Title: A Comparative Validation of a Child Food Frequency Questionnaire using Red Blood Cell Membrane Fatty acids

Keywords children; validation; food frequency; dietary assessment
Short running head: Biomarker Validation study
Type of Manuscript: Original article
Word counts: Abstract 198
Manuscript – 2993
Tables: 1 and 1a Figures: 1a and 1 b

Authors
Burrows, T. [1], Berthon, B [2], Garg, M.L. [3] Collins C.E. [1]
[1] School of Health Sciences, Faculty of Health, University of Newcastle, Newcastle, NSW, Australia, 2308, [2] Department of Respiratory and Sleep Medicine, Hunter Medical Research Institute, John Hunter Hospital, Newcastle, NSW, Australia 2310. [3] School of Biomedical Sciences & Pharmacy, Faculty of Health, University of Newcastle, Callaghan, NSW, Australia

Address of institutions at which the work was carried out
University of Newcastle, Callaghan, NSW, Australia, 2308
Details of roles of each author:
TB and CC were responsible for study design, data collection. MG responsible for fatty acid analysis, BB contributed to data analysis and interpretation. All authors contributed to, read and approved the final manuscript.

Full Author Details
Corresponding author: Dr Tracy Burrows
PhD, BHSc (N&D) APD
School of Health Sciences, Faculty of Health, University of Newcastle,
University Drive, Callaghan, Newcastle, NSW, Australia, 2308
Ph. +61 2 49 217374
Fax +61 2 49 216984

Email Tracy.burrows@newcastle.edu.au

Ms Bronwyn Bertthon
2009: 4th year Bachelor Nutrition and Dietetics student
School of Health Sciences, Faculty of Health, University of Newcastle,
University Drive, Callaghan, Newcastle, NSW, Australia, 2308
Email: Bronwyn.berthton@newcastle.edu.au
2010 Current: BHSc (N&D) APD Department of Respiratory and Sleep Medicine
Hunter Medical Research Institute, John Hunter Hospital, Newcastle, NSW, Australia, 2310 Email:
Bronwyn.berthon@newcastle.edu.au

Prof Manohar Garg
Nutraceuticals Research Group
School of Biomedical Sciences & Pharmacy
University of Newcastle
Callaghan, NSW 2308, Australia
manohar.garg@newcastle.edu.au

Prof Clare Collins
PhD, BSc, Dip Nutr&Diet, Dip Clin Epi, AdvAPD, FDAA
Professor in Nutrition and Dietetics
School of Health Sciences, Faculty of Health, University of Newcastle,
University Drive, Callaghan, Newcastle, NSW, Australia, 2308
Email Clare.Collins@newcastle.edu.au

Corresponding Author and Author to respond to reader requests
Tracy Burrows Email Tracy.burrows@newcastle.edu.au
School of Health Sciences, Faculty of Health, University of Newcastle,
University Drive, Callaghan, Newcastle, NSW, Australia, 2308

Ph. +61 02 49 217374
Fax +61 02 49 216984

Conflicts of Interest:

The authors declare no conflicts of interest

Abstract

Background: There are limited validated tools available for the assessment of dietary intake in pediatric populations. This report describes a comparative validation study of selected fatty acid intakes in children assessed by food frequency questionnaire (FFQ), compared to erythrocyte membrane fatty acids.

Methods: Forty-six overweight and 47 healthy weight children aged 5-12 years (mean ± SD, 9.1 ± 1.3years, body mass index 20.5 ± 4.0) were recruited; dietary fatty acid intakes assessed by parent report using a 135-item semi-quantitative FFQ, were compared with selected child erythrocyte membrane fatty acids assessed from fasting samples using gas chromatography. Spearman's rank correlation coefficients were calculated between fatty acid intake estimates (% of energy) and erythrocyte membrane concentrations (%mol/mol).

Results: Significant correlations were found between dietary and erythrocyte eicosapentanoic acid (EPA) concentration ($r=0.24$, $P<0.05$) with a statistical trend for total omega three (∑n-3) fatty acids ($r=0.22$, $P=0.06$) and linoleic acid ($r= 0.32$, P 0.07) in the healthy weight children only.

Conclusion: Parental report of selected child fatty acid intakes using an FFQ can be used to provide an estimate of child intake of EPA, but further work is required to quantify this relationship for other fatty acids and in other populations.

Introduction

Long chain polyunsaturated fatty acids (PUFAs) including n-3 and n-6 are associated with a variety of health benefits, particularly cardiovascular health (1). Parent n-3 (ALA) and n-6 (18:2) PUFAs cannot be synthesized endogenously, therefore, are exclusively of exogenous origin (2). In contrast,

monounsaturated fatty acids (MUFA) and saturated fatty acids (SFA's) can be synthesized and thus are not suitable as biomarkers in dietary validation studies. Measurement and evaluation of the dietary intakes of children is essential to allow exploration of diet: disease relationships and to identify food patterns that optimize health outcomes. Validation of a dietary intake assessment tool evaluates its accuracy and precision and whether results arising from its use are reproducible or representative of usual intakes (3, 4).

The Australian Child and Adolescent Eating Survey (ACAES) has previously been evaluated in children for validity and reliability (5) (6). Repeat FFQ's were compared with multiple 24 hour recalls in children 10-16 years (5) and parental report of child fruit and vegetable intake using plasma carotenoids (6). Erythrocyte membrane fatty acids are nutritional biomarkers which have been shown to reflect dietary intakes of specific fatty acids consumed in the previous few months in adults and children (7-11). There are limited studies that have assessed a comprehensive range of dietary fatty acid intakes in children (12-14), and even fewer have compared them to red blood cell (RBC) fatty acid biomarkers. Previous studies in children have largely involved participants with metabolic abnormalities or with existing medical conditions (15, 16) or have used plasma fatty acids as the comparator, or have employed dietary methods which may not represent habitual or usual dietary intakes, such as 24hr food recalls (17, 18) that do not reflect similar time periods to the chosen biomarker. Alternatively food frequency questionnaires may have been used which were originally developed for adults and not specifically for use with children (11, 16, 19, 20). The increased prevalence of childhood overweight and obesity internationally (21), coupled with research suggesting that child food intake patterns track through to adulthood (22), has led to an increased interest in exploring the dietary intakes of children (23). Emerging literature suggests that the fatty acid composition of ingested fatty acids is associated with the increased prevalence of obesity (24-26). This makes the assessment of a child's fat intake of high interest, as early detection of high risk dietary behaviors could aid in the development of targeted dietary interventions and early prevention of chronic disease.

The aim of this study was to examine the comparative validity of the Australian Child and Adolescent Eating survey (ACAES) FFQ in assessing essential fatty acid intakes by comparison of estimated dietary fatty acids with erythrocyte membrane fatty acids.

Methods

Subjects

Participants for this analysis were recruited as two independent groups

1. A group of 46 overweight (n=23) and obese (n=23) children, aged 5-12 years from the Hunter Illawarra Kids Challenge Using Parent Support (HIKCUPS) study (27) who had their baseline dietary and blood samples assessed for fatty acid composition (28% of total HIKCUPS sample). Children were selected if the child had a baseline blood collection, their parents had been randomized to receive the nutrition intervention and parents indicated that their children were not taking vitamin supplements. Methods of the HIKCUPS study are published elsewhere (27).

2. Forty-seven children from the healthy weight category defined by sex specific BMI z scores (28) and not taking vitamin supplements were recruited from a similar age range and the same geographic location (Hunter Region, NSW, Australia, population approximately 550 000) as children from the HIKCUPS study. Parents had previously participated in a primary school nutrition study and had agreed to being re-contacted about future research studies (29). Parents were invited to participate in the current study to be controls and undergo the same dietary assessments and data collection and children were only included if written parental consent as well as child assent was provided. All participants from both groups were recruited during April 2005 and July 2006. All children attended a morning assessment session with their parent or care giver for data collection. These sessions were held at the University of Newcastle and a variety of measures were collected as described below. Ethics approval was obtained from The University of Newcastle Human Research Ethics Committee.

Anthropometry

Subjects were weighed in light clothing to the nearest 100g with Tanita HD646 scales (Tanita Corporation, IL) (6). Height was measured using the stretch stature method to the nearest 0.1cm with PE87 portable stadiometers (Mentone Educational Centre, Victoria, Australia) (6). Waist circumferences were measured at the level of the midpoint between the lower costal border and the iliac crest with non-extensible steel tape measures (6). Procedures for anthropometric measures were performed as per the International Society for the Advancement of Kinanthropometry (ISAK) (30). Two measures were taken with a third being collected if there was large a discrepancy between the previous two (6). Body Mass Index (BMI) was calculated (kg/m^2) and a computer program was used to calculate BMI *z*-scores using the LMS methods (31).

Dietary Assessment

Dietary Intake was reported for children by parents or main care givers using the ACAES, a 135 item semi-quantitative food frequency questionnaire (FFQ) with a reporting period of the previous 6 months. There were specific food items for major sources of omega 3 fatty acids including eicosapentanoic acid (EPA) and docasohexaenoic acid (DHA): fish, seafood, nuts, eggs and meat (32). Portion sizes for individual food items were accessed from the Australian Bureau of Statistics (ABS) and unpublished data from the 1995 Australian National Nutrition Survey(33) or the "natural" serving size for common items such as a slice of bread. The frequency options ranged from 'never' to '≥4 times per day' but varied depending on the food item.

Analysis of the FFQ was performed using the Australian AusNut 1999 database (All Foods) Revision 17 and AusFoods (Brands) Revision 5, both of which were accessed through FoodWorks version 4.00.1158, 2005 (Xyris Software, Brisbane, Queensland, Australia) (34). This analysis produced mean daily intakes of energy, macro- and micronutrients. An additional analysis was undertaken using the FoodWorks Fatty Acid Compositional database 2001 (35) to estimate all individual mean daily fatty acid intakes (Table 1a) including EPA and DHA, total SFA, MUFA, PUFA, total (n-3) and very long chain n-3 (VLCn-3) fatty acids.

Blood Sampling & Biochemical Analysis

Blood samples were collected from subjects after an overnight fast by trained phlebotomists in EDTA-coated tubes, with approximately 5ml of blood collected. An accredited pathology service (National Association of Testing Authorities, Australia) firstly analyzed the samples via standard automated techniques for blood lipids including: total cholesterol and cholesterol fractions, triacylglycerol, insulin and C-reactive protein. The samples were then centrifuged to separate plasma from erythrocytes. Aliquots of erythrocyte samples were frozen within 2 hours to -80ºC. Samples were thawed within 6 months and erythrocyte pellets were washed 10 times with cold buffer (50mM Tris-HCL,pH7.5, containing 5mM EDTA and 1mM dithiothreitol), vortexed and centrifuged at 8500xG at 4°C for 10 minutes. After discarding the supernatant the erythrocyte pellet was lyzed four times with cold deionised water, vortexed, centrifuged and stored at -70° until analysis.

A comprehensive range of erythrocyte membrane fatty acids (Table 1a) were analyzed by gas chromatography (GC) based on the methods of Lepage and Roy (1986) (36). Two ml of methanol/toluene (4:1, by vol) was mixed with C19:0 (0.2mg/mL) and BHT (0.12g/L), added to the

erythrocyte pellet and vortexed. Whilst vortexing the sample, 200μL acetyl chloride was added drop-wise to methylate the fatty acids, followed by heating to 100°C for one hour. Samples were allowed to cool, and the reaction was stopped by the addition of 5mL of 6% K_2CO_3 and vortexed. The samples were centrifuged at 3000xG at 4°C for 10 minutes to separate the layers. The upper layer containing toluene and fatty acid methyl esters was transferred to a 2mL glass vial, crimp sealed with a teflon lined cap ready for GC analysis. GC analysis was performed with a 30m x 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 two minutes, increased by 10°C/min to 190°C, held for one minute then increased by 3°C/min up to 220°C which was maintained and run for 30 minutes. The injection volume used was 3μL 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 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 RBC fatty acid analysis is less than 6%.

Statistical Analysis

Energy intakes were assessed to identify subjects with extreme, implausible energy intakes (>16080 kJ/d and <5354 kJ/d (4)). Subjects with implausible energy intakes (n= 5), those without enough blood for fatty acid analysis (n= 11) and incomplete dietary data (n=2) were excluded from the analysis, with a total of 75 subjects included. Data was assessed for normality with the majority of dietary fatty acid intake and erythrocyte fatty acid distributions not normally distributed, thus non-parametric tests were used. Differences between groups and gender were assessed with Kruskal-Wallis tests. Any difference between groups was further tested using the all pairs Tukey Kramer HSD test. Spearman's rank correlation coefficients were calculated between the dietary fat intakes (both as grams /day and % daily energy intake) and erythrocyte concentrations (%mol/mol) for selected fatty acids. Correlations were assessed as poor (<0.2), moderate (0.2-0.6) or strong (>0.6) (37). Multivariate linear regression models were used with a backward stepwise approach to investigate the contribution of individual variables. The model included the potential confounding variables (energy intake, gender, age and as a measure of weight status either weight or BMI). Estimates for total n-3 group erythrocyte fatty acids were calculated by summing values of ά linoleic acid ($C_{18:3n-3}$), EPA ($C_{20:5n-3}$), DPA ($C_{22:5n-3}$) and DHA

($C_{22:6n-3}$). Statistical analysis was completed using SPSS version 15 (SPSS Inc, Chicago, IL, USA). P values <0.05 were considered significant.

Results and Discussion

A total of 75 subjects (53% boys) were included in current analysis. There were no significant differences between anthropometric and metabolic characteristics of boys and girls and results were pooled (Table 1). Dietary intakes and dietary fatty acids are summarized in Table 1 and 1a, and demonstrate that children derived approximately 28% of energy from fat, with 12% from SFA, 10% MUFA and 3% PUFA. Of the total dietary PUFA intake the greatest proportion was from n-6 fatty acids, which is similar to other recent reports in children (38, 39). Seafood, including fresh and canned fish, was the major dietary source of omega 3 fatty acids. For EPA, moderate correlations were found between dietary EPA intake (%total energy intake) and erythrocyte membrane concentration, r= 0.24 (P =0.04) (Figure 1a) and for total n-3 intake (%total energy intake) and erythrocyte membrane concentration, r=0.22 (P= 0.06) (Figure 1b). The EPA values for some of the participants were indeed close to zero. There were no other significant correlations between dietary intake and erythrocyte membrane fatty acids for any other fatty acids analyzed (Table 1a). Results from the multivariate regression demonstrated that none of the confounding variables (energy intake, gender, age, absolute weight or BMI) were statistically significant (P>0.05) when examining the relationship between dietary intake and RBC fatty acid concentrations (%mol/mol). Specifically the relationships with EPA and total n-3 did not change.

In the sub-group categorized as obese, dietary intake of total n-3 (%total energy intake) and erythrocyte fatty acids were significantly and positively correlated, r= 0.66 (P=0.006). A trend was observed between linoleic acid (%total energy intake) and erythrocyte fatty acids in those of a healthy weight, r=0.32 (P= 0.07). Unlike a recent study in young children (14), no trends were seen for DHA (22:6n-3), possibly due to the low estimated dietary DHA intakes (0.06 ± 0.05 g/day), total n-6 fatty acids (6.53± 2.52 g/day) and total n-3 (0.74 ±0.28 g/day) with large standard deviations noted in intakes and total intakes below the suggested Australian and international target dietary intakes for children (40). Australian recommendations advocate 12-20 g/day or 6-10% energy from total n-6 and 0.16-0.43 g/day or 0.08-0.22% energy from LC n-3 (41, 42). Few studies have undertaken a comparative fatty acid intake validation of the essential fatty acids, examining the relationship between

intakes estimated from a food frequency questionnaire with erythrocyte membrane fatty acids within a pediatric population. Correlations were mostly low to moderate but strong for total n-3 fatty acids and were statistically significant. This demonstrates that the ACAES FFQ can provide an estimate of EPA intakes with promising results for n-3 and LA intakes, with further validation studies required in larger and more diverse populations. In this study correlations were found for EPA but not DHA. The correlation between dietary EPA intake and RBC EPA concentrations potentially reflects dietary intakes of ALA (0.73 ± 0.29g/ day), which is de-saturated and elongated to EPA, whereas there is little/ no further conversion of EPA to DHA

It has been reported that strong correlation coefficients cannot be expected for biomarkers of dietary fatty acid intake (2), because erythrocyte fatty acid concentrations are influenced by individual variation in absorption and metabolism of fatty acids and factors affecting metabolic efficiency, including obesity (2).

A limitation of estimating dietary fatty acids intakes includes the fatty acid composition database used to estimate intakes from the FFQ. Food composition and types available do change over time but fatty acid compositions databases are limited in both the number of foods they contain that have been analyzed and the frequency with which they are updated, even though the current study utilized a fatty acid database which has >1000 Australian foods (35). In the current study dietary intakes were assessed using an FFQ which are associated with a respondent bias when investigating the association between dietary and RBC fatty acids (Okuda et al 2009). In the current study the correlations of all subjects may be overestimated because the conditions of overweight/obesity are more prevalent than in the general population. Given these limitations, the proxy parental reports obtained from the ACAES still demonstrated a moderate correlation with total n-3 intake and with EPA intake using erythrocyte fatty acid concentrations as the biomarker. However, the weight status of some children in the study may have influenced the degree of under-reporting, which has been shown to be greater in children and adults who are overweight and obese (2, 43). While some statistically significant correlations were shown for Australian children 5-12yrs , the current work should be undertaken in other ethnically diverse and socio economic populations which merits further investigation due to differences in dietary habits.

The ACAES FFQ retrospectively measured dietary intake over the previous six month period. In contrast, erythrocyte membranes fatty acid concentrations may reflect dietary intake over a shorter period of time (44) (45). In a study examining incorporation of n-3 fatty acids into various tissue samples, the EPA levels in erythrocyte membranes were shown to reflect dietary EPA intakes over the previous couple of months (46). The difference in the reference period for the FFQ and the biomarker may have reduced correlations if dietary intake had changed in the three month period prior to the blood sample collection (7). While a more suitable biomarker of usual fat intake, such as adipose tissue, is able to capture dietary intake over a longer time period, obtaining a specimen from children, it is not likely to be realistic, but could be explored in future validation studies with adults (44). The FFQ asks questions about fish intake but does not distinguish between oily and non-oily fish and did not include foods enriched with n-3 fatty acids such as breads, eggs margarine and milk, which are now commonly available. Even though seafood, omega-3 enriched eggs and meat contain n-3 PUFAs, in Australian children oily fish have been identified as the major source of LC n-3 fatty acids (32)

Conclusions

Biochemical measurement of specific nutrients can be used to evaluate the degree to which reported dietary intakes can be believed as being representative of usual intake. This study reports the comparative validation of a child-specific FFQ against erythrocyte membrane fatty acids. Weak to moderate, but statistically significant correlations were found between parent reported intakes and objectively measured EPA, n-3 and LA membrane fatty acids. Further studies are required to establish the validity across a broader spectrum of fatty acids and in more diverse populations.

References:

1. Connor W. Importance of n-3 fatty acids in health and disease. American Journal of Clinical Nutrition. 2000;71:171S-5S.

2. Arab L. Biomarkers of Fat and Fatty Acid Intake. J Nutr. 2003;133:925S-32S.

3. Beydoun MA, Kaufman JS, Ibrahim J, Satia JA, Heiss G. Measurement error adjustment in essential fatty acid intake from a food frequency questionniare: alternative approaches and methods. BMC Med Res Methodol. 2007;7:41-55.

4. Willett W. Nutritional Epidemiology. 2nd edn ed. New York: Oxford University Press; 1998.

5. Watson J, Collins C, Sibbritt D, Dibley M, Garg M. Reproducibility and comparative validity of a food frequency questionnaire for Australian children and adolescents. International Journal of Behavioral Nutrition and Physical Activity. 2009 11 September;6(62).

6. Burrows TL, Warren JM, Colyvas K, Garg ML, Collins CE. Validation of overweight children's fruit and vegetable intake using plasma carotenoids. Obesity. 2008;17:162-8.

7. Feunekes G I, Van Staveren WA, De Vries JH, Burema J, Hautvast JG. Relative and biomarker-based validity of a food-frequency questionnaire estimating intake of fats and cholesterol. Am J Clin Nutr. 1993;58:489-96.

8. Parra MS, Schnaas L, Meydani M, Perroni E, Martinez S, Romieu I. Erythrocyte cell membrane phospholipid levels compared against reported dietary intakes of polyunsaturated fatty acids in pregnant Mexican women. Public Health Nutr. 2002;5(6a):931-7.

9. Sullivan BL, Williams PG, Meyer BJ. Biomarker Validation of a Long-Chain Omega-3 Polyunsaturated Fatty acid Food Frequency Questionnaire. Lipids. 2006;41(9):845-50.

10. Sun Q, Ma J, Campos H, Hankinson SE, Hu FB. Comparison between plasma and erthrocyte fatty acid content as biomarkers of fatty acid intake in US women. Am J Clin Nutr. 2007;86:74-81.

11. Orton HD, Szabo NJ, Clare-Salzler M, Norris JM. Comparison between omega-3 and omega-6 polyunsaturated fatty acid intakes as assessed by a food frequency questionnaire and erythrocyte membrane fatty acid composition in young children. Eur J Clin Nutr. 2008;62:733-8.

12. Decsi T, Molnar D, Koletzko B. Long-Chain Polyunsaturated Fatty Acids in Plasma Lipids of Obese Children Lipids. 1996;31(3):305-11.

13. Gil-Campos M, Ramirez-Tortosa MC, Larque E, Linde J, Aguilera CM, Canete R, et al. Metabolic Syndrome Affects Fatty Acid Composition of Plasma Lipids in Obese Prepubertal Children. Lipids. 2008;43:723-32.

14. Uusitalo L, Nevalainen J, Salminen I, Ovaskainen M, Kronberg-Kippilä C, Ahonen S, et al. Fatty acids in serum and diet – a canonical correlation analysis among toddlers. Maternal and Child Health. 2011(Nov). DOI: 10.1111/j.1740-8709.2011.00374

15. Colombo C, Bennato V, Costantini D, Valmarana L, Dacco V, Zazzeron L, et al. Dietary and circulating polyunsaturated fatty acid sin cystic fibrosis: are they related to clinical outcomes? J Pediatr Gastroenterol Nutr. 2006 November 2006;43(5):660-5.

16. Vlaardingerbroek H, Hornstra G, de Koning TJ, Smeitink JAM, Baaker HD, de Klerk HBC, et al. Essential polyunsaturated fatty acids in plasma and erythrocytes of children with inborn errors of amino acid metabolism. Mol Genet Metab. 2006;88:159-65.

17. Moilanen T, Rasanen L, Viikari J, Akerblom HK, Nikkari T. Correlation of serum fatty acid composition with dietary intake data in children and young adults. Ann Med. 1992 February 1992;24:67-70,.

18. Guerra A, Demmelmair H, Toschke AM, Koletzko B. Three-year tracking of fatty acid composition of plasma phospholipids in healthy children. Ann Nutr Metab. 2007;51:433-8.

19. Fukushima T, Hojo N, Isobe A, GaoT, Shiwaku K, Yamane Y. Food intake, serum lipids and amino acids of school children in agricultural communities in Japan. Eur J Clin Nutr. 1999;53:207-10.

20. Innis SM, Vaghri Z, King J. n-6 Docosapentaenoic acid is not a predictor of low docosahexaenoic acid status in Canadian pre-school school. Am J Clin Nutr. 2004;80:768-73.

21. Wang Y, Lobstein T. Worldwide trends in childhood overweight and obesity. International Journal of Pediatric Obesity. 2006;1:11-25.

22. Demory-Luce D, Morales M, Nicklas T, Baranowski T, Zakeri I, G. B. Changes in food group consumption patterns from childhood to young adulthood: the Bogalusa Heart Study. Journal of the American Dietetic Association. 2004;104(11):1684-91.

23. Collins CE, Warren JM, Neve M, McCoy P, Stokes BJ. Measuring Effectiveness of Dietetic Interventions in Child Obesity. A Systematic Review of Randomized Trials. Arch Pediatr Adolesc Med. 2006;160:906-22.

24. Allhaud G, Guesnet P. Fatty acid composition of fats is an early determinant of childhood obesity: a short review and opinion. Obesity Reviews. 2004;5:21-6.

25. Karlsson M, Marild S, Brandberg J, Lonn L, Friberg P, Strandvik B. Serum Phospholipid Fattt Acids, Adipose Tissue, and Metabolic Markers in Obese Adolescents. Obesity. 2006;14(11):1931-9.

26. Micellef M, Munro I, Phang M, Garg M. Plasma n-3 polyunsaturated fatty acids are negativly associated with obesity. British Journal of Nutrition. 2009;102(9):1370-4.

27. Jones R, Okely A, Collins C, Morgan P, Steele J, Warren J, et al. The HIKCUPS Trial: a multi site randomised controlled trial of a combined physical activity skill development and dietary modification program in overweight and obese children. BMC Public Health. 2007;7(15).

28. Cole T, Bellizzi M, Flegal M, Dietz W. Establishing a standard definition for child overweight and obesity worldwide: international survey. BMJ. 2000 6th May;320.

29. Finch M, Sutherland R, Harrison M, Collins CE. Canteen purchasing practices of year 1-6 primary school children and assosciation with SES and weight status. Aust N Z J Public Health. 2006;30:247-51.

30. Marfell-Jones M, Olds T, Stewart A, Carter L. International Standards for Anthropometric Assessment. Potchefstroom: South Africa; 2006.

31. Cole T, Pan H. LMS growth computer program. Cambridge: Medica lResearch Council; 2002.

32. Oddy W, Sherriff J, Kendal G, De Klerk NH, Mori T, Blake K, et al. Patterns of fish consumption and levels of serum phospholipid very long chain omega 3 fatty acids in children with and without asthma living in Perth, Western Australia. Nutrition and Dietetics: The Journal of the Dietitians Association of Australia,. 2004;61:31-7.

33. Cook T, Rutishauser I, Allsopp R. The bridging study-comparing results from the 1983,1985 and 1995 Australian national surveys: Commonwealth Department of Health and Aged Care2001.

34. Goodhill C. Nutrient Calculation Software. Brisbane, Australia: Xyris Software; 1998.

35. Mann NJ, Sinclair AJ, Percival P, Lewis JL, Meyer BJ, Howe PRC. Development of a database of fatty acids in Australian foods. J Nutr Diet. 2003;60(1):42-5.

36. Lepage G, Roy GC. Direct transesterification of all classes of lipid in a one step reaction. J Lipid Res. 1986;27:114-20.

37. McNaughton SA, Hughes MC, Marks GC. Validation of FFQ to estimate the intake of PUFA using plasma phospholipid fatty acids and weighed food records. Br J Nutr. 2007;97:561-8.

38. Madden S, Garrioch C, Holub B. Direct Diet Quantification Indicates Low Intakes of (n-3) Fatty Acids in Children 4 to 8 years old. Journal of Nutrition. 2009;139:1-5.

39. Lien V, Clandinin M. Dietary Assessment of Arachidonic Acid and Docosahexaenoic Acid Intake in 4-7 Year-Old Children. Journal of the American College of Nutrition. 2009;28:7-15.

40. Meyer B, Mann N, Lewis J, Milligan G, Sinclair A, Howe P. Dietary Intakes and food sources of omega 6 and omega 3 polyunsaturated fatty acids. Lipids. 2003;38(4):391-8.

41. NHMRC. Report of the NHMRC working party;The role of polyunsaturated fats in the Australian diet. Canberra1992.

42. National Heart Foundation. A review of the relationship between dietary fat and cardiovascular disease. Australian Journal of Nutrition and Dietetics. 1999;56(4S):S5-S22.

43. McPherson RS, Hoelscher DM, Alexander M, Scanlon KS, Serdula MK. Dietary Assessment Methods among School-Aged Children: Validity and Reliability Prev Med. 2000;31:S11-33.

44. Baylin A, Kim MK, Donovan-Palmer A, Siles X, Dougherty L, Tocco P, et al. Fasting Whole Blood as a Biomarker of Essential Fatty Acid Intake in Epidemiologic Studies: Comparison with Adipose Tissue and Plasma. Am J Epidemiol. 2005 November 12 2004;162(4):373-81.

45. Hodson L, Skeaff CM, Fielding BA. Fatty acid composition of adipose tissue and blood in humans and its use as a biomarker of dietary intake. Prog Lipid Res. [Review]. 2008;47:348-80.

46. Katan MB, Deslypere JP, van Birgelen APJM, Penders M, Zegwaard M. Kinetics of the incorpooration of dietary fattyacids into serum cholesteryl esters, erythrocyte membranes, and adipose tissue: an 18-month controlled study. J Lipid Res. 1997;38:2012-22.

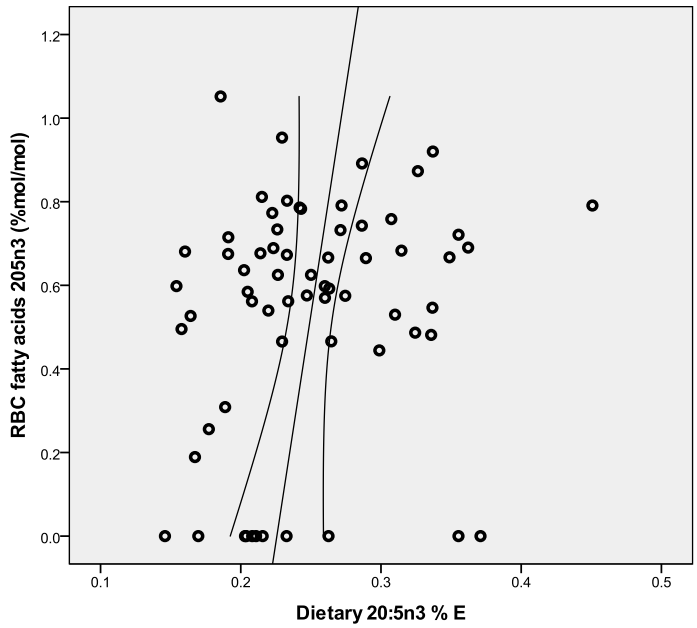

Figure 1a: Correlation between dietary intake of EPA (20:5n-3), as a percentage of total energy intake, and red blood cell membrane as %mol/mol for n= 75 children, (r =0.24, P=0.04). Lines represent the mean and 95% confidence interval

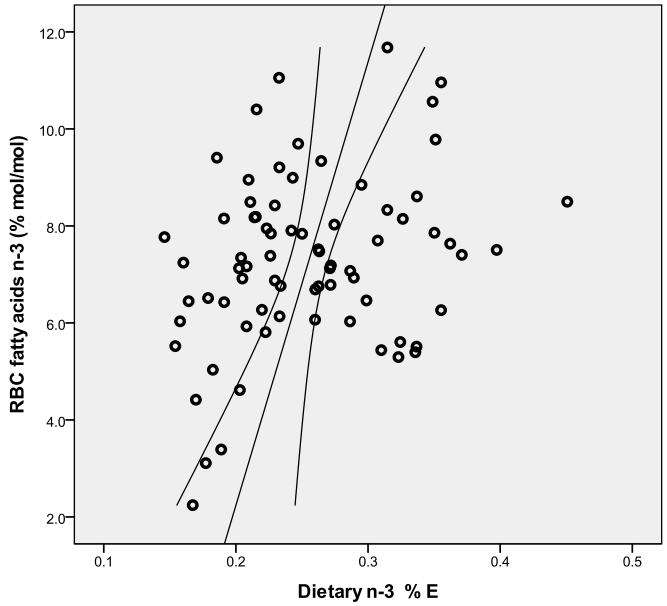

Figure 1b: Correlation between dietary intake of total omega 3 (n-3) as a percentage of total energy intake, and red blood cell membrane as %mol/mol for n= 75 children, (r =0.22, P=0.06). Lines represent the mean and 95% confidence interval

429

430