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Fish intake, erythrocyte n-3 fatty acid status and metabolic health in Danish adolescent girls and boys

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Abstract

Marine n-3 long-chain PUFA (n-3 LCPUFA) may have a beneficial effect on several aspects of the metabolic syndrome (dyslipidaemia, insulin resistance, hypertension and abdominal obesity). The metabolic syndrome is increasing in prevalence during adolescence, but only few studies have investigated the effects of n-3 LCPUFA in adolescence. The present study examines associations between fish intake (assessed by a 7 d pre-coded food diary), erythrocyte (RBC) DHA status (analysed by GC) and metabolic syndrome measures (anthropometry, blood pressure and plasma lipids, insulin and glucose) in 109 17-year-old children from the Copenhagen Birth Cohort Study. Of the children, 8% were overweight or obese and few showed signs of the metabolic syndrome, but all the metabolic syndrome variables were correlated. Median fish intake was 10.7 (interquartile range 3.6–21.2) g/d. Boys tended to have a higher fish intake (P=0.052), but girls had significantly higher RBC levels of DHA (P=0.001). Sex and fish intake explained 37% of the variance in RBC-DHA (P<0.001). After adjusting for confounders, high DHA status was found to be significantly correlated with higher systolic blood pressure (P=0.014) and increased fasting insulin (P=0.018), but no adverse association was observed with the mean metabolic syndrome z-score. Overall, the present study showed the expected association between fish intake and RBC-DHA, which in contrast to our expectations tended to be associated with a poorer metabolic profile. Whether these results reflect the physiological function of n-3 LCPUFA, lifestyle factors associated with fish intake in Denmark, or mere chance remains to be investigated.

Key words: n-3 PUFA: Blood pressure: Insulin sensitivity: Plasma lipid profile

The incidence of metabolic dysregulation is an increasing problem, now even occurring during adolescence. Metabolic dysregulation is characterised by dyslipidaemia, insulin resistance, hypertension and abdominal obesity – the so-called metabolic syndrome – which predisposes toward the development of CVD and type 2 diabetes. Dietary n-3 long-chain PUFA (n-3 LCPUFA) from marine foods have in adults been shown to have a beneficial effect on blood pressure (BP)(1) and blood lipid profile, specifically plasma TAG(2). Furthermore, n-3 LCPUFA have been shown to prevent the development of insulin resistance induced by a high-fat or high-sucrose diet in rodents(3,4). Some studies have shown associations between n-3 LCPUFA and better insulin sensitivity in human subjects(5–6) and recent randomised trials have also shown beneficial effects of fish oil supplementation(7–10). However, the results concerning the relationship between n-3 LCPUFA and insulin sensitivity in human subjects are not at present convincing(11,12). A possible explanation for the inconsistencies could be that the effect may be different in established insulin resistance and during its development.

It is at present believed that infants have higher needs for n-3 PUFA due to their effect on the development of the central nervous system(13). In Denmark, it is recommended that infants ingest a minimum of 1% of their energy (percentage of energy; %E) as n-3 PUFA, as opposed to 0.5 %E from 2 years of age. We have previously shown that fish oil supplementation of 9-month-old infants have a beneficial effect on plasma lipid profile and BP(14). This result surprised us since the infants had an overall low cardiovascular risk profile and the adult studies tend only to find an effect of fish oil in subjects with a high risk profile(15). It is our hypothesis that the results could reflect a greater susceptibility towards effects of n-3 LCPUFA intake in the infant growth phase due to a higher n-3 PUFA need and that effects of n-3 LCPUFA intake would also be detectable in other growth phases and on other characteristics of the metabolic syndrome.

Abbreviations: BP, blood pressure; %E, percentage of energy; FA%, percentage of all fatty acids in the chromatogram; HDL-C, HDL-cholesterol; HOMA-IR, homeostasis model assessment of insulin resistance; LCPUFA, long-chain PUFA; RBC, erythrocyte; SBP, systolic blood pressure.

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In the present study, we examined if n-3 LCPUFA status or intake in adolescents was associated with biomarkers of the metabolic syndrome in a cross-sectional analysis within the prospective Copenhagen Birth Cohort Study. The prospective design enabled us to take prognostic variables (for example, birth weight, duration of breast-feeding and socio-economic factors) into account as well as possible confounding lifestyle factors (diet and physical activity). Furthermore, we examined associations between erythrocyte (RBC) n-3 LCPUFA and fish intake.

Methods

The Copenhagen Cohort Study is a prospective observational study where a random sample of Danish infants born from 1987 to 1988 was followed during the first year of life and later followed up at 10 and 17 years. The inclusion criteria were: parents of Danish origin, singleton birth, 37–42 weeks’ gestation, normal birth weight (10th–90th percentiles for gestational age), no neonatal disease or malformation. Of the 251 infants who fulfilled the inclusion criteria, 145 infants completed the study, fifty-nine of which were in a control group only examined at 9 months, whereas the rest were examined several times during the first year of life. They were all invited to take part in this 17-year follow-up and 109 (75%) agreed to participate. The present study was conducted according to the guidelines laid down in the Declaration of Helsinki and was approved by the Ethics Committee for Copenhagen and Frederiksberg (no. KF 01-226/97). Written informed consent was obtained from all participants and their parents.

Dietary intake was determined in the week before the examination visit by a 7 d food record with pre-coded response categories and open supplementary alternatives as previously described. Intake was registered in household response categories and open supplementary alternatives as examination visit by a 7 d food record with pre-coded all participants and their parents.

Committee for Copenhagen and Frederiksberg (no. KF 01-226/97) conducted according to the guidelines laid down in and 109 (75%) agreed to participate. The present study was examined several times during the first year of life. They were all invited to take part in this 17-year follow-up and 109 (75%) agreed to participate. The present study was conducted according to the guidelines laid down in the Declaration of Helsinki and was approved by the Ethics Committee for Copenhagen and Frederiksberg (no. KF 01-226/97). Written informed consent was obtained from all participants and their parents.

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with their standard errors. Group comparisons (participants vs. non-participants and boys vs. girls) were performed by Student’s t test or, for non-normally distributed variables, by the Mann–Whitney U test. Associations between fish intake (total or individual fish intake parameters) and RBC n-3 LCPUFA were checked by backward multiple regression analysis and the final model was chosen as the one with maximum explained variance and the fewest independent variables. The participants were divided in tertiles according to RBC-DHA status with an even distribution of girls and boys by a separate division of boys and girls and a subsequent pooling of the sex subgroups in each of the tertiles. Associations between RBC-DHA status (high, medium and low as determined by tertiles in the population) and various outcome parameters (diet, body composition parameters, and the other metabolic syndrome variables) were assessed by logistic regression analysis. All models were adjusted for sex, duration of breast-feeding and diet (and body composition for all non-body composition variables) were assessed by logistic regression analysis. The characteristics of the included children are shown in Table 1. The participating children did not differ from the non-participating children with respect to any of the given characteristics (data not shown). Only approximately 8% of the children were overweight or obese according to waist circumference cut-offs or BMI and few exceeded the cut-off for one or more of the other metabolic syndrome criteria: nine had high plasma TAG, eleven had low HDL-C (four of whom also had high TAG), seven had total plasma cholesterol > 5 mmol/l, none were hypertensive, five had SBP > 130 mmHg, and none was hyperglycaemic. Of the children, two had the metabolic syndrome according to the ‘three out of five’ National Cholesterol Education Program definition. There were significant (sex-adjusted) bivariate associations between all of the metabolic syndrome variables.

Table 2 gives the overall fatty acid composition of the RBC of the children. Girls had significantly higher levels of DHA and the PUFA composition generally points to a better Δ6-desaturation capacity with a significant lower ratio between pre- and post-Δ6-desaturase fatty acids (2:4 (SD 0·5) v. 3:0 (SD 0·7) in boys; P<0·001), especially the 22:5n-3:DHA ratio (0·4 (SD 0·1) v. 0·6 (SD 0·1); P<0·001). After adjustment for fish intake, RBC-DHA status was estimated to be 1·0 (SD 0·2) FA% (P<0·001) higher in girls.

Median fish intake was 10·7 (interquartile range 3·6–21·2) g/d and tended to be higher in boys (14·3 v. 8·0 g/d in girls; P=0·052; Mann–Whitney). Overall, fish intake was distributed as follows: 21% of the total amount of fish came from fatty fish for dinner, 25% from dishes with lean fish for dinner, 9% from mixed fish dishes (sushi, etc.), 10% from fatty fish sandwiches for lunch, 10% from sandwiches with lean fish and 14% from seafood – maximally 14% of the fish was breaded. There was a significant association between the intake of fish at 17 years and that at 10 years (r=0·195; P=0·007; n 92) and also a slight tendency towards an association with intake of fish at 9 months of age (r=0·124; P=0·101; n 91).

RBC-DHA was correlated with total fish intake adjusted for sex (r=0·488; P<0·001) and so was the RBC content of n-3 LCPUFA (r=0·341; P=0·001). Of the variance in RBC-DHA,
37% was explained by sex and the last week’s fish intake, with the most significant contributions from lean fish for dinner, tuna, sushi, sole fillet sandwich, fish balls, and fatty fish for dinner. Inclusion of the overall intake of PUFA (essentially equivalent to that of linoleic and α-linolenic acids) did not change this pronouncedly (39% explained variance).

When divided according to RBC-DHA status by sex-adjusted tertiles, the RBC-DHA groups were found to differ significantly with respect to duration of breast-feeding, but not in any of the other prognostic or lifestyle-dependent variables at birth (Table 3). Furthermore, sex-adjusted dietary intake in the high, medium and low RBC-DHA status groups differed significantly with respect to PUFA E% (P=0.041) and there was a tendency towards a difference in the Na intake (P=0.077) and the protein:carbohydrate ratio of the diet (P=0.123). The RBC-DHA groups did not differ significantly with respect to parental BMI (data not shown) or body composition in analysis adjusted for sex, breast-feeding and dietary factors (total energy intake, PUFA E% and protein:carbohydrate) (Table 4). However, after adjusting for sex, breast-feeding, dietary factors and body fat percentage there were significant differences in SBP and fasting insulin and tendencies in HOMA-IR, heart rate and plasma total cholesterol after further adjustment for plasma cholesterol during first year of life (Table 4). The degree of explained variance in the models was 20–30% for the HOMA-IR, about 20% for the blood lipids, and 43% for SBP. The overall outcome of these analyses was not changed by inclusion of physical activity or food patterns (for example, intake of fruits and vegetables). SBP was also associated with RBC-DHA as a continuous variable in a multiple regression analysis including the same adjusting factors as above (B = 1·7 (SEM 0·6) mmHg/FA% (P=0·005) and HOMA-IR tended to be associated with RBC-DHA as well (P=0.052)). However, no association was observed between RBC-DHA and the mean metabolic syndrome z-score (P=0·60). The overall picture and conclusions were the same (although less significant) in similar analyses with RBC-EPA and fish intake.

### Table 2. Fatty acid composition of erythrocytes from 17-year-old boys and girls

<table>
<thead>
<tr>
<th></th>
<th>Boys</th>
<th></th>
<th>Girls</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>Subjects (n)</td>
<td>44</td>
<td>65</td>
<td>41·1</td>
<td>0·55</td>
</tr>
<tr>
<td>SFA</td>
<td>41·9</td>
<td>0·65</td>
<td>41·3</td>
<td>0·90</td>
</tr>
<tr>
<td>MUFA</td>
<td>17·1</td>
<td>0·83</td>
<td>16·8</td>
<td>0·82</td>
</tr>
<tr>
<td>18:2n-6</td>
<td>10·8</td>
<td>1·15</td>
<td>10·2</td>
<td>0·99</td>
</tr>
<tr>
<td>18:3n-3</td>
<td>0·19</td>
<td>0·05</td>
<td>0·18</td>
<td>0·04</td>
</tr>
<tr>
<td>20:2n-6</td>
<td>0·24</td>
<td>0·03</td>
<td>0·26</td>
<td>0·04</td>
</tr>
<tr>
<td>20:3n-9</td>
<td>0·11</td>
<td>0·03</td>
<td>0·11</td>
<td>0·02</td>
</tr>
<tr>
<td>20:3n-6</td>
<td>1·97</td>
<td>0·38</td>
<td>1·94</td>
<td>0·41</td>
</tr>
<tr>
<td>20:4n-6</td>
<td>0·79</td>
<td>0·28</td>
<td>0·74</td>
<td>0·32</td>
</tr>
<tr>
<td>22:4n-6</td>
<td>3·16</td>
<td>0·60</td>
<td>3·21</td>
<td>0·59</td>
</tr>
<tr>
<td>22:5n-6</td>
<td>0·61</td>
<td>0·13</td>
<td>0·70</td>
<td>0·23</td>
</tr>
<tr>
<td>22:5n-3</td>
<td>2·73</td>
<td>0·21</td>
<td>2·31</td>
<td>0·31</td>
</tr>
<tr>
<td>22:6n-3</td>
<td>4·99</td>
<td>1·21</td>
<td>5·82</td>
<td>0·99</td>
</tr>
<tr>
<td>PUFA</td>
<td>41·3</td>
<td>0·93</td>
<td>41·43</td>
<td>0·93</td>
</tr>
<tr>
<td>n-3 Long-chain PUFA</td>
<td>8·62</td>
<td>1·51</td>
<td>9·00</td>
<td>1·23</td>
</tr>
<tr>
<td>n-6:3 PUFA</td>
<td>3·70</td>
<td>0·78</td>
<td>3·50</td>
<td>0·61</td>
</tr>
</tbody>
</table>

*Values are given as the percentages of individual fatty acids relative to the total chromatogram area (FA%).

### Table 3. Diet and lifestyle at 17 years of age and infant characteristics in sex-adjusted tertiles according to erythrocyte DHA status

<table>
<thead>
<tr>
<th>Tertile</th>
<th>Low</th>
<th>Medium</th>
<th>High</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subjects (n)</td>
<td>36</td>
<td>37</td>
<td>36</td>
</tr>
<tr>
<td>Sex (% girls)</td>
<td>58</td>
<td>62</td>
<td>58</td>
</tr>
<tr>
<td>Birth weight (kg)</td>
<td>3·35</td>
<td>0·06</td>
<td>3·50</td>
</tr>
<tr>
<td>Duration of breast-feeding (months)</td>
<td>Median 4</td>
<td>5</td>
<td>9</td>
</tr>
<tr>
<td>25th–75th percentile</td>
<td>1–8</td>
<td>3–8</td>
<td>5–9</td>
</tr>
<tr>
<td>Fish intake at 9 months (g/d)</td>
<td>Median 0</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>HAES physical activity score</td>
<td>4·1</td>
<td>0·2</td>
<td>4·1</td>
</tr>
<tr>
<td>Energy intake (MJ/d)</td>
<td>9·3</td>
<td>0·6</td>
<td>10·2</td>
</tr>
<tr>
<td>Carbohydrate (E%)</td>
<td>53·1</td>
<td>0·7</td>
<td>51·3</td>
</tr>
<tr>
<td>Protein (E%)</td>
<td>13·1</td>
<td>0·3</td>
<td>13·9</td>
</tr>
<tr>
<td>Fat (E%)</td>
<td>30·9</td>
<td>0·8</td>
<td>32·0</td>
</tr>
<tr>
<td>Fish and fish products (g/d)</td>
<td>8</td>
<td>2</td>
<td>16</td>
</tr>
<tr>
<td>Girls (g/d)</td>
<td>8</td>
<td>3</td>
<td>10</td>
</tr>
<tr>
<td>Boys (g/d)</td>
<td>8</td>
<td>2</td>
<td>25</td>
</tr>
<tr>
<td>Meat and poultry (g/d)</td>
<td>136</td>
<td>9</td>
<td>142</td>
</tr>
<tr>
<td>Dairy products (g/d)</td>
<td>461</td>
<td>64</td>
<td>461</td>
</tr>
<tr>
<td>Fruits and vegetables (g/d)</td>
<td>532</td>
<td>48</td>
<td>423</td>
</tr>
<tr>
<td>Cereals and bread (g/d)</td>
<td>210</td>
<td>13</td>
<td>238</td>
</tr>
<tr>
<td>Fats (g/d)</td>
<td>30</td>
<td>3</td>
<td>33</td>
</tr>
<tr>
<td>Sweets and sugar products (g/d)</td>
<td>50</td>
<td>5</td>
<td>44</td>
</tr>
</tbody>
</table>

HAES, habitual activity estimation scale; E%, percentage of energy.
n-3 LCPUFA status. In contrast to our expectations, higher RBC-DHA was not found to be associated with lower markers of the metabolic syndrome, but rather a higher BP and lower HDL-cholesterol (mmol/l) 1·29 0·05 1·46 0·05 1·46 0·07 0·287 TAG (mmol/l) 0·86 0·89 0·87 25th–75th percentile 0·62–1·12 0·61–1·13 0·66–1·06 Mean MS z-score 0·01 0·09 0·04 0·09 0·958 HOMA-IR, homeostasis model assessment of insulin resistance; MS z-score, mean metabolic syndrome z-score; * The statistical analysis of anthropometric variables was adjusted for sex, duration of breast-feeding and diet (percentage of energy from PUFA and proteins/carbohydrates ratio). These covariates were also used in the models for the other metabolic syndrome markers along with body fat percentage, and blood lipids in the first year for plasma lipids and intake of Na for blood pressure.

Discussion

The results of the present study showed that teenage girls have higher levels of DHA in their RBC than boys and that fish intake in both sexes was strongly associated with RBC n-3 LCPUFA status. In contrast to our expectations, higher RBC-DHA was not found to be associated with lower markers of the metabolic syndrome, but rather a higher BP and lower insulin sensitivity compared with adolescents with a lower RBC DHA status. However, after adjustment for confounders, no adverse association was observed with the mean metabolic syndrome z-score.

The positive association between intake of fish and RBC n-3 LCPUFA status was in accordance with our expectations. Intake of fish explained approximately 40% of the variation in RBC-DHA, which is in accordance with that in previous studies, for example, in pregnant women\(^{(21)}\). To our knowledge, no studies have verified the fish intake–RBC biomarker relationship in children, but the present study indicates only minor potential for effect modification by age and sex. The low degree of explained variance may primarily be because the periods of diet registration were short compared with the day-to-day variation in fish intake. Furthermore, the 7 d food record was only semi-quantitative. The uncertainty of the present study is therefore presumed to be mainly related to the dietary registration, while assessment of RBC n-3 LCPUFA is considered a stable measure of fish intake during the last couple of months. We therefore believe that RBC n-3 LCPUFA is the most relevant measure to use when associations with the functional outcomes are examined. Surprisingly, we found that intake of lean fish had a significant impact on DHA status at 17 years. This could be explained by the fact that the fish intake in this group of teenagers was relatively low (approximately half the amount compared with Danish adults) and primarily consumed for dinner (66% was fish for dinner), which typically consists of lean fish in Denmark while fatty fish such as herring and mackerel more often are consumed for lunch. Although the average fish intake in this group of adolescents is low, the range of fish intake was quite wide (total intake of fish ranged between 0 and 120 g/d) and spans the whole spectrum of fish intake that has previously been reported to have beneficial effects. The surprising adverse association between fish intake and some metabolic syndrome markers in the present study may be explained by high fast food intakes (for example, fish and chips) during adolescence. However, that was not supported by the food records. Furthermore, breaded fish filets also contributed to the DHA status, which probably means that the filets were only lightly breaded and not deep-fried, since most of the fat in commercially breaded deep-fried fish products would be SFA, MUFA and n-6 PUFA. It has been hypothesised that intake of linoleic acid relative to that of PUFA may blunt the tissue incorporation of n-3 LCPUFA\(^{(22)}\). However, adjustment for intake of PUFA did not play a role in relation to the association between fish intake and RBC-DHA. This is in agreement with the incorporation...
results from a recent two-factorial trial where we supplemented adult men simultaneously with both n-3 LCPUFA and linoleic acid\(^{[25]}\); nevertheless, such an effect has been shown in plasma from children\(^{[24]}\). Interestingly, this cohort study results show significant associations between fish intake throughout childhood, indicating stability in the dietary pattern and validity in the dietary assessments. This, however, also means that we cannot distinguish between whether the associations we found between status and health reflect a relationship with the present fish intake or a programming effect. The observed association between duration of breast-feeding and DHA status at 17 years of age could be a programming effect but is more likely due to lifestyle associations (between breast-feeding and fish intake). No significant association was observed of breast-feeding on RBC fatty acid composition after adjustment for sex and fish intake (data not shown).

Previous studies have shown that fish oil lowers plasma TAG and BP, although the effect on BP only seems to occur among people who have an unfavourable risk profile\(^{[1]}\) and among infants\(^{[14]}\). Furthermore, it has been shown that n-3 PUFA inhibit development of obesity and insulin resistance induced by a high-fat and -sucrose diet in rodents\(^{[3,4]}\). A high serum n-3 PUFA status has also in adult men been shown to decrease the relative risk for developing the metabolic syndrome over the following 20 years, independent of smoking habits, physical activity and BMI\(^{[25]}\). Much to our surprise, the present cross-sectional study showed a potentially inverse relationship between DHA status and BP and insulin sensitivity in adolescents. A lack of an association might be anticipated due to lack of power, the overall health status of the subjects or too low a dose. However, we have previously in a follow-up study to a randomised controlled trial with fish oil supplementation during lactation seen similar adverse long-term effects on BP\(^{[20]}\), but this is in contrast to the programming effect of perinatal intake of n-3 PUFA observed in rodents\(^{[27]}\) and formula-fed infants\(^{[28]}\). The children in our randomised controlled trial were also healthy Danish adolescents, but the n-3 LCPUFA dose was in the higher end of the population intake range and the difference in intake was limited to a period of the first 4 months of lactation. Most of the children in the present study had BP, plasma lipid profile and markers of glucose homeostasis within the normal range and the overall negative effects were small. The long-term implications may, however, be clinically relevant as these risk markers have been shown to track and a high value could then translate into a clinically increased level later in life. The observed adverse associations could in theory be due to various pollutants in the fish\(^{[29]}\). The fish intake in the examined population was, however, not very high and other studies have also found beneficial association between plasma levels of marine pollutants and CVD risk factors, indicating that the benefits outweigh the potential risks\(^{[30]}\). Fish intake is typically related to a healthy diet and lifestyle and in the present study there was also a positive correlation with intake of fruit and vegetables, indicating that fish consumption is also related to a generally healthy diet among these teenagers, which makes the lack of a beneficial association between RBC-DHA and markers of the metabolic syndrome even more odd. It seems implausible that the active components of a healthy diet should have markedly different physiological effects in teenagers compared with in adults. The adverse relationship between RBC-DHA and BP and markers of insulin sensitivity could, however, be due to hidden confounding factors that we did not measure or that other aspects of the teenage lifestyle were unhealthy – too much food and too little physical activity. Inclusion of the possible confounding factors that we did have information on (for example, physical activity and intake of fruits and vegetables) did not affect the adverse association. We have also taken the protein:carbohydrate ratio in the diet into account, as this has been found to modulate the effect of PUFA in rats\(^{[31,32]}\). This factor played a significant role for some of the outcomes but not as an effect modifier of the effect of DHA status.

There was a significant difference in the RBC fatty acid composition between boys and girls, with higher DHA levels in girls in spite of a lower fish intake. There was also a significant higher RBC incorporation of 22 : 5n-6, the n-6 LCPUFA synthesis endproduct, and a lower RBC Δ6-desaturase substrate-product-ratio, specifically in the ratio between 22 : 5n-3 and DHA. Thus, overall, the sex difference could reflect a higher Δ6-desaturase capacity in girls. This is the first study to show sex differences in PUFA levels in adolescents, but similar differences in fatty acid composition have previously been observed in blood lipids of men and women\(^{[33–35]}\). Furthermore, women have been shown to have a higher rate of conversion from α-linolenic acid to n-3 LCPUFA\(^{[36]}\). Sex differences have also been observed in the tissue fatty acid composition in rats, but Burdge et al. found no sex difference in rat hepatic Δ6-desaturase expression and no association between the proportion of individual PUFA in hepatic phospholipids and Δ6- or Δ5-desaturase expression\(^{[37–39]}\). Genetic differences in fatty acid desaturase (FADS) 1–2, the gene cluster that codes for Δ5- and Δ6-desaturases in man, have been shown to be reflected in tissue PUFA levels, mostly so in the variation in 20 : 2n-6 and 20 : 4n-6 (arachidonic acid)\(^{[40,41]}\). The final LCPUFA synthesis step to DHA and 22 : 5n-6 involves more than a Δ6-desaturation and it could be these other steps that were up-regulated. What ever the mechanism, tissue PUFA composition has been found to be manipulated by sex hormones\(^{[35,36,42]}\). The fact that girls have a higher endogenous capacity for synthesis of DHA could presumably mean that they were less dependent or less affected by intake. This idea is in line with the fact that people with a high genetically determined capacity (a specific allele composition in the FADS2 gene) have been shown to be less affected by intake – for example, the effect of breast-feeding v. formula feeding on intelligence quotient (IQ)\(^{[43]}\). This is among the first studies to examine the relationship between intake of fish and n-3 LCPUFA on markers of health in adolescents. The strength of our cohort study is that it had an initial random selection and a relatively high rate of follow-up (i.e. 75% of all the children who were recruited at birth). However, since it is an observational study we cannot exclude that our findings could be due to
residual confounding or atypical associations between fish intake and lifestyle in this particular group of teenagers. In summary, the results from this cross-sectional analysis in our cohort showed that sex and fish intake explain much of the variance in adolescent RBC-DHA status, boys tend to have a higher fish intake, but girls had higher RBC-DHA status. Furthermore, in contrast to expectations, DHA status was found to be associated with higher BP and lower insulin sensitivity, but not with the overall metabolic syndrome z-score. Whether these results are a mere chance finding or whether they reflect a truly different effect of n-3 LCPUFA during growth needs to be investigated by more studies, preferably intervention studies, on the relationship between n-3 LCPUFA intake and health in adolescence.

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