

**Serum lipid profile and fatty acid composition of erythrocyte phospholipids in children and adolescents with primary hyperlipidemia**

Valeria Deon<sup>1</sup>, Cristian Del Bo<sup>1</sup>, Federica Guaraldi<sup>2</sup>, Giorgio Gargari<sup>3</sup>, Andrea Bosusco<sup>2</sup>, Paolo Simonetti<sup>1</sup>, Patrizia Riso<sup>1\*</sup>, Ornella Guardamagna<sup>2</sup>

*<sup>1</sup>Department of Food, Environmental and Nutritional Sciences, Division of Human Nutrition, Università degli Studi di Milano, Milan, Italy*

*<sup>2</sup>Department of Health, Sciences and Pediatric, Università degli Studi di Torino, Turin, Italy*

*<sup>3</sup>Department of Food Environmental and Nutritional Sciences, Division of Food Microbiology and Bioprocessing, Università degli Studi di Milano, Milan, Italy*

**\*Corresponding author:**

Prof. Patrizia Riso, PhD

Department of Food, Environmental and Nutritional Sciences, Division of Human Nutrition

Università degli Studi di Milano

Via G. Celoria 2, 20133 Milan, Italy

E-mail: patrizia.riso@unimi.it; Phone: +39 0250316726; Fax: +39 0250316721

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## **ABSTRACT**

This study aimed at characterising the fatty acid (FA) composition of red blood cell (RBC) phospholipids in children and adolescents with primary hyperlipidemia, and to ascertain potential association with serum lipid profile and dietary factors. At this purpose, 54 probands aged 6-17 years were recruited. Subjects showed a low omega-3 index (eicosapentaenoic acid, EPA + docosahexaenoic acid, DHA<4%). Compared to males, females had a trend towards lower levels of total monounsaturated fatty acids (MUFA) and MUFA/saturated fatty acids (SFAs) ratio in RBCs. An inverse relationship between MUFA concentration in RBCs and serum cholesterol or HDL-C/triglycerides ratio was found. Omega-6 polyunsaturated fatty acids (n-6 PUFA) were positively associated to serum HDL-C levels, and inversely to dietary cholesterol. Fibre intake was positively associated with MUFA/SFA ratio. In conclusion, we provide the first experimental data on phospholipid FA composition of RBCs in hyperlipidemic children, showing sex differences and an overall low omega 3-index.

## **Introduction**

Hyperlipidemias are disorders of lipoprotein metabolism characterized by an increase in serum total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), or triglycerides (TG). These disorders, both primary (monogenic and polygenic inherited forms) and secondary (related to other diseases) forms, are considered a major cause of atherosclerosis and cardiovascular disease (CVD) (Newman et al. 1986; Ahmed et al. 1998; Groner et al. 2006; Haney et al. 2007).

The atherosclerotic process begins early in life, thus children and adolescents affected by primary hyperlipidemia show a particularly high risk to develop CVD later in life (Durrington 2003), which is mainly secondary to chronic exposure to elevated LDL-C, accumulating in the intima-media of large muscular arteries (Durrington 2003; McGill et al. 2000). Healthy lifestyle, which includes appropriate dietary pattern in agreement with the expert panel guidelines for CV health and risk reduction in children and adolescents (Expert panel ..., 2011), physical activity and weight loss in case of excessive body weight, is the cornerstone in the treatment of hyperlipidemia and represents an important target for CVD prevention (Haney et al. 2007; Catapano et al. 2011). It has been suggested that the lipid composition of RBC membranes can be considered as an additional risk factor in the progression of atherosclerosis and coronary heart disease (Lausada et al. 2007; Tziakas et al. 2010). The fatty acid (FA) composition of red blood cells (RBCs) generally reflects the last three months of dietary fat intake, and it is thought to be a biomarker of the tissue fatty acid status (Sarkkinen et al. 1994; Kuratko et al. 2009; Brigandi et al. 2015).

Since erythrocytes are incapable of de novo phospholipid synthesis, chain elongation or desaturation of fatty acids, the major pathway to renew the RBC phospholipids is the direct exchange from plasma lipoproteins to the erythrocyte (Marks et al. 1960; Farquhar

& Ahrens 1963; Reed 1968; Hodson et al. 2008). Thus, the RBC FA composition is postulated to better and earlier reflect the pathology of lipid metabolism, in respect to lipoprotein changes in blood serum, which are affected by recent food consumption (Sarkkinen et al. 1994; Harris & Von Schacky 2004; Novgorodtseva et al. 2011).

Studies conducted in adults found a correlation between altered FA composition in RBCs and coronary heart disease, arterial hypertension, dyslipidemia and other atherosclerosis-related diseases (Engelmann et al. 1992; Taylor 1994; Antoku et al. 2000; Lausada et al. 2007; Vayá et al. 2009; Ristic-Medic et al. 2009; Tziakas et al. 2010; Novgorodtseva et al. 2011; Jacobs et al. 2014). Harris & Von Schacky (2004) demonstrated that a low content of eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids in the RBC membranes is strongly associated with coronary heart diseases. The total levels of EPA and DHA in RBCs, defined as omega-3 index, was suggested as an additional biomarker of CV risk, and a predictive parameter for morbidity and mortality from CVD (Harris & Von Schacky 2004). Since most of this class of compounds is contained in cell membranes, the index has been also calculated on RBC phospholipids and other cell types; moreover, comparison with data obtained in plasma has been performed (Harris & Thomas 2010; Rice et al. 2016).

Despite the potential impact of knowing the RBC membrane FA composition in patients affected by lipoprotein disorders in terms of risk prediction, studies are still limited and mainly performed in adults. In this regard, trials carried out in children were mainly aimed at characterizing the phospholipid FAs of RBCs in healthy subjects (Laryea et al. 1990; Jakobit et al. 2009; Cortés et al. 2012), or in patients affected by other diseases (Decsi et al. 2002; Vanderjagt et al. 2003; Trebbe et al. 2003; Chen et al. 2004; Bu et al. 2006; Vaisman et al. 2008; Burrows et al. 2011; Perona et al. 2013; Brigandi et al. 2015; Bueno et al. 2015). Furthermore, data on gender difference in RBC composition in

childhood and adolescence have not been adequately investigated.

The aim of the present study is to provide data on the RBC phospholipid FA composition in a population of Italian children and adolescents with primary hyperlipidemia, receiving medical nutrition therapy at least 6 months before the recruitment. Since RBC phospholipid FA composition is influenced by blood lipoprotein profile/concentration changes and diet we also investigated the association with serum biomarkers and dietary parameters.

## **Materials and methods**

### ***Subject enrolment and study design***

Fifty-four children and adolescents with primary hyperlipidemia were recruited among pediatric patients cared at the Department of Health Science and Pediatrics of the University of Turin, after a screening for eligibility. Children and adolescents included in the study were 6-17 years old, affected by familial hypercholesterolemia (FH), familial combined hyperlipidemia (FCHL) or polygenic hypercholesterolemia (PHC). Exclusion criteria were: secondary dyslipidemia; obesity (body mass index (BMI)  $\geq$  90<sup>th</sup> percentile, age and sex matched); renal, endocrine and liver disorders; or chronic diseases requiring drug treatment (i.e. immunologic, neurologic, or oncohematologic disorders). Participants were not on lipid-lowering treatments (including functional foods) in the previous 3 months and were not smoking. Children on diet therapy were selected after demonstrating appropriate compliance with dietary instructions, provided by a trained nutritionist, in the previous 2 months. Recruited subjects and their families were trained by a nutritionist to adhere to a properly dietary regimen, evaluated by a weekly food diary.

Diagnostic criteria of familial hyperlipidemia were based on accepted international standards (Guardamagna et al. 2011). FH was diagnosed in presence of LDL-C  $\geq$  95<sup>th</sup>

percentile, parental LDL-C  $\geq$  190 mg/dL, tendon xanthomas and/ or cardiovascular disease (phenotype IIA). FCHL was diagnosed in children showing TC and/or TG  $>90^{\text{th}}$  age- and sex-specific percentile, with at least one parent affected by hypercholesterolemia, hypertriglyceridemia, or both (IIA, IV, or IIB phenotype, respectively), with concomitant individual and familial lipid phenotype variability. Children with LDL-C levels  $>90^{\text{th}}$  percentile and a family history of dominant inherited hypercholesterolemia, but not fulfilling the biochemical international diagnostic criteria of FH or FCHL were diagnosed with PHC.

All subjects enrolled underwent a medical examination in the morning between 8 and 10 a.m. on the day of recruitment. Physical parameters including height, weight and blood pressure were measured. Height and weight were measured to the nearest 0.1 cm and 0.1 kg respectively (Wunder SA.BI. S.r.l. Italy), with the patients wearing hospital gowns and had bare feet. BMI was calculated as body weight in kilograms divided by height squared in meters ( $\text{kg}/\text{m}^2$ ). Systolic and diastolic blood pressure was measured with a mercury sphygmomanometer during the medical examination. Fasting blood samples were drawn by venipuncture for the analysis of the lipid profile and the FA composition of RBCs.

Children and their parents had received dietary recommendations based on the cardiovascular health integrated lifestyle diet (CHILD-1) for children with identified dyslipidemia, as in the American Academy of Pediatrics (Expert panel ..., 2011). The essential features were: 55% of daily energy from carbohydrate, 15% from protein, 25-30% from total fat (saturated fat  $<10\%$ , MUFA and PUFA 20% of total energy), dietary cholesterol  $<100$  mg/1000 kcal and no more than 200 mg/day, and 10-25 g/day of soluble fibre. In order to examine correct dietary habits, parents of the enrolled children, and probands themselves, were asked to fill in a weekly food diary to be provided at time of

the visit. Detailed instructions on how to collect diet records were comprehensively explained by a nutritionist. The nutritional evaluation of macronutrients and FA content of the diet registered was performed with MètaDieta® Software using the Italian Food Composition Tables (Carnovale & Marletta 2000).

The study protocol complied with the principles of the Declaration of Helsinki, and was approved by the ethics committee of the City of Health and Science University Hospital of Turin (EC:CS377). The protocol and the purpose of the study were carefully explained to the participants and to their parents; a written informed consent was obtained from children's parents before enrolment in the study.

#### ***Blood sample collection and separation***

Venous blood samples of 2.5 ml were drawn into vacutainer tubes containing lithium heparin (to obtain plasma and RBCs) or silicon (to obtain serum). Plasma and serum were separated by centrifugation at 1400 g for 15 min at 4°C. Plasma was stored at -80°C for further analysis, while serum was immediately analysed. The buffy layer of white blood cells was removed using a pipette, and RBCs were washed twice in an equal volume of a physiologic solution (0.9% NaCl, w/v). Two aliquots (0.5 ml) of RBCs were stored at -80°C until the analysis.

#### ***Serum lipid profile***

Serum levels of TC, HDL-C and TG were directly determined by an automatic biochemical analyzer (Olympus AU2700, Japan), while the LDL-C concentration was estimated using the Friedewald formula ( $LDL = TC - (HDL + TG/5)$ ). Non-high density lipoprotein cholesterol (non-HDL-C) was calculated as TC minus HDL-C.

### ***Analysis of FA composition of RBC phospholipids***

Extraction of RBC phospholipids was performed in accordance to the method previously described by Simonetti et al. (2002). Briefly, 1 ml of distilled water, 300 mg of RBC, 2.5 ml of butylated hydroxitoluene (110 µg/ml in methanol), and 5 ml of chloroform were transferred into 10 ml plastic tubes. The mixtures were shaken on vortex for 3 min and centrifuged at 1200 g x for 10 min. The chloroform phase was transferred into 10 ml plastic tubes. An aliquot of 2.5 ml chloroform was added to the tubes containing RBCs to perform the second extraction by vortexing for 3 min, and, after centrifugation at 1200 g x for 10 min, the supernatant was transferred into the corresponding tube. The chloroform phase was eluted on a silica cartridge (Sep-Pack PlusSilica, Waters), which was then evaporated under nitrogen steam. The silica phase was transferred into Pyrex glass tubes, and 2.5 ml of a toluene/methanol (1:4 v/v) mixture and 200 µl of acetyl chloride were added. After 1 h in an oven at 100°C, 5 ml of K<sub>2</sub>CO<sub>3</sub> (6% w/v in water) was added to the silica. After centrifugation at 1200 g x 10 min, the supernatant was transferred into amber glass vials, dried under nitrogen and resuspended in 100 µl of hexane before gas chromatography analysis.

The gas chromatography analysis was performed as described by Ackman (1986), partly modified. Separations were performed with a 30 m 0.32 mm i.d. Omegawax 320 capillary column, under these conditions: initial isotherm, 140 °C for 5 min; temperature gradient, 2 °C/min to 210°C; final isotherm, 210°C for 20 min. The injector temperature was 250°C. Injection volume was 1 µl with a split ratio of 1/100, and the flame ionization detector temperature was 250°C. Carrier and makeup gas were hydrogen and nitrogen, respectively. Fatty acid retention times were obtained by injecting the Omegawax test mix as standard.

### *Statistical analysis*

Statistical analysis was carried out by R statistic software (version 3.1.2). One way repeated-measures analysis of variance (ANOVA) was used to compare the data obtained from subjects stratified by sex and by type of hyperlipidemia; post-hoc analysis of differences between paired data was assessed, when appropriate, by the Least Significant Difference. Differences in serum lipid concentrations, anthropometric data and the proportion of FA composition of RBCs in relation to different lipid disorders was evaluated by non-parametric Wilcoxon-Mann-Whitney test with Benjamini-Hochberg correction. The relationship among variables was assessed by Kendall and Spearman rank, non-parametric correlation tests. This approach was used to achieve the significance level and the trend (direct or inverse) of the data correlation. The level of statistical significance was set at  $p < 0.05$ ; data are presented as a mean and standard deviation (SD).

## **Results**

### *Subjects characteristics*

The main features of the study sample are summarized in **Table 1**. The cohort of children and adolescents included 54 hyperlipidemic subjects (26 F), aged 6-17 years old; 14 were affected by FH (7 F), 21 by FCHL (12 F) and 19 by PHC (7 F). Five subjects were slightly overweight; mean blood pressure levels were in the normal range.

### *Serum lipid profile and RBC phospholipid composition*

Serum lipid profile and RBC phospholipid composition of the patients, are reported in **Table 2**, and classified according to gender and clinical diagnosis. Mean serum lipid parameters exceeded the 90<sup>th</sup> percentiles (age and sex related), except for HDL-C, showing normal concentrations. RBC phospholipid composition was

SFA>PUFA>MUFA, with low omega-3 index value ( $3.76 \pm 1.04\%$ ).

No significant serum lipid concentration difference was observed between males and females, while it was detected in the phospholipid composition of RBCs, as females showed higher concentrations of stearic acid but lower DGLA concentration ( $p= 0.031$  and  $0.050$ , respectively). Moreover, MUFA content and MUFA/SFAs ratio in RBCs was lower in females than males ( $p= 0.052$  and  $0.056$ , respectively).

A further analysis considering the type of hyperlipidemia revealed that children with FH had higher levels ( $p < 0.001$ ) of serum TC, LDL-C and non-HDL-C, and lower serum HDL/LDL ratio if compared to children with FCHL or PHC. Finally, significantly lower levels of vaccenic acid and  $\gamma$ -linolenic acid were detected in FH as compared to PHC children ( $p= 0.036$  and  $0.027$ , respectively).

#### ***Correlation between RBC phospholipid composition and serum lipid profile***

Correlations between serum lipid levels and RBC phospholipid composition are reported in **Table 3**. Lower RBC MUFA levels correlated with higher serum TC levels ( $p= 0.032$ ) and HDL/TG ratio ( $p= 0.025$ ), but lower serum TG levels ( $p= 0.005$ ). A positive correlation between serum HDL-C and PUFA n-6 levels ( $p= 0.048$ ) was also detected.

#### ***Food diary analysis and correlation between dietary factors, RBC phospholipid***

##### ***FAs and serum lipid profile***

Complete and detailed weekly food diaries were obtained from 23 (7 F) out of 54 children, and were used to calculate mean daily energy and macronutrient intake. Results showed a mean daily total energy intake of  $1208 \pm 151$  kcal suggesting a possible overall underestimation of food intake (e.g. in terms of portion size declared). Macronutrients distribution, expressed as percentage of daily energy intake, was:  $16.7 \pm 2.8\%$  protein,

58.6 ± 6.1% carbohydrate and 24.7 ± 6.4% fat (saturated: 7.4 ± 2.1 %, monounsaturated: 10.3 ± 4.8 %, polyunsaturated: 2.6 ± 0.5 %). Estimated cholesterol intake was 136.2 ± 47.2 mg/day, while fibre intake was 14.0 ± 5.3 g/day, confirming previous data (Guardamagna et al. 2013; Guardamagna et al. 2014). Correlations between dietary factors, the RBC phospholipid composition and serum lipid profile are reported in **Table 4**. An inverse association was found between the dietary intake of cholesterol and n-6 PUFA and n-6 LC-PUFA levels in RBC phospholipids ( $p= 0.001$  and  $0.017$ , respectively). The fibre intake was directly associated with RBC MUFA/SFA ratio ( $p= 0.035$ ).

## **Discussion**

To our knowledge, this is the first study aimed at characterizing the FA composition of RBC phospholipids in a population of Italian children and adolescents with primary hyperlipidemia.

The phospholipid composition of RBCs has been suggested as an additional marker to monitor serum lipoprotein profile changes (Harris et al. 2004; Novgorodtseva et al. 2001) and as an outcome parameter in the management of hyperlipidemia, although no reference values exist neither for healthy nor hyperlipidemic adults or children. Alterations of the erythrocyte lipid matrix have been demonstrated in studies performed in adults affected by dyslipidemia and/or with risk factors for coronary heart diseases (Laryea et al. 1990; Engelmann et al. 1992; Taylor 1994; Antoku et al. 2000; Lausada et al. 2007; Vajá et al. 2009; Restic-Medic et al. 2009; Novgorodtseva et al. 2011; Jacobs et al. 2014). For instance, Novgorodtseva et al. (2011) described reduced levels of n-3 PUFA, in particular docosapentaenoic acid (22:5n-3) and DHA (22:6n-3), in dyslipidemic patients as compared to normal subjects. Lausada et al. (2007) documented a PUFA decrease and

SFA concentrations increase in plasma and RBC membranes of patients with coronary heart disease, as compared to controls.

Similar studies have been performed in children with diabetes, attention-deficit hyperactive disorder, autism, and other various disorders, except for hyperlipidemia, thus, making the comparison of our data with other data from literature extremely difficult (Decsi et al. 2002; Vanderjagt et al. 2003; Trebbe et al. 2003; Chen et al. 2004; Bu et al. 2006; Vaisman et al. 2008; Burrows et al. 2011; Perona et al. 2013; Brigandi et al. 2015; Bueno et al. 2015). Results obtained in subjects with disorders seem to differ from those obtained in the relative controls. As an example, Perona et al. (2013) found higher levels of SFAs, and reduced concentrations of MUFAs and n-6 PUFAs in RBC membranes of obese adolescents as compared to normal weight controls. These results are in line with our observations as hyperlipidemic children and adolescents showed comparable SFA and MUFA levels. However, it should be also underlined that values obtained in different studies and different countries can be significantly affected by the dietary habits, thus direct comparison of results is not always possible.

It is recognized that children affected by hyperlipidemia exhibit an increased risk of atherosclerosis and CVD during adulthood (McGill et al. 2000; Durrington 2003). Recently, the PANIC study evaluated the association between plasma FA composition (in triacylglycerol and phospholipid fractions), the estimated desaturase/elongase activities and the cardiometabolic risk in 384 children (Venäläinen et al. 2016). Authors found a significant positive correlation between FAs metabolism and the cardiometabolic risk score for the pediatric population.

The omega-3 index may represent a novel, physiologically relevant and graded risk factor to predict CVD development, thus having a significant clinical utility. A low index ( $\leq 4\%$ ) was associated with a high risk of mortality for CVD and a value  $\geq 8\%$  was

suggested as a reasonable preliminary target for reducing the risk (Harris & Von Schacky 2004; Von Schacky 2010). In the pediatric population, only one other study examined the omega-3 index and concluded that a greater proportion of obese children had a lower index compared with non-obese children (Burrows et al. 2011). In our study, we documented mean omega-3 index <4% in hyperlipidemic children, a factor that contribute to classify them as at future high risk for CVD. Since EPA and DHA derive from essential fatty acids, these results suggest that there is a reduced synthesis or patients did not regularly consume LC-PUFA rich food sources. In this regard, the use of whole dietary strategies and/or specific supplementations (e.g. with PUFA, MUFA or other food bioactives) could be fundamental to improve this index above all in at risk subjects such as hyperlipidemic children.

The present analysis on sex-related differences showed that females had a significantly greater proportion of the SFA stearic acid, and lower proportion of n-6 PUFA, in particular DGLA. Lower DGLA values were also observed in serum glycerophospholipids of healthy young girls when compared with boys (Glaser et al. 2010). Moreover, in females we found a trend towards lower level of MUFA and MUFA/SFA ratio in RBCs with respect to males. Venäläinen et al. (2016) reported that a higher plasma level of SFA myristic acid, MUFA palmitoleic acid and reduced concentrations of n-6 PUFA (linoleic acid) have been correlated with increased cardiometabolic risk in children. Furthermore, higher levels of stearic, palmitic acids and lower levels of linoleic acid in RBC membranes were observed in obese adolescents (Perona et al. 2013) and in hypercholesterolemic adults (Taylor 1994), as compared to healthy controls. Both sex and gender differences in the pathogenesis, progression and manifestation of atherosclerosis and CVD have been well documented for adults (Maas & Appelman 2010; Mosca et al. 2011; Spence & Pilote 2015), while controversial data

have been reported in children (Marelli et al., 2010; Krishnan et al., 2012). In this context, our preliminary findings on sex differences in the pediatric population, associated with higher risk of future coronary diseases, in terms of lipid profile and CVD risks, could be considered by future studies in this field.

Children affected by FH had significantly lower levels of MUFA, vaccenic acid and n-6 PUFA  $\gamma$ -linolenic acid in RBCs, with respect to FCH or PCH patients. These data are consistent with earlier findings in adults demonstrating that subjects with hypercholesterolemia and advanced coronary heart disease exhibited a decreased PUFA level and an increased cholesterol and SFA concentrations in erythrocytes related to the atherosclerotic condition (Taylor 1994; Lausada et al. 2007; Vayá et al. 2009; Novgorodtseva et al. 2011). As far as the pediatric age is concerned, a significantly lower level of vaccenic acid in RBCs was also found in diabetic children, as compared to controls (Decsi et al. 2002).

Correlation analysis performed on the whole study sample (n= 54) showed a positive association between the n-6 PUFA levels in RBCs and serum HDL-C concentrations, which was similar to other observations in young or adults, showing a direct relationship between serum n-6 PUFAs and HDL-C (Ferrucci et al. 2006; Motoyama et al. 2009; Jelenkovic et al. 2014). Furthermore, we observed an inverse correlation between MUFA in RBCs and serum TC concentration and HDL/TG ratio. These results are in agreement with those from previous clinical studies showing that diet rich in MUFA have potential hypocholesterolemic effect (Yu et al. 1995; Gill et al. 2003; Fernandez et al. 2005; Mukuddem-Peterson et al. 2005). In addition, we found a direct correlation between MUFA in RBCs and serum TG. These results are in line with a study conducted in two cohorts of young healthy twins, in which the contribution of genetic and environmental factors at the bases of relationship between FAs and lipoprotein profile have been

performed. Results obtained demonstrated the importance of common genetic factors in determining the phenotypic covariation of n-6 PUFAs and MUFAs with TG and VLDL particles (Jelenkovic et al., 2014). The role of metabolic features and genetic factors on lipid profile is widely studied (Xiang et al. 2007; Matthan et al. 2014; Mayneris-Perxachs et al. 2014; Tosi et al. 2014). For example, recent evidences indicate that FA desaturases play an important role in defining blood and tissue lipid profiles. Polymorphisms in the FA desaturase genes FADS1 and FADS2, that encode respectively for the delta-5 and delta-6 desaturases, have been associated with PUFA levels in both serum phospholipids and RBC membranes (Schaeffer et al. 2006; Malerba et al. 2008; Tosi et al. 2014). Since diet could have also contributed, we tried to correlate dietary factors with lipid profile in both serum and RBCs. A correlation between low intake of dietary cholesterol and high level of n-6 PUFA and n-6 LC-PUFA in RBCs was found. In addition, the fibre intake was directly associated with the MUFA/SFA ratio. These associations have been obtained on a subgroup of children/adolescents involved (23 out of the 54 subjects) and this can represent a possible limitation of the study. However, these results provide additional evidence supporting that a low-cholesterol and fibre-rich diet could contribute to a more favourable RBC phospholipid FA composition in hyperlipidemic children.

Finally, although a limitation of the study is the absence of a normolipidemic control group, our data provides for the first time information about the phospholipid composition of RBCs in a relatively large population of Italian hyperlipidemic children and adolescents.

## **Conclusion**

In conclusion, although preliminary, we provided experimental evidence about differences in the phospholipid composition of RBCs according to patient type of

hyperlipidemia and sex. The low omega-3 index, evaluated on phospholipid RBCs, observed in these children confirms the importance of the quality of dietary FAs intake, particularly in those with primary hyperlipidemia. Further studies should be performed in order to clarify the RBC phospholipid composition differences between hyperlipidemic and normolipidemic children, as well as in relation to sex and diagnosis. Moreover, it would be interesting to study the contribution of specific dietary interventions/regimen on these parameters in children affected by primary hyperlipidemia.

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### **Disclosure statement**

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**Table 1.** Subject characteristics (n= 54)

<b>Parameter</b>	<b>Value</b>
Age, years (range)	11 ± 3 (6-17)
Sex, M/F	28/26
Weight, kg	47.9 ± 16.4
Height, cm	149.1 ± 16.1
BMI, kg/m <sup>2</sup>	20.9 ± 4.0
Systolic blood pressure, mmHg	104.5 ± 10.1
Diastolic blood pressure, mmHg	66.6 ± 7.4

**Notes:** BMI, body mass index. Values are reported as mean ± SD.

1 **Table 2.** Serum lipid concentrations and RBC phospholipid FAs composition in hyperlipidemic children and adolescents (n= 54), according to sex  
 2 and type of hyperlipidemia

Lipid profile	All subjects	Sex			Dyslipidemia			
	n= 54	Male n= 28	Female n= 26	p-value	FH n=14	FCHL n= 21	PHC n= 19	p-value
<i>Serum lipids (mg/dl)</i>								
TC	232.4 ± 55.6	226.0 ± 56.7	239.2 ± 54.8	0.385	288.2 ± 67.1 <sup>a</sup>	214.2 ± 38.3 <sup>b</sup>	211.4 ± 30.8 <sup>b</sup>	< 0.001
TG	90.9 ± 51.9	94.0 ± 53.4	84.9 ± 50.3	0.658	73.6 ± 31.3	102.3 ± 67.6	91.1 ± 42.1	0.282
HDL-C	58.8 ± 15.4	58.6 ± 15.6	60.2 ± 14.6	0.912	55.0 ± 10.6	57.8 ± 19.6	62.7 ± 12.7	0.340
LDL-C	156.3 ± 58.4	149.1 ± 60.9	163.3 ± 56.8	0.355	219.9 ± 69.5 <sup>a</sup>	137.3 ± 36.5 <sup>b</sup>	130.3 ± 28.0 <sup>b</sup>	< 0.001
HDL/LDL ratio	0.4 ± 0.2	0.4 ± 0.2	0.4 ± 0.2	0.502	0.3 ± 0.1 <sup>a</sup>	0.5 ± 0.2 <sup>b</sup>	0.5 ± 0.2 <sup>b</sup>	< 0.001
HDL/TG ratio	0.9 ± 0.5	0.8 ± 0.5	0.9 ± 0.5	0.537	0.9 ± 0.5	0.8 ± 0.5	0.8 ± 0.4	0.812
Non-HDL-C	173.6 ± 59.4	167.4 ± 61.7	179.0 ± 58.1	0.433	233.2 ± 71.0 <sup>a</sup>	156.5 ± 42.5 <sup>b</sup>	148.6 ± 31.4 <sup>b</sup>	< 0.001
<i>RBC phospholipid FAs (%)</i>								
Total SFAs	48.99 ± 2.23	48.66 ± 2.51	49.44 ± 1.84	0.261	49.23 ± 2.87	49.35 ± 1.53	48.43 ± 2.35	0.389
Total MUFAs	18.45 ± 0.91	18.68 ± 0.84	18.12 ± 0.87	0.052	18.07 ± 0.77	18.59 ± 0.86	18.58 ± 1.02	0.197
Total PUFAs	32.55 ± 2.14	32.65 ± 2.55	32.44 ± 1.67	0.729	32.70 ± 2.81	32.06 ± 1.61	32.99 ± 2.10	0.383
MUFAs/SFAs ratio	0.38 ± 0.03	0.39 ± 0.03	0.37 ± 0.03	0.056	0.37 ± 0.03	0.38 ± 0.02	0.39 ± 0.03	0.296
PUFAs/SFAs ratio	0.67 ± 0.08	0.68 ± 0.09	0.66 ± 0.06	0.452	0.67 ± 0.11	0.65 ± 0.05	0.68 ± 0.08	0.391
PUFAs n-3/n-6	0.19 ± 0.06	0.18 ± 0.03	0.20 ± 0.05	0.758	0.19 ± 0.05	0.20 ± 0.07	0.19 ± 0.04	0.871
LC-PUFAs n-3/n-6	0.33 ± 0.09	0.31 ± 0.05	0.34 ± 0.09	0.633	0.33 ± 0.09	0.34 ± 0.11	0.32 ± 0.08	0.822
Omega-3 index	3.76 ± 1.04	3.54 ± 0.55	3.80 ± 1.08	0.770	3.81 ± 0.87	3.84 ± 1.24	3.62 ± 0.95	0.789
<i>Saturated</i>								
14:0 (myristic acid)	0.41 ± 0.08	0.40 ± 0.09	0.42 ± 0.08	0.336	0.42 ± 0.10	0.4 ± 0.07	0.4 ± 0.08	0.789
15:0 (pentadecanoic acid)	0.16 ± 0.03	0.16 ± 0.03	0.17 ± 0.03	0.152	0.16 ± 0.03	0.16 ± 0.02	0.16 ± 0.03	0.963

16:0 (palmitic acid)	24.05 ± 1.38	24.02 ± 1.52	24.08 ± 1.23	0.864	24.4 ± 1.56	24.06 ± 1.10	23.78 ± 1.52	0.625
17:0 (margaric acid)	0.46 ± 0.17	0.46 ± 0.16	0.45 ± 0.19	0.803	0.42 ± 0.22	0.44 ± 0.19	0.51 ± 0.11	0.387
18:0 (stearic acid)	15.47 ± 1.47	15.06 ± 1.40 <sup>a</sup>	15.92 ± 1.44 <sup>b</sup>	0.031	15.61 ± 2.10	15.68 ± 1.16	15.15 ± 1.24	0.593
20:0 (arachidic acid)	0.56 ± 0.15	0.58 ± 0.20	0.55 ± 0.05	0.579	0.54 ± 0.07	0.55 ± 0.05	0.6 ± 0.23	0.610
22:0 (behenic acid)	1.99 ± 0.28	1.98 ± 1.29	1.99 ± 1.27	0.988	1.94 ± 0.32	2.02 ± 0.24	1.98 ± 0.29	0.607
23:0 (tricosanoic acid)	0.31 ± 0.06	0.31 ± 0.05	0.31 ± 0.06	0.935	0.30 ± 0.07	0.32 ± 0.05	0.31 ± 0.05	0.576
24:0 (lignoceric acid)	5.59 ± 0.75	5.7 ± 0.84	5.46 ± 0.63	0.242	5.45 ± 0.83	5.73 ± 0.63	5.54 ± 0.81	0.583
<i>Monounsaturated</i>								
16:1n-9 (hypogeic acid)	0.11 ± 0.03	0.11 ± 0.01	0.11 ± 0.04	0.524	0.11 ± 0.04	0.11 ± 0.03	0.11 ± 0.01	0.934
16:1n-7 (palmitoleic acid)	0.24 ± 0.07	0.24 ± 0.07	0.23 ± 0.07	0.561	0.23 ± 0.08	0.23 ± 0.08	0.25 ± 0.05	0.795
18:1n-9 (oleic acid)	11.09 ± 0.94	11.16 ± 0.89	11.02 ± 1.01	0.569	11.19 ± 1.16	10.95 ± 0.91	11.18 ± 0.83	0.719
18:1n-7 (vaccenic acid)	1.08 ± 0.11	1.07 ± 0.10	1.09 ± 0.12	0.392	1.02 ± 0.07 <sup>a</sup>	1.08 ± 0.11 <sup>ab</sup>	1.12 ± 0.10 <sup>b</sup>	0.036
20:1n-9 (eicosenoic acid)	0.20 ± 0.08	0.21 ± 0.10	0.19 ± 0.02	0.474	0.18 ± 0.02	0.19 ± 0.02	0.22 ± 0.12	0.361
24:1n-9 (nervonic acid)	5.73 ± 0.92	5.9 ± 1.14	5.56 ± 0.56	0.179	5.34 ± 1.11	6.02 ± 0.60	5.70 ± 0.99	0.240
<i>n-6 Polyunsaturated</i>								
18:2n-6 (linoleic acid)	10.70 ± 1.08	10.72 ± 1.06	10.68 ± 1.13	0.916	10.49 ± 0.77	10.78 ± 1.13	10.78 ± 1.24	0.795
18:3n-6 (-linolenic acid)	0.06 ± 0.02	0.06 ± 0.02	0.06 ± 0.02	0.193	0.06 ± 0.02 <sup>a</sup>	0.06 ± 0.02 <sup>a</sup>	0.07 ± 0.02 <sup>b</sup>	0.027
20:2n-6 (eicosadienoic acid)	0.23 ± 0.07	0.24 ± 0.09	0.22 ± 0.05	0.222	0.20 ± 0.06	0.23 ± 0.03	0.26 ± 0.10	0.138
20:3n-6 (dihomo-γ-linolenic acid)	1.91 ± 0.38	2.01 ± 0.46 <sup>a</sup>	1.81 ± 0.24 <sup>b</sup>	0.050	1.75 ± 0.26	1.95 ± 0.37	1.99 ± 0.44	0.137
20:4n-6 (arachidonic acid)	11.05 ± 1.14	10.89 ± 1.29	11.23 ± 0.95	0.270	11.43 ± 1.23	10.84 ± 0.86	11.01 ± 1.33	0.446
22:4n-6 (adrenic acid)	1.93 ± 0.49	1.92 ± 0.53	1.94 ± 0.44	0.867	1.9 ± 0.53	1.76 ± 0.27	2.14 ± 0.58	0.114
Total PUFA n-6	25.89 ± 1.58	26.15 ± 1.37	25.85 ± 1.50	0.815	25.83 ± 1.69	25.62 ± 1.58	26.25 ± 1.51	0.457
Total LC-PUFAs n-6	14.90 ± 1.46	15.07 ± 1.19	14.82 ± 1.64	0.681	15.08 ± 1.65	14.56 ± 1.16	15.14 ± 1.59	0.392
<i>n-3 Polyunsaturated</i>								
18:3n-3 (α-linolenic acid)	0.09 ± 0.04	0.09 ± 0.02	0.09 ± 0.02	0.827	0.09 ± 0.04	0.09 ± 0.04	0.10 ± 0.03	0.291
20:5n-3 (eicosapentaenoic acid)	0.36 ± 0.21	0.38 ± 0.21	0.33 ± 0.22	0.402	0.37 ± 0.29	0.35 ± 0.22	0.36 ± 0.12	0.988

22:5n-3 (docosapentaenoic acid)	1.10 ± 0.20	1.13 ± 0.21	1.07 ± 0.17	0.207	1.08 ± 0.20	1.04 ± 0.19	1.18 ± 0.19	0.130
22:6n-3 (docosahexaenoic acid)	3.40 ± 0.89	3.41 ± 0.96	3.38 ± 0.82	0.888	3.43 ± 0.67	3.49 ± 1.05	3.26 ± 0.86	0.781
Total PUFAs n-3	4.95 ± 1.17	4.68 ± 0.63	5.02 ± 1.21	0.632	4.98 ± 0.99	4.97 ± 1.39	4.90 ± 1.06	0.976
Total LC-PUFAs n-3	4.86 ± 1.17	4.59 ± 0.63	4.93 ± 1.21	0.637	4.89 ± 0.99	4.88 ± 1.39	4.80 ± 1.06	0.970

3 **Notes:** FH, familiar hypercholesterolemia; FCHL, familiar combined hyperlipidemia; PHC, polygenic hypercholesterolemia; TC, total cholesterol;  
4 TG, triglycerides; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; SFAs, saturated fatty acids; MUFAs,  
5 monounsaturated fatty acids; PUFAs, polyunsaturated fatty acids; LC-PUFAs, long chain polyunsaturated fatty acids ( $C \geq 20$ , double bonds  $\geq 3$ );  
6 omega-3 index, sum of EPA + DHA. Values are reported as mean ± SD. <sup>a,b</sup> Data with different letters are significantly different ( $p < 0.05$ ).

7

8 **Table 3.** Correlation between RBC phospholipid FAs and serum lipid concentrations (n= 54)

<i>RBC phospholipid fatty acids</i>	<b>Serum Lipids</b>					
	TC	HDL-C	LDL-C	TG	HDL/LDL	HDL/TG
Total SFAs	0.095	-0.001	0.095	-0.077	-0.051	0.022
Total MUFAs	-0.290*	-0.138	-0.274	0.371*	0.064	-0.308*
Total PUFAs	0.020	0.072	0.021	-0.137	0.030	0.161
MUFAs/SFAs ratio	-0.208	-0.057	-0.210	0.262	0.073	-0.180
PUFAs/SFAs ratio	-0.025	0.029	-0.015	-0.058	0.021	0.084
Total PUFAs n-3	0.104	-0.155	0.151	-0.068	-0.200	0.011
Total PUFAs n-6	0.023	0.275*	-0.028	-0.155	0.198	0.250
PUFAs n-3/n-6	0.085	-0.220	0.151	-0.039	-0.231	-0.043
Total LC-PUFAs n-3	0.105	-0.168	0.159	-0.064	-0.213	0.000
Total LC-PUFAs n-6	0.174	0.207	0.120	-0.197	0.063	0.243
LC-PUFAs n-3/n-6	-0.020	-0.229	0.059	0.006	-0.181	-0.080
Omega-3 index	0.085	-0.203	0.160	-0.080	-0.233	-0.006

9 **Notes:** TC, total cholesterol; TG, triglycerides; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; SFAs,  
10 saturated fatty acids; MUFAs monounsaturated fatty acids; PUFAs, polyunsaturated fatty acids; LC-PUFAs, long chain polyunsaturated fatty acids;  
11 omega-3 index, sum of EPA + DHA. \*Statistical significance after Kendall and Spearman correlations for multiple comparisons ( $p < 0.05$ ).

12

13 **Table 4.** Correlation between dietary factors, RBC phospholipid FAs and serum lipids (n= 23)

	Dietary factors										
	Energy	Protein	Carbohydrate	Total Fat	SFAs	MUFAs	PUFAs	PUFAs n-3	PUFAs n-6	Cholesterol	Fiber
<i>Serum lipids</i>											
TC	-0.265	-0.202	0.302	-0.204	-0.196	-0.221	-0.376	0.011	-0.083	-0.386	-0.210
HDL-C	-0.381	-0.126	0.163	-0.149	0.118	-0.102	-0.034	-0.087	-0.182	-0.254	-0.203
TG	-0.013	-0.101	-0.210	0.249	0.402	0.123	0.181	-0.007	0.099	0.180	0.104
LDL-C	-0.219	-0.243	0.187	-0.079	-0.147	-0.118	-0.339	0.204	-0.027	-0.280	-0.214
HDL/LDL ratio	-0.252	0.012	-0.012	-0.049	0.167	0.017	0.147	-0.081	-0.113	-0.061	-0.074
HDL/TG ratio	-0.289	-0.054	0.282	-0.289	-0.194	-0.147	-0.154	-0.010	-0.145	-0.277	-0.196
<i>RBC phospholipid FAs</i>											
Total SFAs	-0.145	-0.108	0.150	-0.096	-0.297	-0.105	-0.010	-0.350	0.083	0.265	-0.235
Total MUFAs	0.103	-0.137	-0.142	0.123	0.547	0.292	0.409	0.265	0.282	0.360	0.493
Total PUFAs	-0.059	0.174	-0.044	0.049	0.081	-0.047	-0.203	0.277	-0.216	-0.485	-0.012
MUFAs/SFAs ratio	0.193	-0.056	-0.150	0.123	0.504	0.271	0.324	0.340	0.212	0.088	0.525*
PUFAs/SFAs ratio	0.072	0.146	-0.093	0.069	0.164	0.027	-0.108	0.321	-0.128	-0.407	0.131
Total PUFAs n-3	0.360	0.434	-0.397	0.181	-0.039	0.027	0.017	0.105	0.039	0.137	0.135
Total PUFAs n-6	-0.164	-0.105	0.115	0.029	-0.076	-0.054	-0.282	0.201	-0.243	-0.762*	-0.245
PUFAs n-3/n-6	0.363	0.429	-0.409	0.167	-0.059	0.010	0.029	0.078	0.049	0.260	0.150
Total LC-PUFAs n-3	0.360	0.434	-0.397	0.181	-0.039	0.027	0.017	0.105	0.039	0.137	0.135
Total LC-PUFAs n-6	0.002	0.071	-0.201	0.118	0.108	0.051	-0.108	0.225	-0.157	-0.522*	-0.142
LC-PUFAs n-3/n-6	0.368	0.463	-0.390	0.135	-0.083	-0.034	0.007	0.020	0.034	0.390	0.176
Omega-3 index	0.358	0.441	-0.350	0.127	-0.076	-0.032	-0.010	0.081	0.029	0.265	0.154

14 **Notes:** TC, total cholesterol; TG, triglycerides; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; SFAs,  
15 saturated fatty acids; MUFAs monounsaturated fatty acids; PUFAs, polyunsaturated fatty acids; LC-PUFAs, long chain polyunsaturated fatty acids;  
16 omega-3 index, sum of EPA + DHA. \*Statistical significance after Kendall and Spearman correlations for multiple comparisons ( $p<0.05$ ).

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