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# Suboptimal Omega-3 Levels in Australian Adolescents

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**Abstract:** *Objective:* To quantitate the omega-3 status in a cohort of Australian adolescents.

*Design, Setting and Participants:* A cross-sectional descriptive study of 251 apparently healthy adolescents (192 female, 59 male) aged 15-17 years, in year 11, from 10 schools within the Northern Sydney and Central Coast areas of New South Wales. Participants provided a morning non-fasting blood sample *via* finger-prick and written answers to specific demographic and lifestyle questions. Omega-3 index was calculated by adding %EPA and %DHA values in the whole blood. Equivalent erythrocyte omega-3 index values were obtained by using conversion factors (1.33 for EPA and 2.22 for DHA) from published erythrocyte/whole blood values.

*Main Outcome Measures:* Quantitation of the individual, and estimation of the group average, blood omega-3 Index.

*Results:* The blood omega-3 Index for this adolescent cohort ranged from 2.1-22.3 with a mean of 8.3±3.2, and median of 7.8. On average males had a higher omega-3 Index compared to females (10.5±3.7 vs 7.7±2.6,  $p < 0.001$ ). Fifty three percent of adolescents tested had an omega-3 Index below the optimum of >8. Three percent had an Index of <4, placing them in the high risk category for disease.

On average, adolescents from low or medium socioeconomic communities had a significantly lower omega-3 Index compared to those from higher socioeconomic neighbourhoods (mean difference=1.4,  $p=0.018$ ). Overall 20% of boys and 17% of girls reported regularly taking omega-3 supplements. Regular use of omega-3 supplements was associated with a higher average omega-3 Index (9.8±3.7,  $n=44$  compared to 8.0±3.0,  $n=203$ ,  $p=0.001$  in those not taking supplements).

*Conclusion:* This study indicates that Australian adolescents, even when from advantaged homes, have a high probability of below optimum omega-3 levels. As reduced omega-3 levels are linked to conditions of public health concern such as diabetes, asthma and depression, targeted strategies to improve the omega-3 status in the childhood population may be warranted.

**Keywords:** Polyunsaturated fatty acids, child, health, brain, depression.

## INTRODUCTION

The importance of the essential, dietary derived, polyunsaturated fatty acids (PUFAs) omega-6 and omega-3 to human health were first reported by Burr and Burr in two landmark publications almost 90 years ago [1,2]. Though some decades elapsed before PUFAs commanded a reasonable priority in medical research an escalating body of evidence now indicates that adequate levels of both omega-6 and omega-3 PUFAs are required to maintain optimum health throughout life [3].

A western diet typically supplies substantial amounts of omega-6 through ingestion of

manufactured foods containing oils such as corn, safflower, grape-seed, sunflower, peanut and soybean. However this same diet has limited capacity to provide omega-3 [4], suggesting that ingestion of omega-3 PUFAs may not be optimal in either adult or adolescent populations.

This is of concern as research now indicates that sufficient intakes of omega-3 PUFAs is helpful in the reduction of a wide range of chronic medical conditions including cardiovascular disease, depression, anxiety, diabetes, asthma, and rheumatoid arthritis [5-9].

The multiple beneficial effects attributed to omega-3s are due to the involvement of this family of PUFAs, including alpha linolenic acid (ALA), eicosapentaenoic acid (EPA) and docosahexanoic acid (DHA) and their derivatives (eicosanoids, resolvins and protectins), in a diverse range of biochemical pathways and cellular

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functions including metabolism, cell membrane fluidity, intracellular signaling and gene expression [10]. Through these pathways omega-3s influence cell growth and tissue repair and help modulate aspects of inflammation and immunity [11]; functions that are important both to the developing adolescent and mature adult.

While a considerable body of data is available on the omega-3 status of adults, relatively few studies have measured PUFA levels in the child/adolescent; a time of considerable physiological development both systemically and in the central nervous system.

The omega-3 PUFA DHA is the most abundant fatty acid in the human brain. During adolescence the brain goes through a critical stage of neural development [12], where selective synaptic elimination and neural network remodelling may be influenced by environmental factors. DHA has been shown to progressively increase in concentration in the brain from childhood through adolescence, peaking at around 18 years to adult levels before declining in older age [13].

While the functional importance of this is still being elucidated, dietary DHA intake has been associated with alterations in activity in cortical attention networks during sustained attention in healthy boys [14]. In addition, fish consumption (a good source of the omega-3s DHA and EPA) at age 15 has been positively correlated with intelligence at 18 [15]. In fact two separate studies have observed that higher fish intake is associated with more advanced vocabulary and higher grades among adolescents [16,17].

Possibly stemming from its relationship to neural function, omega-3 intake has long been associated with improvements in mood disorders in adults [18]. While limited data is available for adolescents a recent study reported that higher intakes of fish, EPA, and DHA were independently associated with a lower prevalence of depressive symptoms in male adolescents [19,20].

Omega-3 intake is also linked mechanistically with vascular health and therefore risk of both cardio and cerebro-vascular disease [21]. As omega-3 metabolites affect inflammatory activity they are thought to reduce development of pro-atherosclerotic processes. A recent study by O'Sullivan *et al.* observed that in boys, higher omega-3 levels were independently associated with key cardiovascular risk factors such as total cholesterol and diastolic blood pressure suggesting that omega-3

levels may influence cardiovascular health even in the young [22].

Given the relationship between essential PUFA levels and multiple health conditions, assessment of an individual's omega-3 status seems prudent. Block *et al.* showed a significant association between acute coronary syndrome (ACS) case status and blood cell EPA + DHA levels, expressed as a percentage of total membrane fatty acids [23]. This is known as the omega-3 Index. On the basis of these observations the cut points for cardiovascular risk were determined to be <4 = high risk, 4-8 = intermediate risk, >8 = low risk [23]. Harris, has since argued convincingly for the omega-3 Index as both a risk marker and risk factor for cardiovascular disease [24]. More recently work by Baghi *et al.* supports an association between low omega-3 Index (<4) and risk for both cardiovascular and major depressive disorder (MDD) in adults [25].

While a foundation for disease risk based on omega-3 status in adults has been developed, a comparable body of knowledge in relation to omega-3 status and the child/adolescent population is presently absent.

Two fundamental questions need to be answered:

- 1) What is the current status of omega-3 levels in children/adolescents in various locations around the world?
- 2) Do adult cut-points represent relevant ranges for the assessment of disease risk in children/adolescents?

While answers to the second question await further research, initial characterisation of omega-3 levels in the younger age groups has begun. A recent study by O'Sullivan *et al.* in Western Australia reported a mean omega-3 Index for 13 to 15 year old children as  $4.90 \pm 1.04$  (range 1.41-8.42), where 15.6% of adolescents had an Index of <4 putting them in the high risk category [22]. Complementing the work done by O'Sullivan *et al.* the current study reports the measured omega-3 status from 251, 15 to 17 year old, adolescents from the east Australian state of New South Wales.

## METHODS

A cross-sectional survey of 251 participants (192 female, 59 male) aged 15 to 17 years was carried out.

## Morphometric Analysis

The height and weight of each participant was measured using a standard Wedderburn height stick (mm) and Wedderburn body composition analyser BC-420MA. BMI was calculated appropriate to age using the CDC growth charts developed by the National Center for Health Statistics in collaboration with the National Center for Chronic Disease Prevention and Health Promotion (2000).

## Survey Information

Participants provided written responses to questions detailing their personal demographics and use of omega-3 supplements. Socioeconomic status (SES) was determined according to the Socio-Economic Indexes for Areas (SEIFA) published by the Australian Bureau of Statistics according to each participant's resident postcode [26].

## Recruitment and Exclusion Criterion

Participants were recruited from students in year 11 from 10 schools within the Northern Sydney and Central Coast metropolitan areas as part of a larger health study. No apparently healthy student was actively prevented from participating. All participants provided written informed consent signed by both themselves and their legal guardian.

## Blood Sample Collection

A whole blood sample (~200  $\mu$ L) was collected from each participant between 0830 and 1000 hours *via* finger-prick in two heparin anticoagulated capillary tubes and stored at 4-8°C for <4 hours before processing.

## Omega-3 Analysis

A comprehensive range of fatty acids were analyzed by gas chromatography (GC) based on the methods of Lepage and Roy [27]. Methanol/toluene (4:1, by volume) of 2 mL was mixed with C19:0 (0.2 mg/mL) and BHT (0.12 g/L), added to the erythrocyte pellet and vortexed. On vortexing the sample, 200  $\mu$ L acetyl chloride was added drop-wise to methylate the fatty acids, followed by heating to 100 °C for 1 hour. Samples were allowed to cool, and the reaction was stopped by the addition of 5  $\mu$ L of 6% K<sub>2</sub>CO<sub>3</sub> and vortexed. The samples were centrifuged at 3000xg at 4 °C for 10 min to separate the layers. The upper layer containing toluene and fatty acid methyl esters was transferred to a 2 mL glass vial, crimp sealed with a

teflon-lined cap ready for GC analysis. GC analysis was performed with a 30m $\times$ 0.25mm (DB-225) fused carbon-silica column coated with cyanopropylphenyl (J & W Scientific, Folsom, CA, USA). The injector and dejector ports were set at 250 °C and the oven temperature at 170 °C for 2 minute, increased by 10 °C/minute to 190 °C, held for 1 minute then increased by 3 °C/min up to 220 °C, which was maintained and run for 30 minutes. The injection volume used was 3  $\mu$ L and a split ratio of 10:1. A Hewlett Packard 6890 Series Gas Chromatograph with Chemstations version A.04.02 was used in the analysis. The GC was outfitted with a flame (Palo Alto, CA, USA) ionization detector, auto-sampler and auto-detector. The fatty acid methyl ester peaks in the samples were identified by comparing their retention times with those of a standard mixture of fatty acid methyl esters. The coefficient of variation for whole blood fatty acid analysis is <6%.

## Omega-3 Status and Omega-3 Index Comparison

For the purpose of this study the whole blood omega-3 status is defined as the percentage of the long chain polyunsaturated fatty acids, eicosapentanoic acid (EPA) + docosahexanoic acid (DHA) relative to total fatty acid content in the whole blood.

To facilitate comparison with published data on the red cell omega-3 Index, the whole blood omega-3 status as determined in this study was converted to the equivalent omega-3 Index using an average of the conversion factors published by Bell *et al.* for EPA (average of 1.3582 and 1.3186) = 1.3384 and DHA (2.2121 and 2.2202) = 2.2242 [28].

## Statistical Analysis

All statistical analyses were performed using SPSS for windows version 19.0. The means and standard deviations of the Omega-3 Index values represent the estimated values. Levene's test for equality of variances showed that the equality of the variances assumption cannot be rejected for all separate groups (i.e. gender) comparisons. Two sample t-test is used for comparing group means and chi square statistics for comparing categorical variables (i.e. gender versus disease cut points). Statistical significance is reported at 0.05, 0.01 and 0.001 levels.

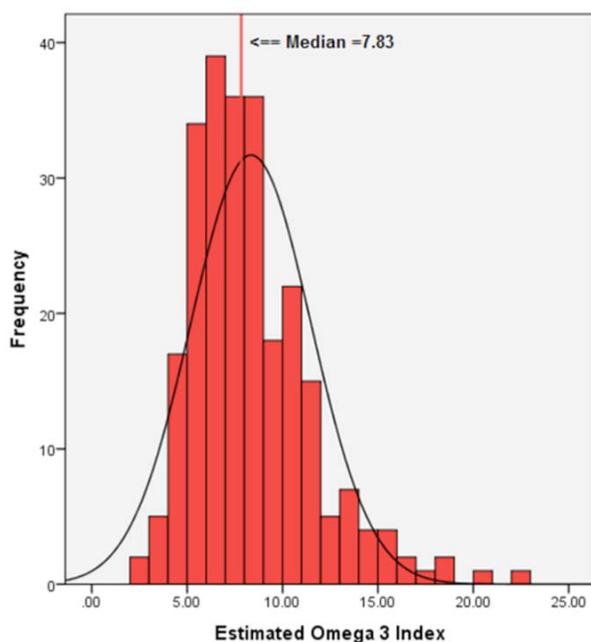
## Ethical Approval

This study was conducted in accordance with the Helsinki declaration. Approval was obtained from the

Human research Ethics Committee (HREC) of the Sydney Adventist Hospital (HREC# 06/08).

## RESULTS

Two hundred and fifty one adolescents from 10 different Sydney upper north shore (90.4%) and Central Coast (9.6%) high schools participated in this cross sectional study. The cohort consisted of 24% males and 76% females aged 15 to 17 years with a group mean BMI of 22.1 (sd=3.7). For boys the BMI ranged from 16.8 to 30.5, mean of 22.2 (sd=3.3) and for girls, 13.1 to 35.6 with a mean of 22.0 (sd=3.8). The omega-3 Index for the group ranged from 2.1 to 22.3 (Figure 1) with a mean of  $8.3 \pm 3.2$  (Table 1).



**Figure 1:** Distribution of whole blood omega-3 levels, expressed as the omega-3 Index, in a cohort of Australian adolescents aged 15-17 years, (Reference line on the X axis shows the median = 7.83).

No significant correlations were observed between BMI and Omega 3 Index for either the group as a whole or male and female subsets.

As shown in Table 1, on average males had a significant 27% higher omega-3 Index compared to females ( $p < 0.001$ ). Adolescents from low or medium socioeconomic communities had a significant 16% lower average omega-3 level compared to those from higher socioeconomic neighbourhoods. Adolescents from homes where English was not the first language showed an 11% greater omega-3 membrane content. Although this was statistically significant, the average difference between the omega-3 indices for the two groups was only 0.9 (Table 1).

As no data is currently available specifying omega-3 levels and risk of pathology in children, the established cut points for risk of disease in adults is used [23].

Table 2 shows that as a group, 53% of adolescents in this cohort have below optimum (i.e.  $< 8$ ) omega-3 levels, with 3% represented in the high risk (i.e.  $< 4$ ) category. Females had on average lower omega-3 levels than males (Table 1) with 62% showing concentrations below the optimum (i.e.  $< 8$ ) and 4% in the high risk ( $< 4$ ) category. In comparison only 24% of male adolescents tested were below the optimum (i.e.  $< 8$ ) and none were in the high risk (i.e.  $< 4$ ) category. The association between the gender and Omega 3 Index disease categories was statistically significant ( $\chi^2 = 26.9$ ,  $df = 2$ ,  $p < 0.001$ ).

A record of self-reported supplement intake was also obtained for each participant. Overall 20% of boys and 17% of girls reported regularly taking omega-3 supplements. Supplement intake was not statistically biased toward either gender. Adolescents reporting regular use of omega-3 supplements had a statistically

**Table 1:**

Demographic		Omega-3 Index Mean(sd)	Omega-3 Index Median	n	p value
All Adolescents (15-17yrs)		8.3(3.2)	7.8	251	--
Gender ***	Male	10.5(3.7)	10.1	59	<0.001
	Female	7.7(2.6)	7.3	192	
1 <sup>st</sup> Language*	English	8.1(3.0)	7.7	177	0.044
	other	9.0(3.4)	8.2	74	
SES*	High	8.5(3.0)	8.1	219	0.018
	Low/medium	7.1(3.9)	6.7	32	

Statistical significance levels \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ .

Table 2:

Omega-3 Index Disease cut-points	% of adolescents represented in each category	% of each gender represented in each category	
		Male (n=59)	Female (n=192)
<4 (high risk)	3	0	4
4-8 (moderate risk)	50	24	58
>8 (low risk)	47	76	38

significant higher whole blood omega-3 status ( $9.8 \pm 3.7$ ,  $n=44$ ) compared to adolescents not taking supplements ( $8.0 \pm 3.0$ ,  $n=203$ ) ( $p=0.001$ ). The higher the omega-3 Index the more likely the adolescent was to be taking a regular omega-3 supplement (Table 3), although the association between taking supplement and risk categories was not significant ( $p=0.074$ ).

Table 3:

Omega-3 Index Disease cut-points	% of adolescents in each category taking omega-3 supplements
<4 (high risk)	0
4-8 (moderate risk)	12
>8 (low risk)	25

## DISCUSSION

Omega-(n)-3 polyunsaturated fatty acids include the shorter chain precursor alpha-linolenic acid (ALA) and the major cell membrane components eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which are particularly rich in neuronal membranes. These have a wide range of effects, from immune-modulation and improved vascular function to synaptic plasticity.

A large body of data from studies in adult populations provides general endorsement of the biological benefits of omega-3. However relatively few studies have quantitatively measured omega-3 incorporation in adolescents.

While dietary estimates have been reported in children [29], the present investigation is one of only two studies world-wide that have reported quantitatively measured omega-3 levels in an adolescent population.

The average omega-3 Index reported for the cohort of 15 to 17 year old adolescents in the present study was higher than that recently reported for a population of West Australian 13 to 15 year olds;  $8.3 \pm 3.2$  vs

$4.9 \pm 1.0$  [22]. While a very significant 53% of adolescents in the present study were still below the optimum omega-3 Index of >8, only 3% of this cohort had an omega-3 Index of <4 placing them in the high risk category for degenerative disease (Table 2). This was considerably less than the 15.5% reported for the West Australian cohort [22]. This discrepancy was unexpected though likely reflects key differences between the two groups. Both adolescent groups represent populations living close to large coastal Australian cities. However the significant geographical separation of the two populations, one on the east and the other the west coast of the continent may be a factor. In addition the majority (87%) of adolescents in the current study were from homes categorised as having high SES while the West Australian cohort were derived from suburbs categorised as medium SES (i.e. Subiaco, WA). Our data indicates that children from higher SES homes have a higher average omega-3 Index (Table 1). This observation is consistent with that reported by O'Sullivan *et al.* who observed a correlation between maternal education, which is linked to SES, and omega-3 Index [22]. A high proportion (i.e. 29%) of adolescents within our east coast cohort were also from migrant families, a demographic that also reported higher average omega-3 levels (Table 1). Finally the mean omega-3 Index recorded in our east Australian cohort is closer to that recently reported for adult populations  $7.0 \pm 1.9\%$  [30],  $6.1 \pm 1.5\%$  [31]. In addition to the points already mentioned it is possible that the higher proportion of older students in our cohort, reflecting a more developed physiology, may also have contributed to a higher average omega-3 Index. Unfortunately no data is currently available for comparison with an international cohort.

Irrespective of the relative difference between the present adolescent cohort and the previous study, the high percentage of adolescents presenting with less than optimal omega-3 Index values may be of some concern. Adolescence is a time of considerable growth and organ development both systemically and within the central nervous system. Omega-3 PUFAs influence a variety of cell/physiological activities including;

membrane fluidity, neural plasticity, vascular function and anti-inflammatory immune signalling. Reduced omega-3 levels are therefore associated with the pathophysiology of disorders such as cardiovascular disease, depression, anxiety, diabetes and asthma [5-9].

While effects on vascular pathology may not be clinically obvious in the adolescent, O'Sullivan *et al.* did report a reduction in cardiovascular risk factors in adolescent boys with increasing omega-3 levels [22]. Consistent with a greater prevalence in females, reduced omega-3 intakes have also been linked to the rise in adolescent melancholia, particularly among females [20]. Multiple mechanisms undoubtedly contribute to the development of depression. However, as adolescence is a critical stage in the expansion of neural networks, lower brain omega-3 levels may negatively influence the neurodevelopment of robust emotion-associated signalling [32].

Ensuring adequate levels of omega-3 incorporation in the developing adolescent seems reasonable to ensure optimum organ growth and development. Both this study and the West Australian study indicate that the majority of Australian adolescents are probably not ingesting sufficient amounts of omega-3 to provide optimal health [22]. This observation is likely to reflect the omega-3 status in adolescent populations of other global locations where western-style nutrition patterns predominate. However this hypothesis awaits further quantitative confirmation.

The data presented in this report is consistent with the proposition that modern urban communities are at risk of omega-3 deficiency [33]. As food security for developed nations like Australia has been achieved in part through the manufacture of edible products with long shelf lives, most packaged items are low in the easily oxidisable omega-3 PUFAs. Therefore as most commonly consumed foods will be low in omega-3 rich PUFAs, recommendation of a high quality supplement is arguably the most effective method of correcting this apparent state of omega-3 fatty acid insufficiency.

## LIMITATIONS

Though the significant observations in this study are statistically valid it is relevant to note that the general applicability of some findings may be affected by two factors unique to this cohort. 1) Participants recruited represent a generally privileged demographic within Australia with the largest percentage of students from homes in the high socioeconomic band. However as

this demographic generally has the education and financial facility to provide a best-case-healthy environment for their children, the significant number of adolescents showing below optimum omega-3 levels even in this cohort suggests these results may underestimate the problem in the wider Australian community. 2) This study cohort also had a higher percentage of girls than boys. Thus as stated previously, the observed gender bias needs to be verified. Finally the clinical relevance of the calculated omega-3 index remains to be verified for an adolescent population as these cut points and calculations are based on research carried out in adults.

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## COMPETING INTERESTS

No relevant disclosures.

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