DHA-enriched re-esterified triacylglycerol fish oil supplementation and oily fish consumption enhance red blood n-3 fatty acid index in Omani pre-adolescent schoolchildren

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ABSTRACT

Dietary habits of Omani population particularly of children and young adults have changed significantly. Consumption of imported calorie-dense foods, vegetable oils, milled and polished grains and carbonated beverages have become the norm. Concomitantly, there has been an exponential increase in the prevalence of non-communicable diseases. The impact of the westernisation of eating habits on children has not been evaluated. We have investigated blood fatty acid profile of male (n=125) and female (n=160) children aged 9 and 10 (9.8±0.4) years enrolled from three state-funded schools. The schools, which are homogenous with respect to socio-economic background of their pupils, were randomised into fish oil (n=98), oily fish (n=82) or control (n=105) group. Subsequently, the children were given during morning tea break for 12 weeks: 1. DHA-enriched re-esterified triacylglycerol fish oil capsule with cheese/salad sandwich (fish oil group), 2. Lightly grilled oily fish with salad (fish group) or 3. Cheese/salad sandwich (control group). At baseline, the males had higher myristic, palmitic and oleic and lower adrenic acids than the females ($p<0.05$). There was no difference in n-3 fatty acid index (4.86±1.95 vs. 5.12±1.67, $p>0.05$) or AA (14.6±1.9 vs. 14.9±1.8, $p>0.05$) between the genders. There was no difference in any of the fatty acids between the three groups at baseline. Post-intervention, the oily fish group had lower n-3 fatty acid index (EPA+DHA, 6.03±1.39 vs. 6.60± 1.63, $p<0.05$) and higher AA (15.2±1.8 vs. 13.7±2.0, $p=0.0001$) and n-3 DPA (1.40±0.27 vs. 1.07±0.22, $p=0.0001$) compared with those who received fish oil capsules. In both the fish oil and oily fish groups, fatty acid index correlated positively with AA ($r=0.394$, $p=0.0001$; $r=0.231$, $p=0.038$) and negatively with total saturated ($r= - 0.816$, $p=0.0001$; $r= - 0.439$, $p=0.0001$) and total mono-unsaturated ($r= - 0.431$, $p=0.0001$; $r= - 0.231$, $p=0.037$) fatty acids. Although seafood is an integral part of traditional Omani cuisine the children had a low level of n-3 fatty acids index. There is a need to address this nutritional insufficiency through school feeding programme, targeted intervention with n-3 fatty acid enriched food products and/or family education programme.

INTRODUCTION

The traditional Omani economy was based on subsistence farming, herding, fishing and trading [1-3] and the diet of the population consisted mainly of whole grain, legumes, fruits, vegetables and fish [4-5]. However, following the discovery and exploitation of oil five decades ago, the country has undergone a major economic transformation [5] and registered a significant progress in health and social care, education and reduction in poverty and mal-nutrition [6]. In the same period of time, the prevalence of non-communicable chronic diseases, such as obesity, diabetes, cancer, high blood pressure and cardiovascular disease [7-8] and the consumption of imported energy-dense foods, vegetable oils, milled and polished grains and carbonated beverages [5, 9-10] have increased considerably. The numerous fast food outlets and restaurants dotted across towns and the supermarket ready meals have and are contributing to the transformation of the traditional dietary pattern. This change in food culture is likely to be enduring as there is evidence that children and adolescents in Oman and other Arab countries have adopted readily Western eating habits [11-12].

Injudicious consumption of contemporary Western pattern diets - high protein and fat of intensively reared land animals, refined sugar, vegetable fat and oils (mainly sunflower, safflower and corn) and low fresh fruits and vegetables and marine foods [13-16] – have played a critical role in the rise of non-communicable chronic diseases [16-18] in the last six decades. This deleterious effect is attributed primarily to an excessive amount of saturated, trans and omega 6 fatty acid and an insufficient omega 3 fatty acid and essential micro-nutrient content of the consumed diet [13, 19-20].

Cell line, experimental animal and human epidemiological and intervention studies demonstrate that the long chain omega 3 polyunsaturated fatty acids directly, or indirectly via their metabolites, play a pivotal role in neuro-visual development, function and rehabilitation [21-24], cardioprotection [25-26], modulation of inflammatory and immune responses [27-29] signal transduction and gene expression [30-32].

In light of the rapidly shifting nutrition transition in Oman, this study investigated fatty acid status of preadolescence schoolchildren before and after intervention with fish oil or oily fish.

MATERIALS AND METHODS

Subjects and Recruitment

Female (n=160) and Male (n=125) healthy schoolchildren aged 9 & 10 years (mean ± sd, 9.8±0.4) were recruited from three public (state) schools in Muscat, Sultanate of Oman. Children who attend these state-funded schools are homogenous with respect to socio-economic background and tribal affiliations. The schools were assigned at random to Oily Fish (n=82), Fish Oil (n=98) or Control (n=105) Group. Subsequently, the children were given a 100-gram lightly grilled fish sandwich (Fish Group), DHA-enriched re-esterified triacylglycerol fish oil (403 mg DHA & 53 mg EPA) and cheese/salad sandwich (Fish Oil Group) or cheese/salad sandwich (Control Group) during morning tea break for twelve weeks. Capsules were supplied free of charge by Efamol Limited, Leatherhead, Surrey, UK. The cooked oily fish used for the study, which were grouper, sea bream, kingfish, emperor and snapper, supplied 243.9±60.5 mg (range 200 - 390) n-3 fatty acids (EPA, DPA and DHA). To help improve consistency, taste, flavour and consequently compliance, a different fish sandwich was prepared daily by professional chefs at the Intercontinental City Hotel, Muscat. Blood specimen, about 8 ml, was collected in EDTA from each child before and after intervention, fractionated into plasma and red cells stored at - 80° C until transporting in dry ice to London for analysis.

Ethical Consideration and Consent

Ethical approval was obtained from the Ethics Committee of the Ministry of Health, Sultanate of Oman (Ref. MH/DGP/R&S/8/2012), and the National Research Ethics Committee North West – Haydock, UK (REC reference no. 12/NW/0760). Informed and signed consent was obtained from the parents/guardians of the children. Study registration number ISRCTN93233285.

Anthropometry and Blood Pressure

Weight (kg), height (cm) and blood pressure (mmHg) were measured with a Seca Electronic Scale 890 (UNISCALE, Seca, Birmingham B5 5QB, UK), a measuring board (Schorr, Weight and Measure, LLC, Olney, Maryland, USA) and a calibrated digital sphygmomanometer, respectively. A clinician (Al Ghannami) and three nurses performed the measurements.

Plasma Lipids

Concentrations of plasma triacylglycerol, total cholesterol, high density lipoprotein cholesterol and low density lipoprotein cholesterol were assayed on an Architect c8000 Analyser (Abbot Diagnostics, Abbot

Park, Il, USA) using reagent kits purchased form Abbot Laboratories (Abbott, Max-Planck-Ring 2, 65205 Wiesbaden, Germany).

Fatty acid analysis

Blood cells - Red blood cell lipids were extracted based on the method of Folch et al [33] by homogenising 0.5 ml of sample in 90 ml chloroform/methanol (2:1 v/v) containing the antioxidant butylated hydroxytoluene (BHT, 0.01% w/v) under nitrogen. Fatty acid methyl esters (FAMEs) were prepared by acid-catalysed transesterfication reaction by heating the extracted lipids in 4 ml methanolic acetyl chloride (15% v/v) in a tightly sealed vial at 70C° for 3 hours after degassing with nitrogen. The FAMEs were extracted with petroleum spirit, dried and then re-dissolved in heptane for separation by a gas chromatograph (HRGC MEGA 2 series, Fisons Instruments, Italy) fitted with a BPX 70 capillary column (60m x 0.25mm ID, 0.25μ film). Hydrogen was used as carrier gas at a flow rate of 2.3 ml/min and the injector and detector temperatures were set at 250 and 280°C, respectively. The initial oven temperature which was 60°C increased at 5ºC/min to 160°C, maintained for 5 min, and then increased at 3ºC/min to 205°C and kept constant for 12 minutes. The eluted peaks were identified with fatty acid methyl ester standard mixture certified for quality (Supelco® 37 Component FAME Mix. U47885-U, Sigma-Aldrich, Dorset, UK), GC-MS authenticated fatty acid methyl esters prepared from lipid extract of vegetable seed oils containing alpha-linolenic, gamma-linolenic and stearidonic acids, and bovine brain L-A-phosphatidylethanolamine Type 1 (Sigma-Aldrich, Dorset, UK). Peak areas were computed with EZChrom Ellite, version 3.2 (Scientific software, Pleasant, CA, USA).

Fish – Lightly grilled fish (muscle), 100 gram, homogenised thoroughly and a 2-gram representative sample extracted and methylated as described above. Heptadecanoic acid (C17:0) was used as an internal standard for quantification.

DATA ANALYSES

The continuous variables are expressed as mean and standard deviation (mean± sd). Statistical differences between the data of the female and male students at baseline, and between the three groups at post-intervention were evaluated with an independent (unpaired) t-test and a one-way analysis of variance (ANOVA), respectively. The Bonferroni pairwise multiple comparison post-hoc test was performed when the ANOVA F-test indicated significance. A univariate regression analysis was used to assess relationships between the major fatty acids. P values of less than 0.05 were deemed statistically

significant. Analyses were conducted with IBM SPSS statistics, version 22 (IBM Corporation, New Orchard Road, Armonk, New York 10504-1722, USA)

RESULTS

Demographics and baseline blood pressure and lipids

Age, height, weight, systolic and diastolic blood pressure, plasma triglycerides and HDL, LDL and total cholesterol levels of the children prior to the intervention are presented in table 1. There was no difference in any of the aforementioned variables between the genders ($p>0.05$).

Baseline red blood cell fatty acids

The male students had a higher level of myristic (C14:0), palmitic (C16:0) ($p<0.05$) and oleic (C18:1n-9, $p<0.01$) and lower adrenic (22:4n-6, $p<0.05$) acids compared with their female counterparts. The percentages of the other saturated, mono-unsaturated, n-6 and n-3 fatty acids and DHA sufficiency index (DHA/N-6 DPA) were not different between the two groups ($p>0.05$, table 2). There was no difference in any of the fatty acids between the three groups at baseline. The n-3 fatty acid index (EPA+DHA) correlated positively with arachidonic ($r=0.258$, $p=0.004$) and total n-6 ($r=0.504$, $p=0.0001$) and inversely with total saturated ($r= - 0.765$, $p=0.0001$) and mono-unsaturated ($r= - 0.684$, $p=0.0001$) fatty acids in the male children. Similarly, in the girls, arachidonic ($r= 0.169$, $p=0.030$), total n-6 ($r=0.340$, $p=0.0001$), total saturated ($r= - 0.677$, $p=0.0001$) and mono-unsaturated ($r= - 0.484$, $p=0.0001$) fatty acids and diastolic blood pressure ($r= - 0.207$, $p=0.049$) and body weight ($r= - 0.224$, $p=0.005$) were associated with n-3 fatty acid index.

Post-intervention red blood cell fatty acids, blood pressure and plasma lipids

Red blood cell total lipid fatty acid composition of the children after intervention with fish oil or oily fish for twelve weeks is shown in table 3. The control group compared with those who received oily fish and fish oil capsules had higher levels of lignoceric (24:0), nervonic (24:1n-9), docosapentaenoic (DPA, 22:5n-6) ($p<0.05$) and lower eicosapentaenoic (EPA, 20:5n-3), decosapentaenoic (DPA, 22:5n-3), docosahexaenoic (DHA, 22:6n-3) and total n-3 fatty acids, n-3 metabolites, n-3 index and 18:1 dimethyl acetals ($p= 0.0001$). Palmitic and total saturated fatty acids, DHA, DHA sufficiency index and n-3 index were lower ($p<0.05$) and AA, n-6 metabolites and n-3 DPA higher ($p=0.0001$) in the oily fish group than in the children supplemented with fish oil capsules. There was no post-intervention gender difference in any of the fatty acids within the same group.

In the fish oil group, n-3 index correlated positively with arachidonic (r=0.394, p=0.0001), adrenic (r=0.394, p=0.0001), n-6 DPA (r=0.562, p=0.0001) and total n-6 (r=0.414, p=0.0001) and negatively with palmitic (r= -0.762, p=0.0001), total saturated (r= -0.816, p=0.0001), palmitoleic (r= -0.384, p=0.001), oleic (r= -0.347, p=0.002) and total mono-unsaturated (r= - 0.431, p= 0.0001) fatty acids. Similarly, there was a correlation between n-3 index and arachidonic (r=0.231, p=0.038), oleic (r= -0.271, p=0.008) and total saturated (r= -0.439, p= 0.0001) and mono-unsaturated (r= -0.213, p=0.037) fatty acids in the children who received oily fish.

Post-intervention blood pressure, plasma triglycerides and LDL-cholesterol, HDL-cholesterol and total cholesterol values of the children are presented in table 4. Systolic and diastolic blood pressure (p=0.0001) and plasma triglycerides ($p<0.05$) but not HDL-cholesterol, LDL and total cholesterol ($p>0.05$) were lower in the children supplemented with fish oil compared with those who were fed oily fish. There was no gender-related difference in plasma triglycerides and HDL-, LDL- and total-cholesterols between the control and oily fish children ($p>0.05$). The boys in the fish oil group had lower triglyceride and higher HDL cholesterol levels than their female counterparts ($p<0.05$).

DISCUSSION

The primary focus of this study was the n-3 long chain polyunsaturated fatty acids which are thought to be limiting nutrients in Westernised dietary patterns. The sum of red blood cell DHA and EPA percentages, n-3 fatty acid index, was used to assess status.

There was no difference in levels of the fatty acids between the groups at baseline. This finding reflects the socio-economic homogeneity of the children of the three schools."

Previous studies have reported higher levels n-3 long chain polyunsaturated fatty acids in women than in men [34] this distinction is attributed to hormonal effect [35, 36]. In the current study, although the female children had slightly elevated levels of DHA, n-3 metabolites (EPA, n-3 DPA and DHA) and n-3 fatty acid index compared with their male counterparts, the differences were not statistically significant. This was predicable since the children were prepubescent aged 9 and 10 years and the gender-related variation in oestrogen concentration [35, 36] would not be high enough to have a measurable influence on the activity of delta 6 and 5 desaturases. Consistent with the afore-stated prediction, the delta 6

desaturase index (18:3n-6/18:2n-6) values of the boys and girls were similar (0.011±0.003 vs 0.010±0.004).

In keeping with the country's long maritime history and coastline, fish is an integral part of the Omani traditional diet. Therefore, the low mean baseline red blood cell n-3 fatty acid index of the children, which is broadly comparable to those of healthy Guatemalan [37] Danish [38] and American [39] children of a similar age group, suggests the consumption of fish might have declined among the preadolescent Omani perhaps in favour of the widely available subsidised processed foods [10]. Children aged 2 to 8 from Tanzania [40] and aged 3 to 8 from European countries [41] had a similar and a lower n-3 fatty acid index, respectively, compared to the level found in the current study. For comparative purposes, the whole blood n-3 fatty acid index values of the aforementioned studies is scaled up by a factor 1.6. This multiplication factor is calculated from our red blood and whole blood fatty acid data of healthy children and adults (n=120). It is unclear why the European children had a low n-3 fatty acid index. However, we have reported significant drops in levels of DHA and EPA of dried filter paper blood spot samples stored at room temperature for 8 weeks and at 4 °C for a longer period [42].

The most striking and indeed puzzling finding was the very low n-3 fatty acid index (<2%), which was unrelated to improper specimen collection and processing, in a small number of seemingly healthy children with no history of mental or neurological disorders (figure 1).The short and long-term implications of this finding is not apparent; nevertheless, it is of concern because of the critical role of n-3 long chain fatty acids, particularly in visual and neuronal development, function and brain protection [43-47]. In addition, in adults, there is evidence that patients with psychiatric conditions and individuals at a higher risk of unexpected death from a heart disease have red blood cell n-3 fatty acid index of less than 4% [48]. These observations are pertinent for children since early life is though to be the genesis of a number of non-communicable chronic diseases (psychiatric and cardiovascular) [49-54]. Indeed, O'Sullivan and colleagues have reported that n-3 fatty acid index correlates with reduced cardiovascular disease risk factors in adolescent boys [55].

After 12 weeks supplementation, compared with the controls, red blood cell n-3 fatty acid index increased by 27.2% and 39.2% in the oily fish and fish oil groups, respectively. The observed differential increase was a reflection of intake which was 150 to 200 mg/day n-3 long chain

polyunsaturated fatty acids in the oily fish and 456 mg/day in the fish oil children. A pilot test conducted on a small number of children prior to the start of supplementation revealed the maximum amount of oily fish that they can consume in a single meal was 100 grams containing the aforementioned amount of n-3 long chain pufa. Although red blood cell n-3 fatty acid index of the children increased with both oily fish (6.03±1.39%) and fish oil (6.60±1.63%) supplementation it did not reach the cardioprotective high value target (≥8%) for adults proposed by Harris and von Schacky [56]. The reason for this is not evident; however, it could be the 12 week supplementation period was short, the 456 mg/day given to the children was insufficient or both. Consistent with our findings, Block and colleagues [53] have reported 2/3 servings of oily fish a week, 2/3 standard fish oil capsules a day or 2 or more servings of oily fish a week plus standard fish oil capsules did not raise the level of red blood cell n-3 fatty acid index to ≥8%. The American Heart Association (AHA) guidelines have suggested adults need to consume 500 to 1000 mg of docosahexaenoic and eicosapentaenoic acids from oily fish to help reduce the risk of coronary heart disease [56, 58]. It is plausible children and adults may respond differently to n-3 fatty acid supplementation; however, from the current study, it appears the lower intake, 500 mg/day, suggested by AHA is unlikely to increase n-3 fatty acid index to the proposed optimal target [56] in adults.

The fish oil group compared with their counterparts who received oily fish and with baseline had significantly lower levels of systolic (−9.1% and −5.1%) and diastolic (−10.3% and −5.8%) blood pressure and plasma triglycerides (−15.4% and −8.3%). Conversely, in contrast to previous findings [55-56], oily fish did not seem to have beneficial effects on any of the above blood parameters in the current study. A plausible explanation for the discrepancy might be that the dose of omega 3 fatty acids, 150 to 200 mg/day, given to the group was too low to have a discernable impact. There is a strong association between childhood and adulthood blood pressure [61, 62]; therefore, early supplementation of children with n-3 long chain polyunsaturated fatty acids might help to reduce the risk of development of essential hypertension later in life.

With the exception of palmitolic acid which was lower in the children who were given oily fish there was no difference in any of the other saturated and mono-unsaturated fatty acids between the three groups. This finding was not unexpected as the oily fish and fish oil supplement used in the study contained saturated and unsaturated fatty acids. N-3 fatty acid index correlated inversely with total

saturated (figure 2) and mono-unsaturated (figure 3) fatty acids in the oily fish and fish oil groups. . This perhaps to be expected since these fatty acids are displaced from membrane by both eicosapentaenoic and docosahexaenoic acids [63, 64].

In agreement with previous studies fish oil supplementation increased n-3 metabolites and n-3 fatty acid index and concomitantly reduced arachidonic [63, 65-67], dihomo gamma linolenic and n-6 docosatetraenoic acids. In contrast, oily fish did not decrease the level of the aforementioned n-6 fatty acids. Fish species from the warm-water of Gulf of Oman and Indian Ocean contain appreciable amounts of linoleic, dihomo gamma linolenic and arachidonic acids and this may have counterbalanced the n-6-reducing effect of docosahexaenoic and eicosapentaenoic acids. Paradoxically, although arachidonic, dihomo gamma linolenic acid and n-6 docosatetraenoic acids were displaced by fish oil supplementation, n-3 fatty acid index correlated positively with total n-6 fatty acids (figure 4) and arachidonic acid (figure 5). This finding corresponds with the result of our previous study that indicated significant direct relationships between docosahexaenoic and arachidonic acids in red cell phosphoglycerides of British and Korean pregnant women and their offspring at birth, cord blood [68]. Similarly, Payet and others have reported that the consumption of docosahexaenoic acid acid-enriched egg induces accretion of arachidonic acid in erythrocytes of elderly patients [69]. Luxwolda et al have found a bell curve relationship between erythrocyte n-3 fatty acid index and arachidonic acid level [70]. It appears the incorporation of n-3 and n-5 long chain polyunsaturated fatty acids into cell membrane is highly regulated and the balance between the two fatty acid families is critical for orderly structural organisation and function of cellular and subcellular membranes. Indeed, there is evidence that the relative composition of the major fatty acid classes (SFA, MUFA and PUFA) in membrane lipids of all tissues is strongly regulated over very large variation in diet composition [71].

Tribal and socio-economic homogeneity, narrow age range, consistency of the sandwiches prepared in the schools by the chiefs of the International Hotel, and compliance monitoring by teachers were some of the strengths of the study. Sandwiches/capsules were handed out daily by appointed teachers who ensured that it is consumed by the children. Learning ability and health-related behaviour of the children with the very low n-3 fatty acid index, post-intervention blood pressure of the control group for logistic reasons, and dietary fatty acid intake of the whole group because of the incompleteness of the food composition database were not assessed. In addition, in a sub-group of children, it would have been

important to assess if a longer period of supplementation increases the level of n-3 fatty acid index. These limitations must be taken in account in future research.

This study provides evidence that Omani pre-adolescents have a low level n-3 fatty acid index that can be ameliorated by fish oil supplementation or a consumption of oily fish. Epidemiological and intervention studies indicate these fatty acids are critical for visual and neurological development and function, reduction of cardiovascular and inflammatory disease risk factors and healthy ageing. There is a need to tackle this problem through school feeding programme, targeted intervention with n-3 fatty acid enriched food products and/or family nutrition education programme.

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HIGHLIGHTS

- Red blood fatty acid profile of Omani pre-adolescent school children was investigated before and after intervention with oily fish or DHA enriched fish oil capsules for twelve weeks.

- Seafood is an integral part of Omani traditional diet but the children had a low level of n-3 fatty acid index at baseline.

- There was no gender related difference in EPA, DHA or AA at baseline.

- Both oily fish and fish oil capsules increased the level of red blood cell n-3 fatty acid index.

- Red blood cell n-3 fatty acid index correlated positively with AA and inversely with total saturated and total mono-unsaturated fatty acids in the oily fish and fish oil groups.

AUTHORSHIP

A. Al-M coordinated the implementation of the study and liaised with ministries, schools and parents; **E. S.** processed samples, conducted laboratory analysis and collated data; **H. S. Al-O** contributed significantly with the conception of the study, acquisition of funds and the refining of the proposal and study protocol; **I. S. H.** contributed substantially to the conception and design of the study, writing of the proposal and enrolment and follow up of the children; **K. G (PI)** contributed to the conception, design and implementation of the study, analysed and interpreted data and drafted the manuscript; **P.C** contributed significantly in study design, protocol formulation and acquisition of fish oil supplement; **S. M. Al-S** assisted significantly with the implementation of study, data collation and statistical analysis; **S. S. Al-G r**ecruited, screened, enrolled and followed the children, liaised with teachers and parents, collected and processed samples and acquired data; **Y. M.** participated substantially in writing of the proposal, data collation, analyses and interpretation and manuscript drafting.

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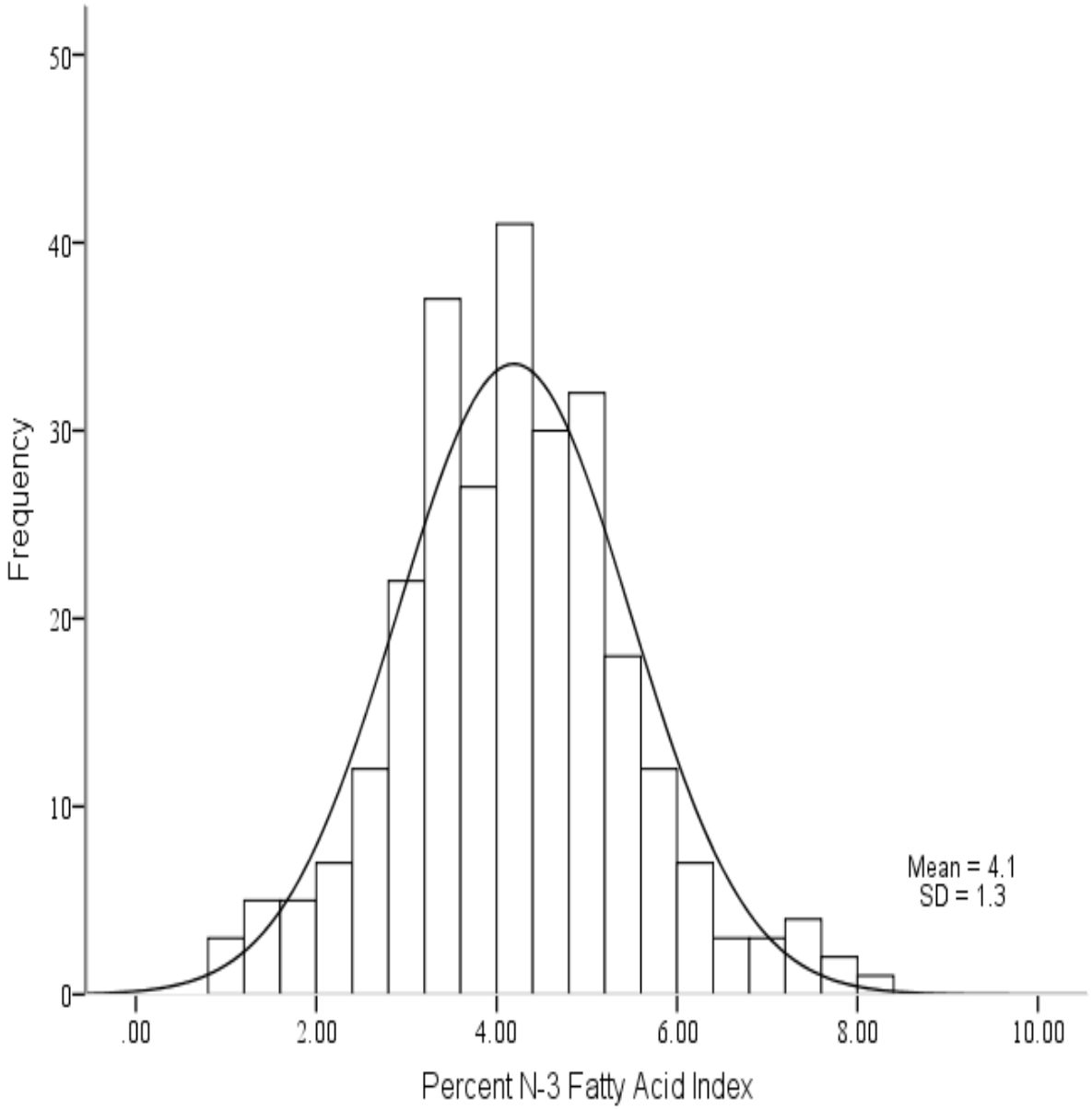
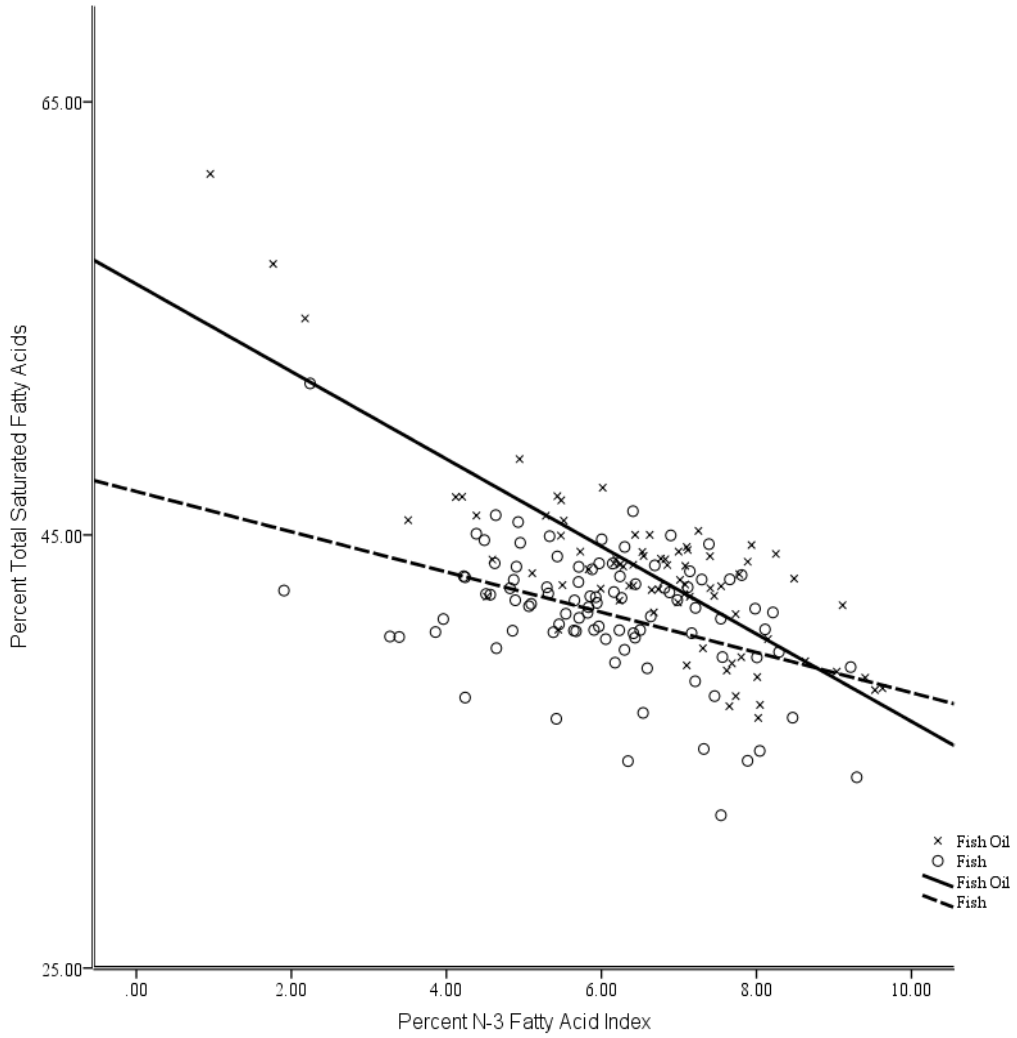
Figure 1 – Baseline Percent N-3 Fatty Acid Index Distribution



N-3 Fatty Acid Index – (%EPA+DHA)

Figure 2 – Post-intervention Relationship between Total Saturated Fatty Acids and N-3 Fatty Acid Index (Fish oil group r = - 0.816, p<0.0001; Fish group r = - 0.439, p<0.0001)



N-3 Fatty Acid Index – (%EPA+DHA)

Figure 3 – Post-intervention Relationship between Total Monounsaturated Fatty Acids and N-3 Fatty Acid Index (Fish Oil Group r = - 0.431, p<0.0001; Fish Group r = - 0.231, p <0.05)

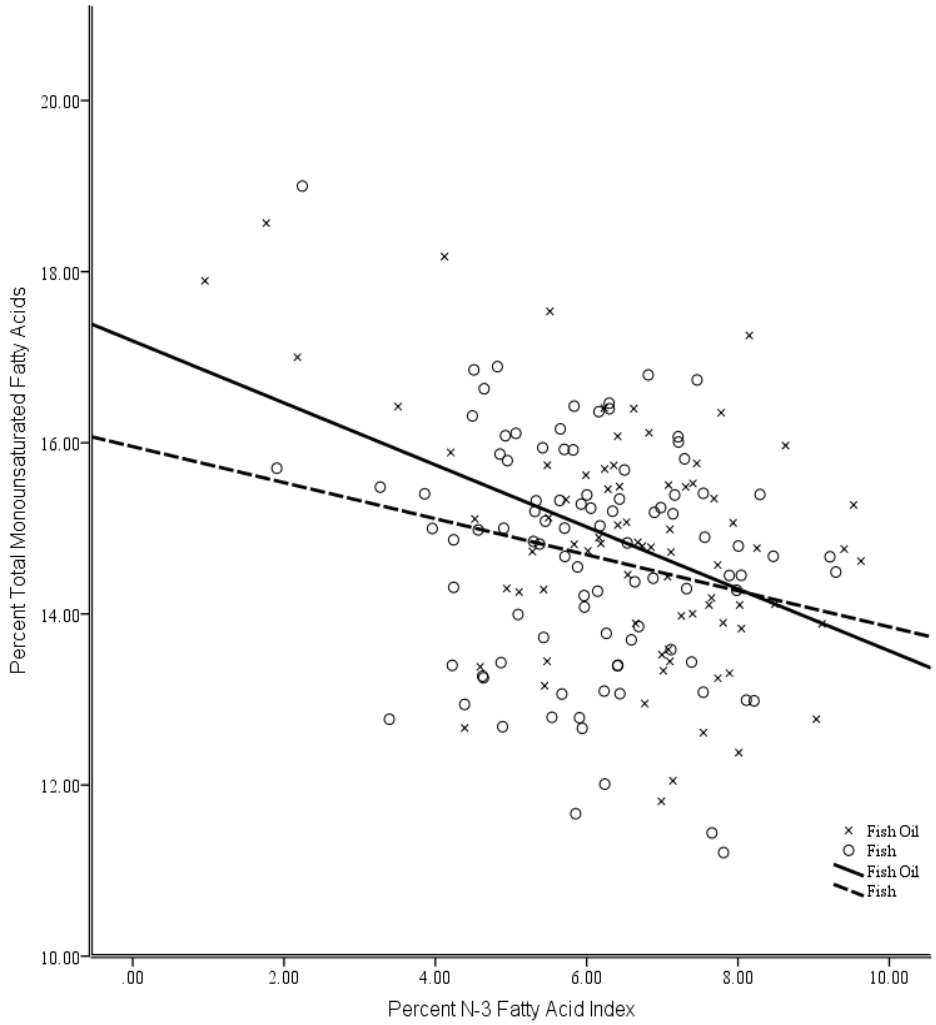
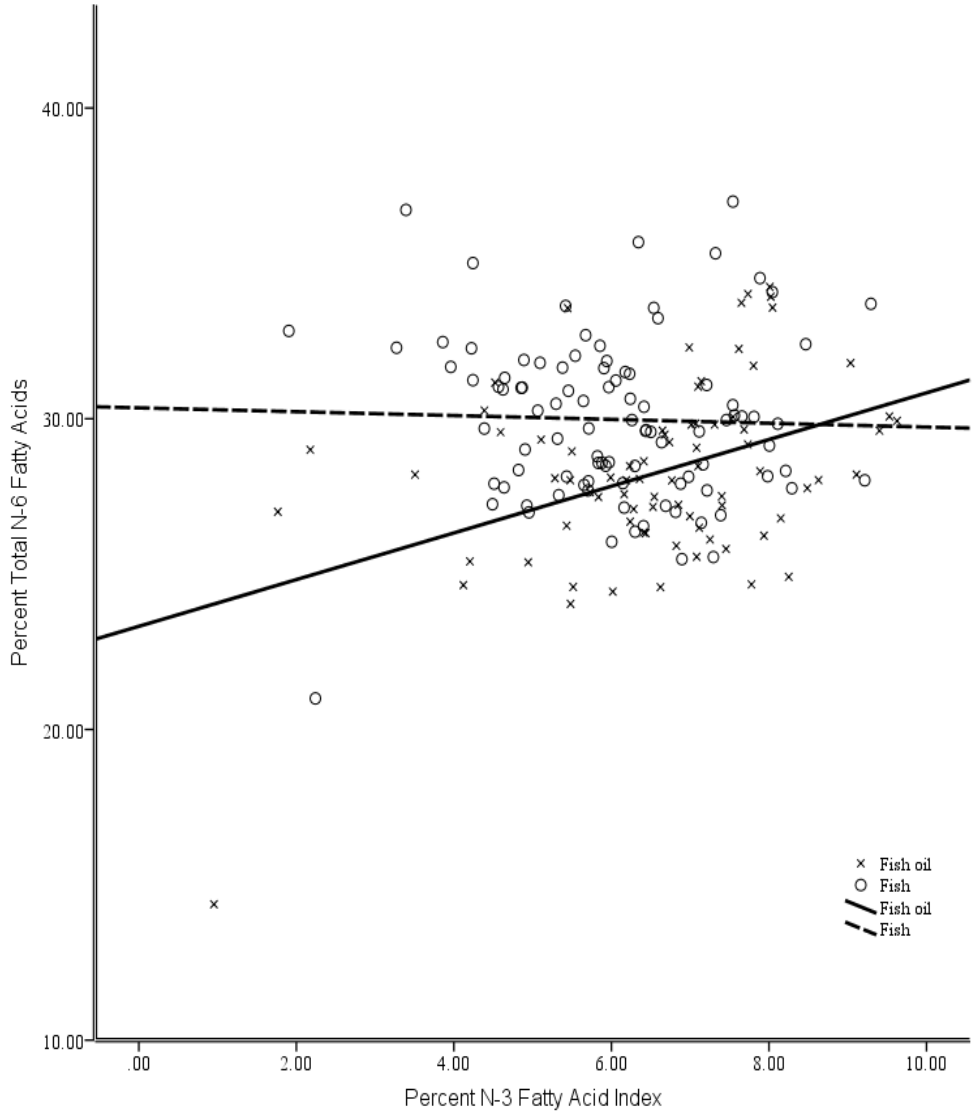
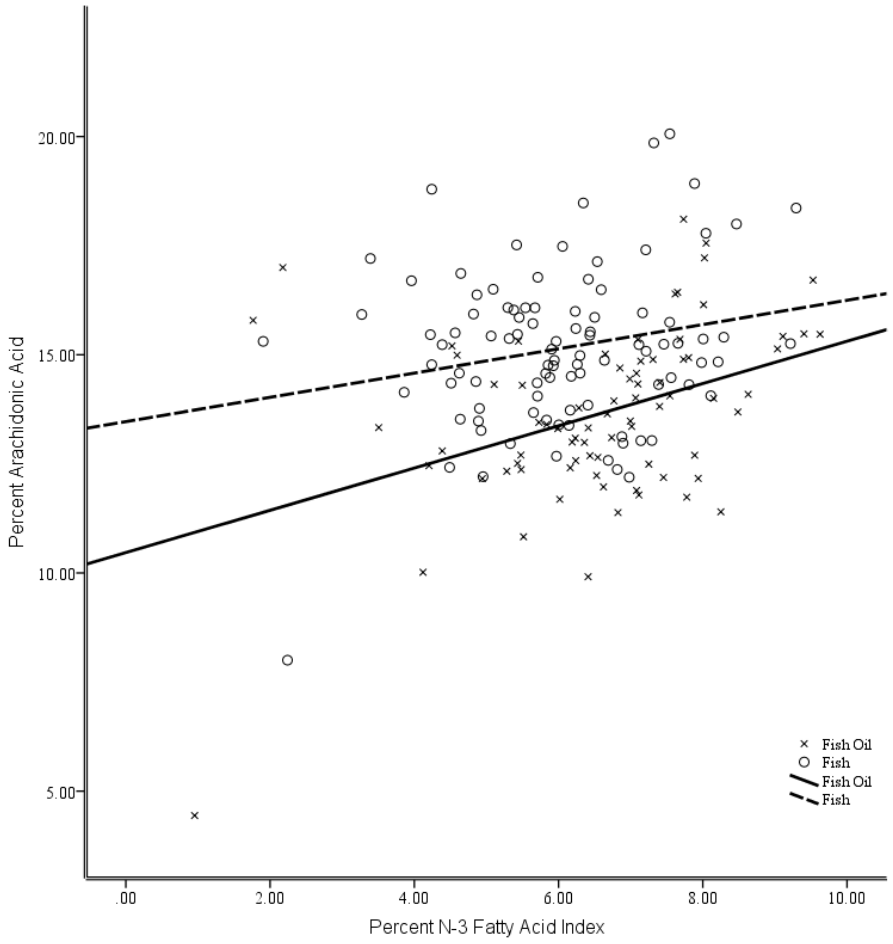


Figure 4 – Post-intervention Relationship between Total N-6 Fatty Acids and N-3 Fatty Acid Index (Fish Oil Group r = 0.414, p<0.0001; Fish Group r = - 0.032, p>0.05)



N-3 Fitty Acid Index – (%EPA+%DHA)

Figure 5 – Post-intervention Relationship between Arachidonic Acid and N-3 Fatty Acid Index (Fish Oil Group r = 0.394, p<0.0001; Fish Group r = 0.213, p<0.05)



N-3 Fatty Acid Index - (%EPA +DHA)

Table 1: Baseline characteristics preadolescent Oman children aged 9 & 10 years

Characteristics	**Boys & Girls (n=285), mean ±sd**	**Boys (n=125) mean ±sd**	**Girls (n=160) mean ±sd**
Age (year)	9.8±0.4	9.8±0.4	9.7±0.4
Weight (kg)	29.6±7.5	29.3±6.6	29.9±8.2
Height (cm)	133.3±6.6	133.3±6.3	133.3±6.8
Systolic BP (mmHg)	107.2±10.4	107.5±9.5	106.9±10.6
Diastolic BP (mmHg)	63.8±10.3	63.8±9.9	63.8±10.6
Triglycerides (mmol/L)	0.61±0.3	0.59±0.30	0.63±0.26
Total Cholesterol (mmol/L)	4.32±0.68	4.28±0.71	4.35±0.65
HDL- Cholesterol (mmol/L)	1.38±0.32	1.41±0.34	1.36±0.31
LDL-Cholesterol (mmol/L)	2.66±0.55	2.61±0.58	2.71±0.53

Table 2: Mean (±sd) percent red blood cell fatty acid composition of Omani preadolescent school children at baseline

Fatty acids	Boys & Girls (n=285)	Boys (n=125)	Girls (n=160)
14:0	0.17±0.10	0.18±0.10*	0.16±0.09
16:0	23.5±4.3	24.2±4.5*	22.9±4.1
18:0	15.9±1.4	15.9±1.5	15.9±1.3
20:0	0.36±0.11	0.35±0.11	0.36±0.10
22:0	1.58±0.49	1.56±0.52	1.59±0.48
24:0	3.42±.1.1	3.44±1.1	3.40±0.99
∑ Saturates	44.9±5.9	45.6±6.5	44.3±5.3
16:1n-7	0.17±0.07	0.17±0.07	0.17±0.07
18:1n-7	0.63±0.10	0.62±0.11	0.64±0.10
18:1n-9	11.6±1.5	11.9±1.6**	11.4±1.3
20:1n-9	0.16±0.05	0.15±0.06	0.16±0.05
24:1n-9	2.39±0.72	2.33±0.69	2.44±0.74
∑ Monoenes	15.0±1.9	15.2±2.1	14.8±1.7
18:2n-6	9.31±1.5	9.23±1.5	9.36±1.5
18:3n-6	0.10±0.03	0.10±0.03	0.09±0.03
20:2n-6	0.18±0.06	0.18±0.06	0.19±0.05
20:3n-6	1.33±0.37	1.33±0.41	1.34±0.33
20:4n-6	14.7±1.8	14.6±1.9	14.9±1.8
22:4n-6	2.84±0.87	2.71±0.90*	2.94±0.83
22:5n-6	0.67±0.24	0.66±0.25	0.68±0.22
N-6 Metabolites	19.9±2.7	19.5±2.70	20.1±2.7
∑ N-6	29.2±3.5	28.8±3.8	29.5±3.3
18:3n-3	0.05±0.07	0.05±0.06	0.05±0.08
20:5n-3	0.36±0.14	0.39±0.13	0.35±0.14
22:5n-3	0.99±0.33	0.97±0.37	1.00±0.30
22:6n-3	3.63±1.45	3.50±1.57	3.78±1.35
N-3 Index	4.10±1.32	3.89±1.65	4.20±1.41
N-3 Metabolites	4.98±1.79	4.86±1.95	5.07±1.64
∑ N-3	5.00±1.80	4.84±1.96	5.12±1.67
16:0 DMA	2.45±0.49	2.49±0.52	2.41±0.47
18:0 DMA	3.33±0.56	3.26±0.56	3.38±0.55
18:1 DMA	0.43±0.11	0.42±0.12	0.44±0.10

Male vs. Female * P<0.05, ** P<0.01

N-3 Index – (% EPA+% DHA.), DMA – Dimethyl acetal,

N-6 metabolites - ∑ (18:3n-6, 20:2n-6, 20:3n-6, 20:4n-6, 22:4n-6, 22:5n-6)

N-3 metabolites - ∑ (20:5n-3, 22:5n-3, 22:6n-3)

Table 3: Mean (±sd) percent red blood cell fatty acids of Omni school children given re-esterified triacylglycerol fish oil capsules, oily fish or a habitual snack for 12 weeks

Fatty acids	Control (n=102)	Oily Fish (n=77)	Fish oil (n=96)
14:0	0.10±0.07	0.11±0.10	0.12±0.08
16:0	21.1±3.6	19.8±3.6[a]	21.5±3.8
18:0	16.2±0.99	16.2±1.1	16.4±1.1
20:0	0.32±0.09	0.33±0.07	0.32±0.06
22:0	1.57±0.24	1.53±0.27	1.53±0.29
24:0	3.71±0.54[g]	3.45±0.58	3.46±0.73
∑ Saturates	42.9±3.4	41.4±2.9 [a]	43.3±4.0
16:1n-7	0.13±0.06	0.13±0.08	0.12±0.05
18:1n-7	0.63±0.11	0.61±0.09	0.59±0.09
18:1n-9	11.5±1.1	11.3±1.2	11.4±1.2
20:1n-9	0.14±0.09	0.15±0.04	0.14±0.04
24:1n-9	2.65±0.47[g]	2.53±0.51	2.46±0.51
∑ Monoenes	15.0±1.3	14.7±1.4	14.8±1.4
18:2n-6	9.77±1.3	9.64±1.3	10.0±1.2
18:3n-6	0.04±0.03	0.04±0.02	0.03±0.02
20:2n-6	0.15±0.05	0.17±0.05[b]	0.16±0.04
20:3n-6	1.44±0.27	1.45±0.31	1.35±0.25
20:4n-6	14.8±2.1	15.2±1.8 [c]	13.7±2.0 [e]
22:4n-6	3.00±0.57	2.87±0.59	2.42±0.53[f]
22:5n-6 (N-6 DPA)	0.74±0.19[g]	0.67±0.17	0.65±0.14
N-6 Metabolites	20.1±2.7	20.3±2.4	18.3±2.5[f]
∑ N-6	29.9±3.1	30.0±2.7 [c]	28.3±3.0 [e]
18:3n-3	0.03±0.02	0.04±0.02	0.03±0.02
20:5n-3	0.25±0.13[h]	0.41±0.19	0.36±0.12
22:5n-3	1.17±0.29[h]	1.40±0.27 [c]	1.07±0.22
22:6n-3	4.49±1.43[h]	5.63±1.24 [d]	6.25±1.53
N-3 Index	4.74±1.53[h]	6.03±1.39 [d]	6.60±1.63
N-3 Metabolites	5.91±1.69[h]	7.42±1.57	7.59±1.82
∑ N-3	5.95±1.70[h]	7.46±1.58	7.62±1.82
16:0 DMA	1.97±0.31	2.00±0.31	1.90±0.29
18:0 DMA	3.26±0.41	3.46±0.46 [b]	3.37±0.49
18:1 DMA	0.41±0.09[h]	0.48±0.09	0.48±0.08
DHA/N-6 DPA	6.49±2.71	8.77±2.79 [b]	9.86±2.12[f]

Oily Fish vs. Control & Fish oil ([a] $p < 0.05$), vs. Control ([b] $p < 0.05$) and vs. Fish Oil ([c] $p<0.0001$, [d] $p<0.05$)
Fish Oil vs. Control ([e] $p<0.001$) and vs. Oily Fish & Control ([f] $p<0.0001$)
Control vs. Oily Fish & Fish Oil ([g] $p<0.05$, [h] $p< 0.0001$)

DMA – Dimethyl acetal, N-6 metabolites - ∑ (18:3n-6, 20:2n-6, 20:3n-6, 20:4n-6, 22:4n-6, 22:5n-6)
N-3 metabolites - ∑ (20:5n-3, 22:5n-3, 22:6n-3)

Table 4: Mean (±sd) blood pressure, plasma triacylglycerol and HDL cholesterol, LDL cholesterol and total cholesterol of Omni pre-adolescent school children after intervention with DHA-enriched re-esterified fish oil or oily fish

	Control			Oily Fish			Re-esterified Triacylglycerol Fish Oil		
Parameters	All (n=102)	Male (n=46)	Female (n=56)	All (n=96)	Male (n=46)	Female (n=50)	All (n=77)	Male (n=34)	Female (n=43)
Systolic BP (mmHg)	–	–	–	111.9±9.6	111.9±9.1	111.8±10.0	101.7±7.5**	102.1±6.7	101.4±7.9
Diastolic BP (mmHg)	–	–	–	67.0±11.7	66.6±11.6	67.0±11.9	60.1±7.9**	60.0±5.5	60.0±7.3
Triacylglycerol (mmol/L)	0.64±0.35	0.59±0.37	0.67±0.33	0.65±0.23	0.64±0.24	0.65±0.22	0.55±0.28*	0.48±0.27$	0.60±0.28
Total Cholesterol (mmol/L)	4.47±0.65	4.52±0.65	4.44±0.65	4.49±0.71	4.57±0.80	4.43±0.63	4.50±0.64	4.49±0.57	4.52±0.70
HDL Cholesterol (mmol/L)	1.39±0.29	1.41±0.28	1.38±0.31	1.41±0.33	1.46±0.33	1.37±0.32	1.44±0.32	1.55±0.35$	1.36±0.28
LDL Cholesterol (mmol/L)	2.80±0.54	2.83±0.56	2.75±0.52	2.79±0.54	2.81±0.66	2.76±0.44	2.81±0.55	2.71±0.49	2.89±0.59

* $p<0.05$ Fish oil vs. Fish and vs. Control (All), ** $p< 0.0001$ Fish oil vs. Fish (All); $ $p<0.05$ Male vs. Female (Fish oil group)