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Structural and Compositional Changes in Erythrocyte Membrane of Obese Compared to Normal-Weight Adolescents

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Abstract Unhealthy dietary habits are key determinants of obesity in adolescents. Assuming that dietary fat profile influences membrane lipid composition, the aim of this study was to analyze structural changes in the erythrocyte membrane of obese compared to normal-weight adolescents. The study was conducted in a group of 11 obese and 11 normal-weight adolescent subjects. The lipid profile, lipid peroxidation and acetylcholinesterase enzyme (AChE) activity were analyzed by conventional methods. The structural properties of reconstituted erythrocyte membrane were characterized by X-ray diffraction. Erythrocyte membrane from obese adolescents had a lipid profile characterized by a higher cholesterol/phospholipid ratio, an increase in saturated fatty acid and a decrease in monounsaturated and n-6 polyunsaturated fatty acid concentrations. Differences in lipid content were associated with changes in the structural properties of reconstituted membranes and the oxidative damage of erythrocyte membrane. The lower oxidative level shown in the obese group (0.15 ± 0.04 vs. 0.20 ± 0.06 nmol/mg for conjugated diene concentrations and 2.43 ± 0.25 vs. 2.83 ± 0.31 nmol/mg protein for malondialdehyde levels) was related to a lower unsaturation index. These changes in membrane structural properties were accompanied by a lower AChE activity (1.64 ± 0.13 vs. 1.91 ± 0.24 nmol AChE/[min mg protein]) in the obese group. The consequences of unhealthy dietary habits in adolescents are reflected in the membrane structural properties and may influence membrane-associated protein activities and functions.

Keywords Acetylcholinesterase activity · Adolescent · Erythrocyte membrane structure · Lipid peroxidation · Obesity

Introduction

The number of obese children has grown considerably in developed countries in recent years (Sinha and Kling 2009). Due to the importance of the problem, there is growing research on the relationship between lifestyle and obesity in adolescence (Dietz 1998; Collins et al. 2010). In the teenaged population, genes play a role, like in adults (Jacobsson et al.

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2012). However, the dietary lipid profile is also a factor that affects body weight gain, inducing obesity and associated diseases. Thus, the influence of an obesogenic environment is widely accepted as a significant cause for the increase of weight in the adolescent population (Symonds et al. 2009).

It has been demonstrated that dietary fat intake influences the fatty acid composition of cell membranes (Barceló et al. 2009). Changes in fatty acid profiles have an impact on membrane structural characteristics and may influence membrane protein functions that take part in relevant physiological activities. For example, alterations in the cell membrane lipid composition of hypertensive subjects affect signaling proteins that participate in the control of blood pressure (Escriba et al. 2003). On the other hand, lipid peroxidation under conditions of oxidative stress can also contribute to alterations in membrane structure/function (Jacob and Mason 2005). In obese patients, a high metabolic pro-oxidant status of erythrocytes alters membrane properties and has been associated with obesity-related pathologies (Cazzola et al. 2004). It was argued that dietary fat might influence cellular metabolism through an effect on membrane lipid composition, but the study did not examine in detail how the diet affects membrane structure in the context of obesity development (Pan et al. 1994). There is a lack of experimental data relating cell metabolism and membrane structure. However, if cell membrane properties are affected by environmental factors, the dietary profile of adolescents can influence structural membrane characteristics and associated protein functions. Thus, the global result might be a cellular metabolic rearrangement that accompanies obesity. This possibility might represent an additional effect on the disease to be considered.

The aim of this study was to analyze the structural properties of erythrocyte membranes from obese and normal-weight adolescent subjects and correlate them with the oxidative effect derived from free radical damage. Acetylcholinesterase enzyme (AChE) activity was also examined as a membrane protein and a marker of the nonneuronal cholinergic system in human erythrocytes. Our findings provide information regarding (1) the lipid profile, lipid peroxidation and AChE activity of erythrocyte membranes from obese adolescents and (2) how differences in lipid composition are associated with changes in the structural properties of reconstituted erythrocyte membranes from obese and normal-weight adolescents.

Materials and Methods

Materials

Lipid standards, cholesterol (Cho), 1,2-dipalmitoyl-*sn*-glycero-3-phosphatidylethanolamine, 1,2-diacyl-*sn*-glycero-3-phosphocholine, 1,2-diacyl-*sn*-glycero-3-phospho-L-serine, *N*-acyl-4-sphingenyl-1-*O*-phosphorylcholine and lysophosphatidylcholine as well as the chemical compounds *N*-(2-hydroxyethyl) piperazine-*N*′-(2-ethanesulfonic acid) sodium salt (HEPES), acetylthiocholine iodide, 5,5′-dithiobis-2-nitrobenzoic acid and 5,5′-dithiobis-2-nitrobenzoic acid were purchased from Sigma-Aldrich (Madrid, Spain). Solvents (HPLC grade) were from Romil (Cambridge, UK). The LiChrospher diol column was from Merck (Darmstadt, Germany), and the Supelcowax 10 capillary silica column was from Sigma-Aldrich.

Study Design

The present work is part of a major project designed to evaluate the clinical and molecular parameters that characterize the prevalence of obesity among adolescents in the province of Granada, Spain (González-Jiménez et al. 2012). Its aim was to analyze the structural and compositional properties and oxidative damage of erythrocyte membranes of obese and normal-weight adolescents. For this purpose, 22 subjects (12–16 years old) without a history of metabolic disorders were recruited from the public school in the city of Guadix (Granada, Spain). All participants belonged to the same community (a city of 21,000 inhabitants), attended the same high school and had similar dietary and cultural habits, physical activity and socioeconomic level. Subjects were classified as obese, using the body mass index (BMI) score and following the indications of the Obesity Task Force, according to Cole et al. (2000). Eleven subjects were obese (BMI >30 kg/m^2 and percentile >97) and 11 normal-weight (BMI = 20–25 kg/m^2 and a range percentile 5–75). The volunteers' parents gave written informed consent to participate in the study following the protocol approved by the Institutional Committee on Human Research (Hospital San Cecilio, Granada, Spain). All procedures were in accordance with the institutional and national ethical standards for human experimentation and the Helsinki Declaration of 1975 (revised in 2000).

Blood Sample Collection and Erythrocyte Membrane Preparation

Venus blood samples were taken via a cubital vein catheter after overnight fasting and used for erythrocyte membrane preparations. Blood samples were collected in the presence of EDTA and immediately processed in order to reduce oxidation processes. Erythrocyte membranes were isolated by centrifugation as described previously (Ruíz-Gutiérrez et al. 1996). Membrane pellets were washed twice with 110 mM $MgCl_2$, sedimented at

1,750 g for 5 min and immediately stored at −80 °C under nitrogen until use.

Lipid Classes and Fatty Acid Methyl Ester Analysis

Membrane lipids were extracted with chloroform:methanol (2:1, v:v), according to the method of Folch et al. (1957). Lipid classes were analyzed by HPLC (2690 Alliance; Waters, Milford, MA), as described previously (Perona and Ruiz-Gutierrez 2004), using a LiChrospher diol column (250 × 4.6 mm, 5-μm particle size; Merck) and a light-scattering detector (ELSD 2420, Waters). Fatty acid methyl esters were quantified with a Hewlett-Packard 5890 series II gas chromatograph equipped with a flame ionization detector (Hewlett-Packard, Avondale, PA) and a Supelcowax 10 capillary silica column (60 m and 0.25 mm internal diameter; Sulpelco, Bellefonte, PA), according to recognized protocols (Ruiz-Gutierrez et al. 1992).

Erythrocyte Membrane Oxidation Analysis

Conjugated Diene Measurement

The concentration of erythrocyte-conjugated dienes was measured according to the method of Reilly and Aust (1999). Membrane lipid extract (1 g/subject) dissolved in 1.5 ml cyclohexane was quantified by measuring the absorbance at 233 nm using an extinction coefficient of 2.52×10^4/Mcm. All data are mean values of three independent experiments performed in duplicate.

Malondialdehyde Assay

Membrane lipid peroxidation was analyzed by a colorimetric assay kit (Calbiochem, San Diego, CA) to measure malondialdehyde (MDA) levels. Experimental procedures were performed according to the manufacturer's instructions. MDA–erythrocyte samples and MDA standards of known concentration were placed in glass tubes containing the chromogenic agent methyl-2-phenylindole (10.3 mM) in acetonitrile:methanol (3:1, v:v). After the addition of HCl 12 N, samples were incubated at 45 °C for 1 h. MDA–erythrocyte levels were measured at 586 nm and calibrated against the standard curve. All data are mean values of three independent experiments.

MDA–Protein Adduct Assay

MDA–protein adducts were determined by an enzyme immunoassay (Cell Biolabs, San Diego, CA). Erythrocyte samples (10 μg protein/ml) were probed with an anti-MDA antibody, followed by a horseradish peroxidase-conjugated secondary antibody. The MDA protein adduct content was quantified from a MDA–BSA standard curve.

AChE Activity Assay

Erythrocyte AChE activity was quantified by a spectrophotometric method based on the enzymatic hydrolysis of acetylthiocholine iodide (Ellman et al. 1961). Measurements were performed at 37 °C in hemolysate samples (100 μg protein). Enzymatic activity was analyzed in 1 M Tris–HCl buffer (pH 7.4), containing 5 mM EDTA and 0.5 mM 5,5′-dithiobis-2-nitrobenzoic acid. The reaction was initiated by addition of 10 mM acetylthiocholine iodide and monitored at 412 nm. Enzyme activity data are mean values of two independent experiments.

Erythrocyte Model Membrane Preparation

Total lipids of erythrocyte membranes, extracted with chloroform/methanol (2:1, v:v; Folch et al. 1957), were used to reconstitute erythrocyte model membranes. Multilamellar lipid vesicles (MLVs), 15 % (w/w), were prepared in 10 mM HEPES, 100 mM NaCl and 1 mM EDTA (pH 7.4, HEPES buffer), following established procedures (Funari et al. 2003). Lipid mixtures were hydrated, thoroughly homogenized with a pestle-type minihomogenizer (Sigma) and vortexed to obtain homogeneous samples. Suspensions were submitted to seven temperature cycles (heated to 70 °C and cooled to −20 °C). Samples were stored under argon at −80 °C until use. They were equilibrated at 4 °C for 24 h before analysis.

X-Ray Diffraction Analysis

Small- and wide-angle synchrotron radiation X-ray scattering (SAXS and WAXS) experiments were conducted on the Soft Condensed Matter beamline A2 at the storage ring Doris III of HASYLAB (DESY, Hamburg, Germany) using radiation of 0.15 nm wavelength. The scattering patterns or images were collected by an MAR 165 2D detector. Samples were loaded into a temperature-controlled holder, heated from 10 to 60 °C and then cooled to 10 °C at a scan rate of 1 °C/min. The system was equilibrated for 10 min at each temperature before measurements. For SAXS data analysis, a circular sector from each image was integrated using A2tool, software written in-house (by A. Rothkirch), producing 1D scattering curves. The WAXS data analysis was as described previously (Funari et al. 2003). The positions of the observed peaks were converted into distances, d, after calibration with the standards rat tendon tail and poly(ethylene terephthalate) for the SAXS and WAXS regions, respectively. Interplanar distances, d_{hkl}, were calculated according to the equation

Table 1 Erythrocyte membrane lipid composition

Lipid class (molar%)	Normal-weight	Obese
CE	3.62 ± 1.62	2.95 ± 1.37
TG	0.34 ± 0.23	0.37 ± 0.26
Cho	43.44 ± 3.14	48.63 ± 6.65*
MG	2.66 ± 1.02	2.59 ± 1.08
PL	49.72 ± 4.85	45.57 ± 5.79
C/PL	0.89 ± 0.13	1.11 ± 0.33*

Data are expressed as mean values ± SD from three independent analyses for each sample of obese ($n = 11$) or normal-weight ($n = 11$) subjects

CE cholesterol esters, *TG* triglycerides, *Cho* cholesterol, *MG* monoglycerides, *PL* phospholipids

* $p < 0.05$, obese versus normal-weight

$$s = 1/d_{hkl} = (2\sin\theta)/\lambda,$$

where s represents the scattering vector, 2θ the scattering angle, λ (0.15 nm) the X-ray wavelength and *hkl* the Miller indexes of the scattering planes.

Statistical Analysis

Statistical analysis was performed with the software package SPSS Statistics (21.0; SPSS, Inc., Chicago, IL). The Shapiro–Wilk W test was applied to assess the normal distribution of experimental data. Statistical significance was analyzed by the unpaired Student's t test and Pearson's coefficient for correlations. Data are reported as mean ± standard deviation (SD). Differences were considered statistically significant at $p \leq 0.05$.

Results

Membrane Lipid Composition

The lipid composition of erythrocyte membranes from the obese and normal-weight groups is shown in Table 1. The Cho, but not the phospholipid (PL), content was significantly higher in obese subjects compared to normal-weight participants. Therefore, the Cho/PL ratio was superior in the obese group. Table 2 illustrates the fatty acid composition. Differences were observed in the percentage of saturated (SFA, palmitic and stearic), monounsaturated (MUFA, oleic) and n-6 polyunsaturated (PUFA, linoleic and arachidonic) fatty acids between obese and normal-weight adolescent subjects. In the obese group, the SFA content was considerably higher. There was an important decrease in the n-6 PUFA and the n-9 MUFA percentage, while the n-3 fatty acid content and the n-6/n-3 ratio were scarcely altered. The significantly lower n-6/n-3 ratio shown by the obese participants was mostly attributable to a minor amount of linoleic and arachidonic acids. The global effect of these changes was a reduction in the membrane unsaturation index (UI) that characterized the erythrocyte membrane of obese adolescents.

Table 2 Erythrocyte membrane fatty acid composition

Fatty acid (wt%)	Normal-weight	Obese
14:0	0.42 ± 0.21	0.52 ± 0.23
14:1 n-5	2.08 ± 0.42	1.87 ± 0.43
16:0	17.96 ± 1.02	20.76 ± 2.20***
16:1 n-9	0.49 ± 0.30	0.46 ± 0.14
16:1 n-7	1.25 ± 0.29	1.08 ± 0.33
18:0	17.73 ± 1.37	20.93 ± 2.87**
18:1 n-9	19.74 ± 2.73	17.37 ± 2.45*
18:2 n-6	10.17 ± 1.50	8.38 ± 1.25**
18:3 n-6	2.34 ± 0.77	2.87 ± 1.85
18:3 n-3	2.34 ± 0.77	2.05 ± 1.06
20:0	1.01 ± 0.39	0.84 ± 0.23
20:2 n-6	1.44 ± 0.40	1.51 ± 0.61
20:3 n-6	1.77 ± 0.39	1.51 ± 0.33
20:4 n-6	12.32 ± 1.53	10.80 ± 0.94*
20:4 n-3	0.52 ± 0.28	0.63 ± 0.10
20:5 n-3	0.91 ± 0.48	1.20 ± 0.59
22:5 n-6	3.26 ± 0.62	2.80 ± 0.68
22:4 n-3	1.09 ± 0.22	1.00 ± 0.38
22:5 n-3	1.65 ± 0.54	1.39 ± 0.28
22:6 n-3	3.34 ± 0.78	3.73 ± 0.82
SFA	36.73 ± 1.26	42.91 ± 4.73***
MUFA	23.05 ± 2.81	20.40 ± 2.94*
PUFA n-6	31.30 ± 2.84	27.71 ± 1.98**
PUFA n-3	8.91 ± 1.17	8.98 ± 1.38
UFA/SFA	1.63 ± 0.19	1.36 ± 0.25*
n-6/n-3	3.56 ± 0.53	3.15 ± 0.51
UI	1.56 ± 0.07	1.42 ± 0.12**

Data are expressed as mean values ± SD from three independent analyses for each sample of the obese ($n = 11$) or the normal-weight ($n = 11$) group

SFA saturated fatty acids, *MUFA* monounsaturated fatty acids, *PUFA* polyunsaturated fatty acids, *n-6/n-3* PUFA n-6 to PUFA n-3 ratio, *UFA/SFA* unsaturated to total saturated fatty acids ratio, *UI* unsaturation index, calculated as the sum of each unsaturated fatty acid concentration multiplied by its double bond number and divided by the total unsaturated fatty acid concentration

* $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$, obese versus normal-weight

Membrane Oxidation

The concentrations of erythrocyte-conjugated dienes and MDA levels in the obese and normal-weight groups are depicted in Fig. 1. Obese subjects had lower erythrocyte-conjugated dienes (0.15 ± 0.04 vs. 0.20 ± 0.06 nmol/mg,

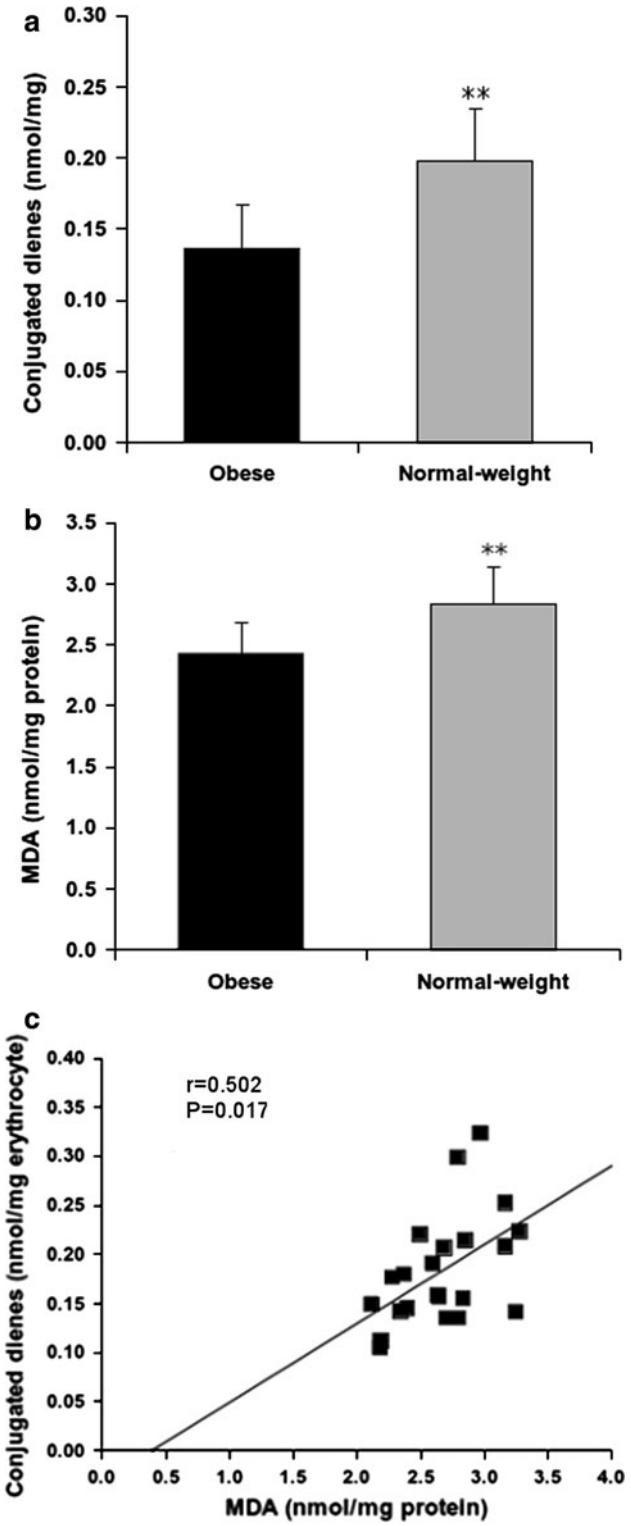

Fig. 1 Erythrocyte membrane lipid oxidation products: **a** conjugated dienes, **b** malondialdehyde (MDA) levels and **c** correlation between erythrocyte-conjugated dienes and MDA values. Correlation parameters and significance were calculated with the Pearson correlation method. Values are expressed as mean ± standard deviation. $^{**}p < 0.01$ obese versus normal-weight (unpaired Student's *t* test)

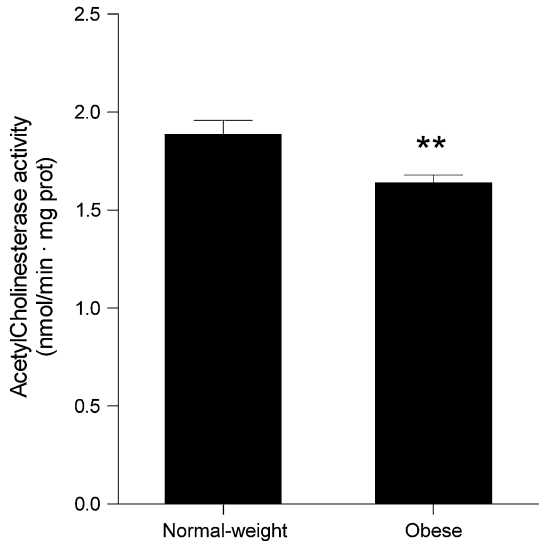

Fig. 2 Erythrocyte membrane acetylcholinesterase activity at 37 °C. Values are expressed as mean ± standard deviation. $^{**}p < 0.01$ obese versus normal-weight (unpaired Student's *t* test)

$p = 0.040$) and erythrocyte–MDA (2.43 ± 0.25 vs. 2.83 ± 0.31 nmol/mg protein, $p = 0.003$) levels. Both markers correlated ($r = 0.50$, $p = 0.02$). The MDA–protein adducts assay did not show differences between the two adolescent groups (data not shown). Correlation analyses between the lipid peroxidation data and the membrane fatty acid component were also performed. Conjugated diene values correlated positively with linoleic acid concentrations ($r = 0.75$, $p = 0.01$) in the control group and negatively with stearic ($r = 0.75, p = 0.01$) and oleic ($r = 0.74$, $p = 0.02$) acids in the obese group. In turn, the MDA marker was significantly related with PUFA ($r = 0.41$, $p = 0.02$) and the SFA/PUFA ($r = 0.51$, $p = 0.03$) and 16:0–18:2 ($r = 0.62$, $p = 0.02$) ratios in both groups. Linoleic acid was revealed as a key fatty acid regarding peroxidation via direct correlation with peroxidation markers.

Erythrocyte AChE Enzyme Activity

AChE activity was measured as an indicator of the non-neuronal cholinergic system. The enzyme activity levels are shown in Fig. 2. The obese group showed lower AChE activity compared to the normal-weight group (1.64 ± 0.13 vs. 1.91 ± 0.24 nmol AChE/[min mg protein], $p = 0.007$).

Supramolecular Organization and Lipid-Phase Behavior of Reconstituted Erythrocyte Model Membranes

Erythrocyte membranes reconstituted with the total cell lipid extract isolated from each subject were analyzed by

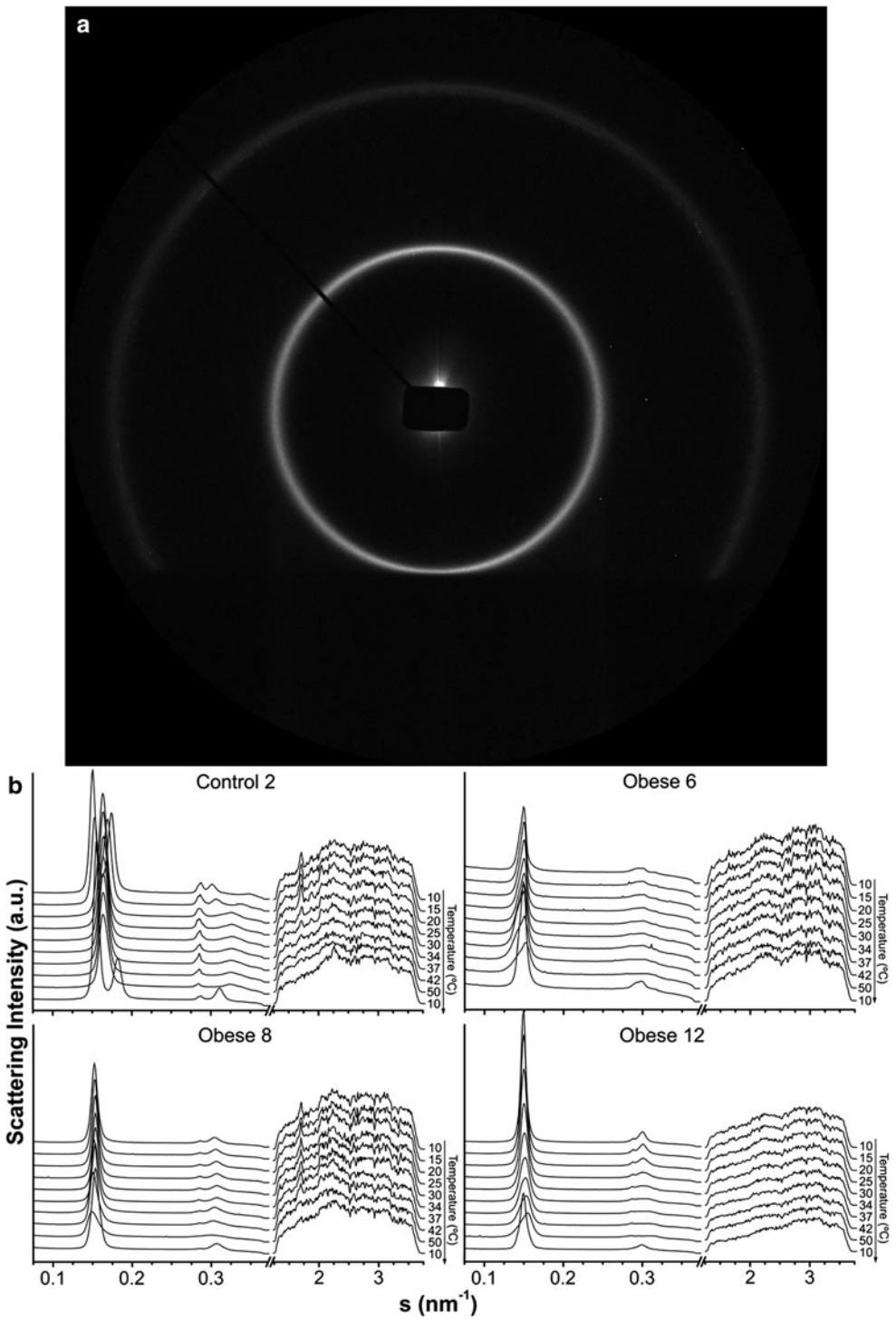

Fig. 3 **a** A representative experimental scattering pattern of reconstituted erythrocyte membranes at 30 °C. *Lines* represent ordered scattering from the lamellar liquid crystalline phase (L_α). **b** Linear plots of the X-ray diffraction patterns of reconstituted membranes: normal-weight (*upper panels*) and obese (*lower panels*) adolescent subjects. Pattern sequences were acquired after 10-min sample equilibration at each temperature. Successive diffraction patterns were collected for 15 s. The L_α phase was identified by two diffraction peaks in the SAXS. The peak at $s = 0.289\ \text{nm}^{-1}$ was assigned to a crystalline Cho structure. Diffraction peaks in the WAXS patterns indicate the presence of crystalline structures not identified

X-ray diffraction. Figure 3a illustrates that membranes displayed a clear X-ray diffraction pattern composed by a lamellar L_α phase. Each sample had a particular pattern that could be taken as a fingerprint of the erythrocyte membranes. Figure 3b depicts representative peak diffraction plots of the MLV (15 % w/w) reconstituted

Table 3 Structural properties of erythrocyte reconstituted model membranes

Samples[a]	d^{20} (nm)	d^{37} (nm)	d^{Cho} (nm)
C-1	6.08	6.05	3.5
C-2	6.25	6.19	3.5
C-3	6.50	6.50	§
C-4	6.30	6.26	3.5
C-5	6.02	5.98	ND
C-6	6.14	6.08	3.5
C-7	6.47	6.42	3.5
C-8	6.23	6.21	3.5
O-1	6.67	6.68	3.5
O-2	7.00	7.12	3.5
O-3	6.79	6.83	§
O-4	6.49	6.49	3.5
O-5	6.68	6.71	3.5
O-6	6.71	6.60	3.5
O-7	6.91	6.95	§
O-8	6.93	6.91	§

[a] Normal-weight ($n = 11$; C) and obese ($n = 11$; O) subjects

§ Cho diffraction peak with low intensity, *d* repeat distance of a lamellar L_α phase at 20 and 37 °C, d^{Cho} repeat distance of the cholesterol crystalline structure, *ND* peak not detected

erythrocyte membranes. The obese and normal-weight groups had comparable structural characteristics. The SAXS patterns showed two main diffraction peaks, corresponding to the first and second orders of the lamellar L_α phase, and a third peak at $s = 0.289$ nm^{-1} assigned to a crystalline Cho structure. All samples exhibited reversible thermotropic behavior on cooling. In some patterns, there were indications of a possible coexistence of two structural systems in the temperature range 40–50 °C (probably two lamellar phases), but the weak diffraction peaks did not allow us to draw any conclusion. Several controls (e.g., samples C-2, C-18 and C-19) displayed a peculiar diffraction pattern, presenting the coexistence of two L_α phases at low temperature that melted into one at 20 °C, and then the system proceeded as a simple lamellar phase. The diffraction peaks in the WAXS were related to the presence of unidentified crystalline structures, probably Cho or unknown mixtures containing Cho. Table 3 shows a summary of the structural parameters. Reconstituted erythrocyte membranes from both groups had an L_α phase with a *d* value in the range 6–7 nm. In spite of small differences between the two groups, membranes from the obese had a *d* value ~0.5 nm significantly higher ($p < 0.001$) than that of normal-weight subjects, indicating a higher overall membrane width in obese adolescent subjects. The increase in the repeat spacing value observed must be associated with the sum of the thickness of the lipid bilayer plus the thickness of the water layer between bilayers.

Discussion

Despite the amount of data existing on obesity, relatively little information is available concerning the adolescent population. Food habits and lifestyle seem to be important factors in the development of obesity in adolescence. If the diet can induce changes in the lipid profile of cell membranes (Barceló et al. 2009), dietary habits in obese adolescents can affect structural membrane properties and related functions. In this sense, we designed a study to compare the structural properties of the erythrocyte membrane from obese and normal-weight adolescent subjects, assuming that the erythrocyte membrane lipid profile could be a dietary biomarker (Poppitt et al. 2005).

Our data show that the membrane lipid profile in obese adolescents is characterized by an increase in SFA and a decrease in MUFA and n-6 PUFA fatty acids, leading to a significant reduction in the UI that most likely could be a consequence of a diet enriched in saturated fats (Poppitt et al. 2005). Indeed, the PL fatty acid profile was associated with a lower membrane susceptibility to lipid peroxidation compared to normal-weight subjects. Among unsaturated fatty acids, significantly lower linoleic acid content was observed in the membrane of obese subjects, which correlated with the level of lipid peroxidation determined by the conjugated dienes and the MDA methods. The level of membrane peroxidation must not be solely attributed to linoleic acid but also to the presence of conjugated linoleic acid (CLA). However, since the average dietary intake of CLA from food is low (estimated in 0.3 g/day in Europe according to the EFSA Panel on Dietetic Products, Nutrition and Allergies [NDA] 2010) and dietary CLA intake is poorly incorporated into membrane PLs (Martins et al. 2011), it is very unlikely that the content of CLA in erythrocyte membranes could significantly alter the levels of conjugated dienes measured, changing the positive correlation observed between linoleic acid and membrane propensity to oxidation. The relevance of linoleic acid in erythrocyte membranes of adolescent subjects is in contrast with a previous study that showed lower n-3 PUFA content and higher oxidative damage in erythrocyte membranes of overweight and obese women (Cazzola et al. 2004). There are scarce data regarding the lipid profile of obese children and adolescents (Warensjo et al. 2006), mostly corresponding to the plasma lipid fraction (Agostoni et al. 1994; Gil-Campos et al. 2008). Some common features reported are an alteration in linoleic acid metabolism (Reynoso et al. 2003) and a high contribution of n-6 PUFA. Such changes in the lipid profile appear to be an early marker of the

metabolic syndrome in children at prepubertal age (Gil-Campos et al. 2008). On the other hand, the high levels of palmitic acid found in membrane PLs of obese adolescents might be related to the progression of insulin resistance in these individuals (Reynoso et al. 2003; Haag and Dippenaar 2005). Palmitic acid is a substrate for the synthesis of ceramides, which seems to play a role as mediators in lipid-induced insulin resistance (Corcoran et al. 2007) and in the metabolic disease of obesity (Summers 2006). Finally, another significant result was an increase in the Cho content and the Cho/PL ratio in obese adolescents. Cho affects the overall membrane structure and the associated features of fluidity and lipid peroxidation (Cazzola et al. 2004; Ohvo-Rekilä et al. 2002). Structural properties of reconstituted model membranes may be considered as a fingerprint of the individual erythrocyte membrane. The obese adolescent group had a significant augment in the membrane *d*-space value that must be related to changes in membrane lipid composition in addition to an increase in the water layer between bilayers. Since Cho modulates the membrane lipid order and the lateral packing of PL acyl chains (Ohvo-Rekilä et al. 2002), the rise in the Cho/PL ratio of obese adolescents is expected to reduce the motional order of the PL acyl chains and to increase the membrane width, as shown in this study. Indeed, the increase in the packing density of the acyl chains can promote lipid peroxidation in less fluid environments. In turn, lipid peroxidation can affect membrane structure by inducing the formation of discrete membrane-restricted Cho domains (Jacob and Mason 2005), as we have also observed.

Structural changes in the erythrocyte membrane of obese adolescents can affect membrane functions and have physiological implications. For example, an increase in the Cho/PL ratio may result in morphological adjustments. Changes in the lipid profile affecting bulk lipid fluidity and membrane structural properties may have an effect on the organization of specialized membrane lipid domains (Kamata et al. 2008), lipid–protein interactions and the localization and/or activity of membrane-associated proteins (Villar et al. 1996; Mohandas and Gallagher 2008). Several functions related to AChE activity in the erythrocyte membrane have been described (Lopes de Almeida and Saldanha 2010; Prall et al. 1998; Carvalho et al. 2009). In the context of our work, we can speculate that the lower AChE activity observed in obese adolescents could be related to a decrease in the enzyme content and/or activity that may also be influenced by protein interactions in which the membrane structure participates. This part of the work deserves further study of other membrane proteins to relate changes in membrane protein function and obesity.

Our present data constitute a first study focused on obese adolescent subjects. The limited number of individuals does not allow us to make generalizations to the whole population of adolescents. But the study clearly shows that the consequences of unhealthy dietary habits, such as a high intake of fat and SFA, are reflected in the erythrocyte membrane lipid composition and the structural properties of the reconstituted model membranes. The demonstration that dietary habits have an effect on erythrocyte membrane properties and may influence membrane protein activities suggests that such interrelations are of physiological significance and may have implications in the development of diseases associated with obesity in adolescents.

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Conflict of interest The authors declare that there are no conflicts of interest.

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