data are provided in Table 2. As assessed by the CES-D, more than half (53/91) of patients had moderate to severe depression and when measured by HAM-D, about one third were not depressed (28/91), 47/91 had mild depression and 16/91 had moderate to severe depression. There was no difference between men and women in depression status.

Long chain omega-3 polyunsaturated fatty acid status

The mean erythrocyte membrane levels of EPA, DPA and DHA were 0.8 ± 0.3%, 2.4 ± 0.4% and 4.0 ± 0.9% respectively. The mean Omega-3 Index was 4.8 ± 1.0% (ranging from 3.1 to 7.6%) and the mean erythrocyte membrane level of total LCn3PUFAs was 7.2 ± 1.1%. Stratifying the Omega-3 Index by the severity of depression indicated that patients with higher depression scores, measured by either CES-D or HAM-D, tended to have a higher Omega-3 Index (Table 3).

Associations between membrane fatty acids, angina symptoms and depressive symptoms

There were no significant relationships between depression measures and any of the individual LCn3PUFAs (data not shown) or between the Omega-3 Index and either self-reported (CES-D; r =

0.152, P = 0.150) or clinician-rated (HAM-D; r = 0.098, P = 0.357) depression. No significantly different associations were observed when data were split into those taking or not taking antidepressant medication.

There were no significant associations between Omega-3 Index and angina status measured by either SAQ or CCSA (Table 4). There were weak to moderate negative correlations between depression scores and angina status, indicating that better angina status was associated with less depression (Table 4).

Discussion

The principal finding of this study was that while worse symptoms of angina were associated with greater severity of depression the Omega-3 Index was not associated with either. Thus, our hypotheses were not supported.

The relationships between blood levels of LCn3PUFAs and depression remain controversial in the literature. Many studies have found lower LCn3PUFA levels in erythrocyte membranes [28], total serum [29], plasma [43] and plasma phospholipids [30, 44] in depressed patients and inverse relationships between these LCn3PUFA biomarkers and depression status, while others have found no such relationships [42, 45-47]. These discrepancies may be due to differing demographic characteristics of the samples evaluated (including severity of depressive symptoms), type of cardiovascular disease, the method of depression measurement and the component of blood extracted for the assessment of LCn3PUFA content. Whilst the content of LCn3PUFAs in blood samples is a useful and accessible biomarker of LCn3PUFA status, the fraction of blood used for analysis is important. Erythrocyte membrane content is considered to be the most reliable, biologically relevant, and long-term marker of dietary LCn3PUFA intake, with the Omega-3 Index reflecting the erythrocyte membrane content of EPA and DHA and having shown to be inversely associated with risk for CHD mortality [48].

The mean Omega-3 Index in the present study was 4.8 ± 1.0%. This is comparable with levels reported in other Australian studies, where values ranged from 3.97 to 5.78% [42, 49] in healthy controls. Milte et al reported similar omega-3 levels in people with mild cognitive impairment (MCI) and age matched healthy controls (HC) (mean ± SEM ; MCI 5.49 ± 0.13% vs HC 5.78 ± 0.17%) [42]. By comparison the mean Omega-3 Index in an American population with acute coronary syndrome and depression was 2.9%, compared with 3.3% in those with acute coronary syndrome but without depression [28]. The Omega-3 index was also found to be lower in a German population with major depressive disorder (3.9%), compared to healthy controls (5.1%) [27]. It may be that omega-3 status only influences depression below a threshold Omega-3 Index of ~ 4-5%, and thus the omega-3 status of patients in the present study was too high to see any effect. Dietary differences across countries,

and/or the way in which depression has been diagnosed, may account for some of the differences in findings between studies.

Rates of depression vary widely across the world [50] and the intake of long chain omega 3 varies between countries [51], with several including Australia [52] and the USA [53] reporting that population intakes are below the recommended levels. A recent study in the USA examined plasma LCn3PUFA concentrations as a biomarker assessment of LCn3PUFA status in a nationally representative cohort (NHANES) and reported that nearly the whole population had levels below those associated with cardiovascular protection [54]. Whilst the plasma levels differ from the Omega-3 Index, these findings are nevertheless supportive.

Different dietary patterns including pro-inflammatory diets have been linked to increased risk of depression [55], [56] although a recent meta-analysis highlighted the variability in findings [57]. The association between fish consumption specifically and risk of depression was recently reviewed with results from a meta-analysis concluding that high fish consumption was associated with reduced risk of depression, the pooled risk ratio of depression for the highest versus lowest consumption of fish being 0.83 (95% CI 0.74 to 0.93) [58]. However, when studies were separated by continent, not all followed the same pattern; there were significant associations in studies conducted in Europe but not in those conducted in North America, South America, Asia and Oceania. This heterogeneity may result from differences in fish type, fish preservation and cooking styles or from differences in methods used to report intake. Cultural and socioeconomic differences across countries in these continents are also an important consideration when considering diet patterns and stimuli of depressive symptoms [59].

Another difference between the findings of this report and previous studies is that the majority of previous studies have compared depressed and non-depressed groups of patients rather than assessing the relationship between LCn3PUFA status and severity of depressive symptoms within a sample with depression. It might be expected to be more difficult to demonstrate a relationship between

LCn3PUFAs and depression within a more homogenous population than between two different populations, and in particular in a population that for the most part was not severely depressed. In two studies reporting no association between blood levels of LCn3PUFAs and depression status [45, 46] the authors argued that a significant association may be evident only in clinical or severe levels of depression. This is partially supported by a study in a Dutch community population (n = 241), which did not find an association between levels of LCn3PUFAs in plasma phospholipids and depression status in the full sample but found a strong inverse relationship ($r = -0.465$, $P = 0.005$) in those participants who reported clinically relevant depressive symptoms (CES-D $\geq$ 16, n = 35) [60]. A meta-analysis of trials conducted in populations with major depression or trials conducted in other populations found that beneficial effects of LCn3PUFAs on depressive symptoms were only evident in populations with major depression [61]. The patients in the present study were screened for depression status by CES-D (CES-D $\geq$ 16), which was considered as clinically relevant level of depressive symptoms. However, when the patients were assessed using the clinician rated tool, almost one third (28 of 91) did not reach the clinically relevant level (HAM-D $\geq$ 8). Moreover, more than half of the patients (53) had moderate to severe depressive symptoms measured by CES-D whereas less than one fifth (16) had moderate to severe depressive symptoms when they were assessed using the HAM-D instrument (Table 2). HAM-D may be a more sensitive and specific tool than CES-D and truly reflect the depression status of these patients. Thus, the lack of a relationship between LCn3PUFA status and depression in the present study may have been due to only a small proportion of the sample having clinically relevant or severe depression. Future studies should aim to recruit populations with a broader range of depressive symptoms to explore this further. Nevertheless, despite the low ratings of depression, we found that worse angina status was associated with higher depression scores. These findings are consistent with previous studies which have reported that patients with higher depression scores (measured by CES-D) suffered more frequent angina (measured by SAQ) [5]. However, there were no associations between angina status and the Omega-3 Index.

A limitation of the present study that might have contributed to the absence of a significant relationship between LCn3PUFA status and depression or angina symptoms is insufficient statistical

power. Using the correlation coefficient obtained from testing the association between CES-D depression scores and the Omega 3 Index (r = 0.152), a post hoc power analysis [62] found that 266 participants would be required to detect a significant relationship. However, despite not being statistically significant, because the coefficient (r = 0.152) only explains approximately 2% of the variance in depression scores (CES-D), it is unlikely to be clinically meaningful. Common factors associated with LCn3PUFAs and depression are age, gender, race, education, hypertension, diabetes, BMI, medications and smoking status [28]. These variables may influence the relationship between LCn3PUFAs and depression, which might not only reduce the statistical power, but also confound the effects of LCn3PUFAs on depression. Some of these variables were recorded (Table 1), however given the small sample size in the present study, no analyses controlling for these potential confounders were performed. Increasing the size of the sample and performing analyses controlling for these factors may have assisted in finding a significant relationship between blood levels of LCn3PUFAs and depression status.

A strength of this study was the determination of omega-3 status by measuring the Omega-3 Index. However a limitation was the narrow distribution of the Omega-3 index in the study population. The minimal variability of the Omega-3 Index in this group may have limited the ability to detect a relationship between the Omega 3 index and depression. It is possible that associations may exist between omega 3 status and depression when a population with a broader range of omega 3 intakes are considered. Future studies should also look at relationships between changes in omega 3 erythrocyte content and changes in depression in controlled supplementation studies.

Conclusion

In summary, the Omega-3 Index was not associated with the depression scores measured by HAM-D or CES-D in depressed cardiac patients who have low fish / omega-3 intake. The absence of significant relationships may be due to the small sample size or relatively low severity of depression in the present sample. The relationship between LCn3PUFAs and depression in patients with heart disease remains unclear.

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References

1. Chauvet-Gélinier J-C, Trojak B, Vergès-Patois B, Cottin Y, Bonin B. Review on depression and coronary heart disease. Arch Cardiovasc Dis. 2013;106(2):103-110.

2. Van der Kooy K, van Hout H, Marwijk H, Marten H, Stehouwer C, Beekman A. Depression and the risk for cardiovascular diseases: systematic review and meta analysis. Int J Geriatr Psychiatry. 2007;22(7):613-626.

3. Huffman JC, Celano CM, Beach SR, Motiwala SR, Januzzi JL. Depression and cardiac disease: epidemiology, mechanisms, and diagnosis. Cardiovasc Psychiatry Neurol. 2013;2013:695925.

4. Schweinhardt P, Kalk N, Wartolowska K, Chessell I, Wordsworth P, Tracey I. Investigation into the neural correlates of emotional augmentation of clinical pain. NeuroImage. 2008;40(2):759-766.

5. Arnold SV, Spertus JA, Ciechanowski PS, Soine LA, Jordan-Keith K, Caldwell JH, et al. Psychosocial modulators of angina response to myocardial ischemia. Circulation. 2009;120(2):126-133.

6. Kris-Etherton PM, Harris WS, Appel LJ, Committee ftN. Fish consumption, fish oil, omega-3 fatty acids, and cardiovascular disease. Circulation. 2002;106(21):2747-2757.

7. Colquhoun D, Ferreira-Jardim A, Udell T, Eden B, the Nutrition and Metabolism Committee of the Heart Foundation. Review of evidence: Fish, fish oils, n-3 polyunsaturated fatty acids and cardiovascular health. Online: National Heart Foundation of Australia; 2008. Available from: http://www.heartfoundation.org.au/SiteCollectionDocuments/Fish-FishOils-revie-of-evidence.pdf.

8. Salem N, Jr., Litman B, Kim H-y, Gawrisch K. Mechanisms of action of docosahexaenoic acid in the nervous system. Lipids. 2001;36(9):945-959.

9. Sinclair AJ, Begg D, Mathai M, Weisinger RS. Omega 3 fatty acids and the brain: Review of studies in depression. Asia-Pac J Clin Nutr. 2007;16 (Suppl 1):391-397.

10. Sinn N, Howe PRC. Mental health benefits of omega-3 fatty acids may be mediated by improvements in cerebral vascular function. Biosci Hypotheses. 2008;1(2):103-108.

11. Chalon S, Delion-Vancassel S, Belzung C, Guilloteau D, Leguisquet A-M, Besnard J-C, et al. Dietary fish oil affects monoaminergic neurotransmission and behavior in rats. J Nutr. 1998;128(12):2512-2519.

12. Robinson RG, Jorge RE. Post-Stroke Depression: A Review. The American journal of psychiatry. 2015:appiajp201515030363.

13. Spalletta G, Bossu P, Ciaramella A, Bria P, Caltagirone C, Robinson RG. The etiology of poststroke depression: a review of the literature and a new hypothesis involving inflammatory cytokines. Mol Psychiatry. 2006;11(11):984-991.

14. Tousoulis D, Plastiras A, Siasos G, Oikonomou E, Verveniotis A, Kokkou E, et al. Omega-3 PUFAs improved endothelial function and arterial stiffness with a parallel antiinflammatory effect in adults with metabolic syndrome. Atherosclerosis. 2014;232(1):10-16.

15. Song TJ, Chang Y, Shin MJ, Heo JH, Kim YJ. Low levels of plasma omega 3-polyunsaturated fatty acids are associated with cerebral small vessel diseases in acute ischemic stroke patients. Nutrition research (New York, NY). 2015;35(5):368-374.

16. Chowdhury R, Stevens S, Gorman D, Pan A, Warnakula S, Chowdhury S, et al. Association between fish consumption, long chain omega 3 fatty acids, and risk of cerebrovascular disease: systematic review and meta-analysis. BMJ (Clinical research ed). 2012;345:e6698.

17. Block RC, Harris WS, Reid KJ, Sands SA, Spertus JA. EPA and DHA in blood cell membranes from acute coronary syndrome patients and controls. Atherosclerosis. 2008;197(2):821-828.

18. Sala-Vila A, Guasch-Ferre M, Hu FB, Sanchez-Tainta A, Bullo M, Serra-Mir M, et al. Dietary alpha-Linolenic Acid, Marine omega-3 Fatty Acids, and Mortality in a Population With High Fish Consumption: Findings From the PREvencion con DIeta MEDiterranea (PREDIMED) Study. Journal of the American Heart Association. 2016;5(1).

19. Marchioli R, Marfisi RM, Borrelli G, Chieffo C, Franzosi MG, Levantesi G, et al. Efficacy of n-3 polyunsaturated fatty acids according to clinical characteristics of patients with recent myocardial infarction: insights from the GISSI-Prevenzione trial. Journal of cardiovascular medicine (Hagerstown, Md). 2007;8 Suppl 1:S34-37.

20. Tavazzi L, Maggioni AP, Marchioli R, Barlera S, Franzosi MG, Latini R, et al. Effect of n-3 polyunsaturated fatty acids in patients with chronic heart failure (the GISSI-HF trial): a randomised, double-blind, placebo-controlled trial. Lancet (London, England). 2008;372(9645):1223-1230.

21. Wen YT, Dai JH, Gao Q. Effects of Omega-3 fatty acid on major cardiovascular events and mortality in patients with coronary heart disease: a meta-analysis of randomized controlled trials. Nutrition, metabolism, and cardiovascular diseases : NMCD. 2014;24(5):470-475.

22. Rizos EC, Ntzani EE, Bika E, Kostapanos MS, Elisaf MS. Association between omega-3 fatty acid supplementation and risk of major cardiovascular disease events: a systematic review and meta-analysis. Jama. 2012;308(10):1024-1033.

23. Nestel P, Clifton P, Colquhoun D, Noakes M, Mori TA, Sullivan D, et al. Indications for Omega-3 Long Chain Polyunsaturated Fatty Acid in the Prevention and Treatment of Cardiovascular Disease. Heart, lung & circulation. 2015;24(8):769-779.

24. Salachas A, Papadopoulos C, Sakadamis G, Styliadis J, Voudris V, Oakley D, et al. Effects of a low-dose fish oil concentrate on angina, exercise tolerance time, serum triglycerides, and platelet function. Angiology. 1994;45(12):1023-1031.

25. Solomon SA, Cartwright I, Pockley G, Greaves M, Preston FE, Ramsay LE, et al. A placebo-controlled, double-blind study of eicosapentaenoic acid-rich fish oil in patients with stable angina pectoris. Curr Med Res Opin. 1990;12(1):1-11.

26. Vacek JL, Harris WS, Haffey K. Short-term effects of omega-3 fatty acids on exercise stress test parameters, angina and lipoproteins. Biomed Pharmacother. 1989;43(5):375-379.

27. Baghai TC, Varallo-Bedarida G, Born C, Hafner S, Schule C, Eser D, et al. Major depressive disorder is associated with cardiovascular risk factors and low Omega-3 Index. J Clin Psychiat. 2011;72(9):1242-1247.

28. Amin AA, Menon RA, Reid KJ, Harris WS, Spertus JA. Acute coronary syndrome patients with depression have low blood cell membrane omega-3 fatty acid levels. Psychosom Med. 2008;70(8):856-862.

29. Frasure-Smith N, Lesperance F, Julien P. Major depression is associated with lower omega-3 fatty acid levels in patients with recent acute coronary syndromes. Biol Psychiat. 2004;55(9):891-896.

30. Parker G, Heruc G, Hilton T, Olley A, Brotchie H, Hadzi-Pavlovic D, et al. Low levels of docosahexaenoic acid identified in acute coronary syndrome patients with depression. Psychiat Res. 2006;141(3):279-286.

31. Bloch MH, Hannestad J. Omega-3 fatty acids for the treatment of depression: systematic review and meta-analysis. Mol Psychiatry. 2012;17(12):1272-1282.

32. Sublette ME, Ellis SP, Geant AL, Mann JJ. Meta-analysis of the effects of eicosapentaenoic acid (EPA) in clinical trials in depression. J Clin Psychiat. 2011;72(12):1577-1584.

33. Grosso G, Pajak A, Marventano S, Castellano S, Galvano F, Bucolo C, et al. Role of omega-3 fatty acids in the treatment of depressive disorders: a comprehensive meta-analysis of randomized clinical trials. PloS one. 2014;9(5):e96905.

34. Harris PJ, Behar VS, Conley MJ, Harrell FE, Jr., Lee KL, Peter RH, et al. The prognostic significance of 50% coronary stenosis in medically treated patients with coronary artery disease. Circulation. 1980;62(2):240-248.

35. Zich JM, Attkisson CC, Greenfield TK. Screening for depression in primary care clinics: The CES-D and the BDI. Intl J Psychiat Med. 1990;20(3):259-277.

36. Spertus JA, Winder JA, Dewhurst TA, Deyo RA, Prodzinski J, McDonell M, et al. Development and evaluation of the Seattle Angina Questionnaire: a new functional status measure for coronary artery disease. J Am Coll Cardiol. 1995;25(2):333-341.

37. Selzer A, Cohn K. Functional classification of cardiac disease: a critique. Am J Cardiol. 1972;30(3):306-308.

38. Goldman L, Hashimoto B, Cook EF, Loscalzo A. Comparative reproducibility and validity of systems for assessing cardiovascular functional class: Advantages of a new specific activity scale. Circulation. 1981;64(6):1227-1234.

39. Radloff LS. The CES-D scale: A self-report depression scale for research in the general population. Appl Psych Meas. 1977;1(3):385-401.

40. Hamilton M. A rating scale for depression. J Neurol Neurosur Ps. 1960;23:56-62.

41. Zimmerman M, Martinez JH, Young D, Chelminski I, Dalrymple K. Severity classification on the Hamilton Depression Rating Scale. J Affect Disord. 2013;150(2):384-388.

42. Milte CM, Sinn N, Street SJ, Buckley JD, Coates AM, Howe PRC. Erythrocyte polyunsaturated fatty acid status, memory, cognition and mood in older adults with mild cognitive impairment and healthy controls. Prostaglandins Leukot Essent Fatty Acids. 2011;84(5-6):153-161.

43. Feart C, Peuchant E, Letenneur L, Samieri C, Montagnier D, Fourrier-Reglat A, et al. Plasma eicosapentaenoic acid is inversely associated with severity of depressive symptomatology in the elderly: Data from the Bordeaux sample of the Three-City Study. Am J Clin Nutr. 2008;87(5):1156-1162.

44. Tiemeier H, van Tuijl HR, Hofman A, Kiliaan AJ, Breteler MM. Plasma fatty acid composition and depression are associated in the elderly: The Rotterdam Study. Am J Clin Nutr. 2003;78(1):40-46.

45. Appleton KM, Gunnell D, Peters TJ, Ness AR, Kessler D, Rogers PJ. No clear evidence of an association between plasma concentrations of n-3 long-chain polyunsaturated fatty acids and depressed mood in a non-clinical population. Prostaglandins Leukot Essent Fatty Acids. 2008;78(6):337-342.

46. Jadoon A, Chiu C-C, McDermott L, Cunningham P, Frangou S, Chang C-J, et al. Associations of polyunsaturated fatty acids with residual depression or anxiety in older people with major depression. J Affect Disord. 2012;136(3):918-925.

47. Ruusunen A, Virtanen JK, Lehto SM, Tolmunen T, Kauhanen J, Voutilainen S. Serum polyunsaturated fatty acids are not associated with the risk of severe depression in middle-aged Finnish men: Kuopio Ischaemic Heart Disease Risk Factor (KIHD) study. Eur J Nutr. 2011;50(2):89-96.

48. Harris WS, von Schacky C. The omega-3 index: A new risk factor for death from coronary heart disease? Prev Med. 2004;39(1):212-220.

49. Milte CM, Coates AM, Buckley JD, Hill AM, Howe PRC. Dose-dependent effects of docosahexaenoic acid-rich fish oil on erythrocyte docosahexaenoic acid and blood lipid levels. Br J Nutr. 2008;99(5):1083-1088.

50. Ferrari AJ, Somerville AJ, Baxter AJ, Norman R, Patten SB, Vos T, et al. Global variation in the prevalence and incidence of major depressive disorder: A systematic review of the epidemiological literature. Psychological Medicine. 2013;43(3):471-481.

51. Harika RK, Eilander A, Alssema M, Osendarp SJ, Zock PL. Intake of fatty acids in general populations worldwide does not meet dietary recommendations to prevent coronary heart disease: a systematic review of data from 40 countries. Annals of nutrition & metabolism. 2013;63(3):229-238.

52. Meyer B. Australians are not Meeting the Recommended Intakes for Omega-3 Long Chain Polyunsaturated Fatty Acids: Results of an Analysis from the 2011–2012 National Nutrition and Physical Activity Survey. Nutrients. 2016;8(3):111.

53. Papanikolaou Y, Brooks J, Reider C, Fulgoni VL, 3rd. U.S. adults are not meeting recommended levels for fish and omega-3 fatty acid intake: results of an analysis using observational data from NHANES 2003-2008. Nutrition journal. 2014;13:31.

54. Murphy RA, Yu EA, Ciappio ED, Mehta S, McBurney MI. Suboptimal Plasma Long Chain n-3 Concentrations are Common among Adults in the United States, NHANES 2003-2004. Nutrients. 2015;7(12):10282-10289.

55. Khosravi M, Sotoudeh G, Majdzadeh R, Nejati S, Darabi S, Raisi F, et al. Healthy and Unhealthy Dietary Patterns Are Related to Depression: A Case-Control Study. Psychiatry investigation. 2015;12(4):434-442.

56. Sanchez-Villegas A, Ruiz-Canela M, de la Fuente-Arrillaga C, Gea A, Shivappa N, Hebert JR, et al. Dietary inflammatory index, cardiometabolic conditions and depression in the Seguimiento Universidad de Navarra cohort study. Br J Nutr. 2015;114(9):1471-1479.

57. Rahe C, Unrath M, Berger K. Dietary patterns and the risk of depression in adults: a systematic review of observational studies. Eur J Nutr. 2014;53(4):997-1013.

58. Li F, Liu X, Zhang D. Fish consumption and risk of depression: a meta-analysis. Journal of epidemiology and community health. 2016;70(3):299-304.

59. Jacka FN, Cherbuin N, Anstey KJ, Butterworth P. Dietary patterns and depressive symptoms over time: examining the relationships with socioeconomic position, health behaviours and cardiovascular risk. PloS one. 2014;9(1):e87657.

60. Schiepers OJ, de Groot RH, Jolles J, van Boxtel MP. Plasma phospholipid fatty acid status and depressive symptoms: Association only present in the clinical range. J Affect Disord. 2009;118(1-3):209-214.

61. Appleton KM, Hayward RC, Gunnell D, Peters TJ, Rogers PJ, Kessler D, et al. Effects of n–3 long-chain polyunsaturated fatty acids on depressed mood: Systematic review of published trials. Am J Clin Nutr. 2006;84(6):1308-1316.

62. Faul F, Erdfelder E, Buchner A, Lang AG. Statistical power analyses using G*Power 3.1: Tests for correlation and regression analyses. Behav Res Methods. 2009;41:1146-1160.

Figure 1: The Consort diagram showing patient recruitment for this cross-sectional analysis. CES-D = Epidemiological Studies Depression Scale

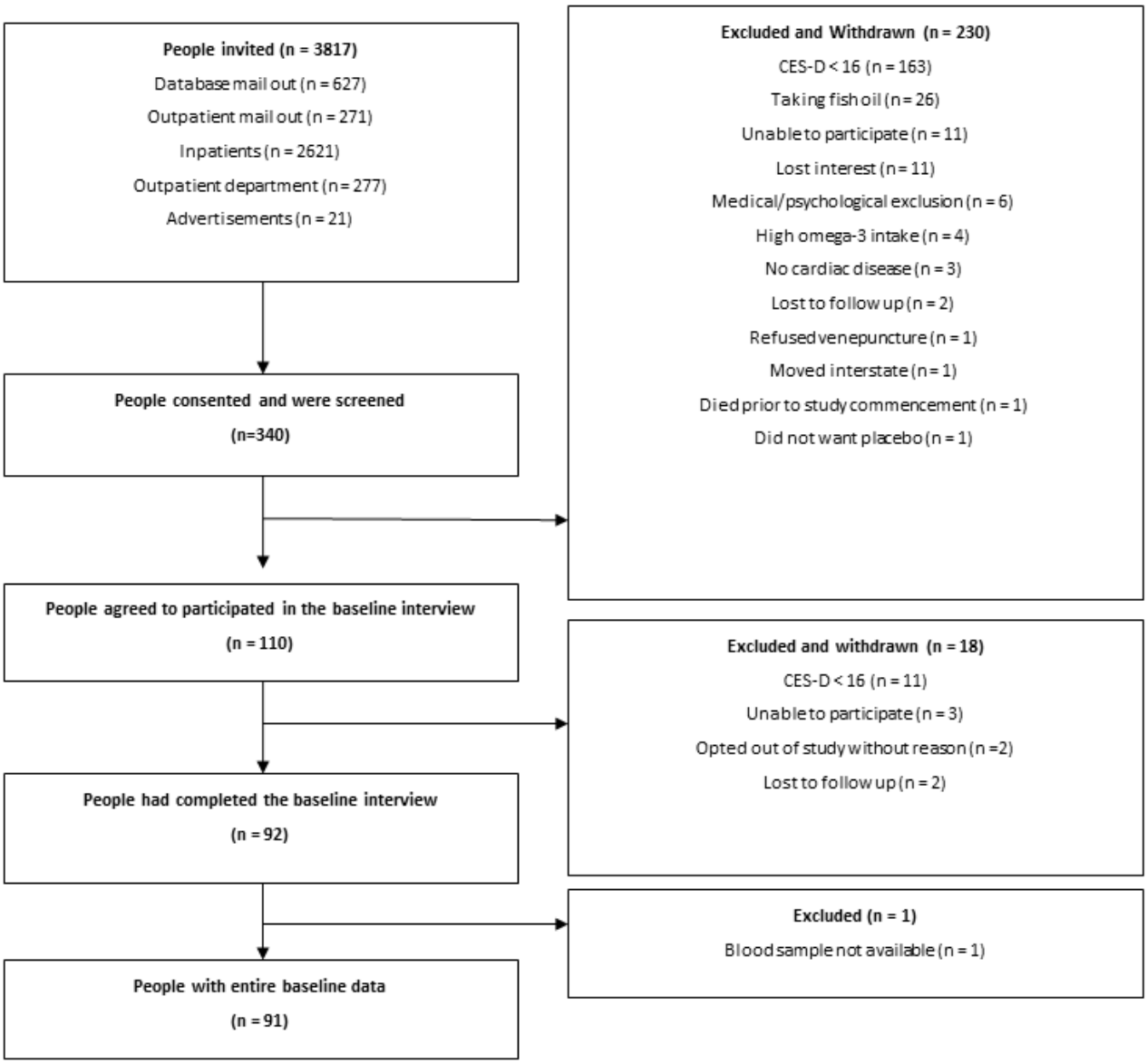

Table 1: Characteristics of the Patients (n = 91)

Variables	Mean ± SD or n (%)
Age (Years)	59.2±10.3
BMI (kg/m^2) $^+$	30.5±5.9
Gender	
Male	65 (71%)
Female	26 (29%)
Type of Heart Disease	
Coronary Heart Disease	71 (78%)
Heart Failure	20 (22%)
Stable Angina	46 (51%)
Smoking Status	
Non-smokers	20 (22%)
Current Smoker	25 (27%)
Past Smoker	46 (51%)
Hypertension	50 (55%)
Type 2 Diabetes	30 (33%)
Family History of Premature cardiovascular disease	
Yes	45 (49%)
No	41 (45%)
Unknown	5 (6%)
Medication History	
Aspirin	66 (75%)
Clopidogrel	32 (35%)
Nitrates	42 (46%)
Calcium Channel Blockers	47 (52%)
Beta Blockers	31 (34%)

ACEI	46 (51%)
ARB	15 (16%)
Diuretics	32 (35%)
Digoxin	13 (14%)
Antiarrhythmic agents	6 (7%)
Statins	68 (75%)
Fibrates	4 (4%)
Benzodiazepines	18 (20%)
SSRI	19 (21%)
Tricyclic antidepressant	9 (10%)
Other Antidepressant	18 (20%)

ACEI = Angiotensin Converting Enzyme Inhibitor, ARB = Angiotensin Receptor Blocker, BMI = Body Mass Index, SD = Standard deviation, SSRI = Selective Serotonin Re-uptake Inhibitor,+ n = 90

Table 2: Depression status of patients (n = 91)

Variable	n	Mean ± SD
CES-D		29.2 ± 8.8
Mild (≥ 16 and ≤ 26)	38	21.1 ± 3.4
Moderate to severe (> 26)	53	35.1 ± 6.7
HAM-D		11.0 ± 5.7
None (0 – 7)	28	5.2 ± 1.4
Mild (8 – 16)	47	11.2 ± 2.6
Moderate (17 – 23)	13	19.7 ± 1.8
Severe (≥ 24)	3	25.3 ± 1.2

CES-D = Center for Epidemiological Studies Depression Scale, HAM-D = Hamilton Depression Scale, SD = Standard Deviation

Table 3: The Omega-3 Index stratified by depression scores (n =91)

Depression scores	n	Omega-3 Index (Mean ± SD)	P value
CES-D			
Mild (≥ 16 and ≤ 26)	38	4.6 ± 1.0	0.085
Moderate to severe (> 26)	53	4.9 ± 1.0	
HAM-D			
None (0 – 7)	28	4.6 ± 0.8	
Mild (8 – 16)	47	4.8 ± 1.1	0.817
Moderate (17 – 23)	13	4.9 ± 1.0	
Severe (≥ 24)	3	5.6 ± 0.5	

CES-D = Center for Epidemiological Studies Depression Scale, HAM-D = Hamilton Depression Scale, SD = Standard deviation

Table 4: Correlations between the Omega-3 Index, depression status and angina study

	Omega-3 Index		HAM-D		CES-D	
	r	P value	r	P value	r	P value
SAQ						
Angina Frequency	-0.005	0.963	-0.313	0.003	-0.273	0.009
Physical Limitation	0.167	0.149	-0.374	0.001	-0.262	0.022
Disease Perception	-0.003	0.976	-0.364	0.000	-0.371	0.000
Treatment Satisfaction	0.139	0.192	-0.338	0.001	-0.285	0.007
CCSA*	-0.002	0.986	0.308	0.003	0.257	0.015

CCSA = Canadian Cardiovascular Society Classification for Angina Pectoris, CES-D = Center for Epidemiological Studies Depression Scale, HAM-D = Hamilton Depression Scale, SAQ = Seattle Angina Questionnaire. *Spearman's rank-order correlation coefficient (rho)reported for relationships with CCSA. Statistically significant at $p<0.05$.