

Changes of Plasma Lipids and Erythrocyte Membrane Fluidity in Psoriatic Children

G. FERRETTI, O. SIMONETTI, A. M. OFFIDANI, L. MESSINI, B. CINTI, I. MARSHISEPPE,
G. BOSSI, AND G. CURATOLA

*Clinica Dermatologica [O.S., A.M.O., L.M., G.B.], Istituto di Biochimica [G.F., G.C.], Laboratorio Analisi [B.C.],
Medicina del Lavoro [I.M.], Università degli Studi di Ancona, Ancona, Italy*

ABSTRACT. Psoriasis has been associated with an abnormal plasma lipid metabolism, and changes of erythrocyte membrane lipid composition and fluidity have been shown in adult patients. To investigate whether the alterations of plasma lipids appear also in pediatric patients, we have studied plasma lipids and lipoproteins in 15 prepubertal children affected by mild-to-moderate psoriasis with respect to healthy controls. The patients showed higher levels of plasma total cholesterol (4.44 ± 0.78 versus 4.03 ± 0.58 mmol/L), a significant increase of cholesterol associated with HDL (1.39 ± 0.26 versus 1.13 ± 0.28 mmol/L, $p = 0.02$), and a significant decrease of the ratio LDL cholesterol to HDL cholesterol (1.73 ± 0.6 versus 2.46 ± 0.8 , $p = 0.02$). By using fluorescence polarization of 1,6-diphenyl-1,3,5-hexatriene, we have shown a significant increase in fluidity in erythrocyte membrane of psoriatic children that was associated with a slight, but not significant, decrease in the cholesterol to protein ratio (422 ± 127 versus 503 ± 117 nmol/mg). No significant changes of phospholipid fatty acid composition have been shown, in disagreement with previous studies in adult patients. Our results support the relation between childhood psoriasis and plasma lipid changes, which are likely related to the slight compositional changes in erythrocytes. However, the observed abnormalities are expressed differently in children than in adults. (*Pediatr Res* 33: 506-509, 1993)

Abbreviations

DPH, 1,6-diphenyl-1,3,5-hexatriene
HDL-C, cholesterol associated with high-density lipoprotein
LDL-C, cholesterol associated with low-density lipoprotein
P_f, fluorescence polarization
TC, total cholesterol
TG, triglyceride
VLDL-C, cholesterol associated with very low-density lipoprotein

Psoriasis is a chronic inflammatory skin disease characterized by an accelerated turnover of epidermal cells and an incomplete differentiation in lesional epidermis. The etiology of psoriasis is unknown, but genetic, metabolic, and immunologic mechanisms have been proposed (1). Various disorders of the plasma lipid and lipoprotein pattern that include an increase in TG in VLDL and a decrease in HDL-C have been shown in adult

patients with respect to controls (2). Valquist *et al.* (3) have also observed modifications of the fatty acid composition of plasma TG, phospholipids, and cholesterol esters in which lower content of linoleic and linolenic acids and higher levels of dihomogamma-linolenic acid have been observed.

At the membrane level, modifications of phospholipid fatty acid composition with a significant increase in arachidonic acid have been shown in the plasma membrane of the skin cells in the psoriatic lesion (4). The abnormalities of fatty acid composition are not confined to epidermal cells but have been observed also in adipose tissue (3) and in erythrocytes in adult patients (5), suggesting that perturbation of lipid metabolism may be a generalized phenomenon in psoriasis. However, at present, it has not been established whether the lipid alterations are primary events or a consequence of an abnormal metabolism of the inflamed skin.

The studies on metabolic and functional alterations in psoriasis have been mostly limited to adult patients. Less attention has been paid to pediatric subjects, although psoriasis has an incidence of about 0.1% to 3% in childhood (6).

In this report, we have studied plasma lipids and lipoproteins in prepubertal patients with a duration of psoriasis ranging from 2 to 9 y. To investigate whether modifications of plasma lipoproteins may be associated with changes of erythrocyte membrane composition and fluidity, we studied fatty acid composition, cholesterol content, and fluidity in erythrocyte membrane of psoriatic children. In previous studies (7), we correlated changes in the lipid composition of circulating lipoproteins with abnormalities in erythrocyte membrane lipids and fluidity. We suggested that erythrocyte membrane could represent a sensitive model to reveal complex alterations of lipid metabolism, because mature red blood cells lack lipid biosynthetic pathways (8) and therefore depend on exchange processes with circulating lipoproteins for their membrane stability (9).

MATERIALS AND METHODS

Patients. We studied, with parental consent, 15 prepubertal patients (seven females and eight males, mean age 9.4 ± 2.2 y) with psoriasis. In all patients, the disease was moderate and of nummular type, and less than 25% of the skin surface was involved at the time of examination. None of the patients had received any systemic or topical medication for at least 2 wk preceding the study. The duration of disease ranged from 2 to 9 y. None of the patients had a history of cardiovascular disease or familial hyperlipidemia or known diabetes mellitus, and all had normal laboratory tests for liver and renal function.

The control group consisted of 16 healthy, nonobese children (six females and 10 males, mean age 9 ± 2.3 y) who periodically were checked at the Dermatological Institute of the University of Ancona. They were not taking any drugs and were known to have had normal plasma cholesterol and TG values on at least one previous occasion. Patients and controls came from the same

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Correspondence: Dott. Gianna Ferretti, Istituto di Biochimica, Facoltà di Medicina e Chirurgia, Università degli Studi di Ancona, Via Ranieri, 60131 Ancona, Italia.

region of Italy and had similar nutritional habits, which were evaluated by a questionnaire concerning their dietary habits.

The body mass index (*i.e.* weight in kg divided by the square of height in meters), taken as obesity index, was similar in both groups (mean value 17.65 ± 2.8 kg/m² in controls and 17.73 ± 2.2 kg/m² in psoriatic patients).

Preparation of erythrocyte membranes and study of membrane lipid composition. Five mL of blood were drawn from each subject, who had fasted for 12 h overnight. Blood was put into heparinized tubes and used for the preparation of erythrocyte membranes. Erythrocytes were washed three times with isotonic saline (0.15 M NaCl, 10 mM Tris HCl, pH 7.4), and the buffy coat and plasma were removed each time. Erythrocyte membranes were prepared by hemolyzing the washed erythrocytes in 5 mM phosphate buffer, pH 8, according to the method of Steck and Kant (10). Membrane protein content was estimated by the method of Lowry *et al.* (11) using BSA as standard.

Lipids were extracted from erythrocyte membranes by chloroform/methanol (2:1, vol/vol) followed by chloroform/methanol/H₂O (60:30:20, vol/vol/vol) (12). Cholesterol content was assayed enzymatically using cholesterol oxidase (13). The fatty acid methyl esters were chromatographed by reverse-phase HPLC. All chromatographic equipment was from Kontron (Munich, Germany). The system comprised a model 420 pump, a 735 LC programmable UV detector, and a 1459 integrator. Solvent was acetonitrile/tetrahydrofuran/0.1% H₃PO₄ 50.4:21.6:28 (vol/vol/vol), flowing at 1 mL/min. A 3- μ m Supelcosil LC8 15-cm \times 4.6-mm inside diameter column (Supelco, Bellefonte, PA) was the analytical column, and a stainless steel guard column (2-cm \times 4.6-mm inside diameter) packed with pellicular reversed-phase material was used. Detection of fatty acid methyl esters was performed at 215 nm at an attenuation factor of 6. Fatty acid methyl esters were identified by chromatography with authentic standards and quantified using the chromatographic conditions previously described. The sum of all peak areas of the fatty acids identified was taken as 100%.

Fluorescence polarization measurements. The hydrophobic fluorescent probe DPH (Aldrich Chemical Co., Germany) was used to investigate membrane fluidity in erythrocyte membranes. DPH, from a 2×10^{-3} M stock solution in tetrahydrofuran, was added to one volume of erythrocyte membranes containing about 100 μ g/mL of membrane protein to give a final DPH concentration of 10^{-6} M (14).

Steady state fluorescence polarization measurements of DPH were performed using a Perkin-Elmer MPF66 spectrofluorimeter (Norwalk, CT) equipped with two glass prism polarizers. Excitation and emission wavelengths were 365 and 430 nm, respectively. The steady state P_f was obtained from the fluorescence intensities parallel (I_{||}) and perpendicular (I_⊥) to the polarization direction of excitation light using the following equation: $P_f = (I_{||} - I_{\perp} \times g) / (I_{||} + I_{\perp} \times g)$ where g is an instrumental correction factor. Fluorescence values were also corrected for light scattering contributions by means of unlabeled membrane suspensions.

Analytical methods. TC and TG were routinely determined using enzymatic methods (15, 16) and diagnostic kits supplied from Boehringer (Mannheim, Germany) on a Synchron C5 analyzer (Beckman, Germany). An HDL fraction of plasma was obtained by the precipitation method (17) using Boehringer kits, and the levels of HDL-C were determined by the same method as in whole plasma.

LDL-C and VLDL-C were determined using the formula of Friedewald (18), in which VLDL-C can be calculated as plasma TG divided by 5 and LDL-C is calculated as total cholesterol - (HDL-C + VLDL-C). It is assumed that, in fasted subjects a good correlation exists between values obtained by the method of Friedewald *et al.* (18) and those obtained according to the Lipid Research Clinic Program methodology (19). Amounts of apo B and apo A1 were assayed by the electroimmunoassay procedure (20).

Statistics. All the results are expressed as mean \pm SD. Statistical

differences between data from psoriatic patients and controls were determined according to the Mann-Whitney test using a computer program (Stat View II for Personal Computer Macintosh, Apple, Berkeley, CA).

RESULTS

Plasma lipids and apoproteins. The mean value of plasma TC was higher in psoriatic children than in controls, but the difference was not statistically significant (Table 1). Moreover, a significant increase in HDL-C ($p = 0.02$) and a significant decrease in the ratio of LDL-C to HDL-C ($p = 0.02$) were observed (Table 1). The aforementioned plasma lipid changes were not correlated with the duration of the disease (data not shown). No relevant modification of lipoprotein and apoprotein levels could be observed concerning psoriasis (Table 2).

In control subjects, the levels of apo B were positively correlated with plasma TC ($r = 0.59$, $p < 0.03$); moreover, the levels of apo A1, the major peptide of HDL lipoproteins, were positively correlated with HDL-C ($r = 0.89$, $p < 0.002$). In psoriatic children, similar to the results of controls, apo A1 was positively correlated with HDL-C ($r = 0.88$, $p < 0.001$), and apo B correlated with TC ($r = 0.91$, $p < 0.001$). However, abnormalities in the lipid-apolipoprotein relation are supported by the significant increase in the TC:apo B ratio ($p = 0.003$) and the significant decrease in the apo A1:HDL-C ratio ($p = 0.003$) in psoriatic children compared with the controls (Table 2).

Erythrocyte membranes. The mean value of P_f of DPH in erythrocyte membranes of controls was 0.316 ± 0.009 . The corresponding value in psoriatic children was slightly but significantly decreased (0.304 ± 0.007 , $p = 0.01$) (Table 3) and indicates an increase in membrane fluidity in psoriatic subjects.

To investigate whether the changes of membrane fluidity are related to modifications of membrane lipids, we studied the phospholipid fatty acid composition and the cholesterol:protein ratio in erythrocyte membranes of psoriatic children and control children. The mean percentage values of the various fatty acids in erythrocyte membranes of controls were in agreement with the values reported by other authors in adult patients (21). No relevant changes of fatty acid composition have been shown in erythrocytes of psoriatic children; the level of linoleic acid (18:2) was lower (20.5 ± 1 versus 21.35 ± 1.37 , Table 4), but the difference was not significant. Moreover, the cholesterol:protein ratio was lower in erythrocyte membranes of psoriatic patients (422 ± 127 versus 503 ± 117 nmol/mg), but the difference was not significant (Table 3).

DISCUSSION

Psoriasis in middle-aged patients has been associated with alterations of lipid metabolism as evidenced by changes of plasmatic parameters with respect to control subjects (2). Elevated levels of TG in both VLDL and LDL, as well as a higher VLDL-C have been reported in adult psoriatics; these abnormalities are more pronounced in patients with severe psoriasis (2). Contrasting results have been observed about serum TC; it has been reported to be raised (22), normal (23), or lowered (24). No data are available so far in the literature about the plasma lipid and lipoprotein pattern in psoriatic children.

An increase in TC associated with a significant increase in HDL-C and a significant decrease in the LDL-C:HDL-C ratio have been shown in psoriatic children. The levels of apo A1 and apo B were not modified with respect to the controls, in agreement with Aguilar Martinez (25), in adult patients. Despite the absence of significant changes of the mean apoprotein values, there was a significant increase in the ratio of TC to apo B in patients, and the ratio of apo A1/HDL-C was significantly decreased with respect to the controls. All these results suggest modifications of plasma lipids and the plasma lipid-apolipoprotein relationships in psoriatic children.

At the membrane level, we have shown an increase in fluidity

Table 1. Plasma lipids in psoriatic children and their matched healthy controls*

	Controls	Patients	p
TC (mmol/L)	4.03 ± 0.58 (n = 16)	4.44 ± 0.78 (n = 14)	NS
TG (mmol/L)	0.80 ± 0.22 (n = 16)	0.88 ± 0.56 (n = 10)	NS
VLDL-C (mmol/L)	0.36 ± 0.10 (n = 16)	0.38 ± 0.12 (n = 10)	NS
LDL-C (mmol/L)	2.69 ± 0.46 (n = 14)	2.79 ± 0.67 (n = 10)	NS
HDL-C (mmol/L)	1.13 ± 0.28 (n = 14)	1.39 ± 0.26 (n = 14)	0.02
LDL-C/HDL-C	2.46 ± 0.8 (n = 14)	1.73 ± 0.6 (n = 10)	0.02

* Values are mean ± SD.

Table 2. Plasma lipoproteins and apoproteins in psoriatic children and healthy controls*

	Controls (n = 6)	Patients (n = 15)	p
α-Lipoproteins (%)	30 ± 4	34 ± 7	NS
β-Lipoproteins (%)	42 ± 2	48 ± 6	NS
Pre-β-Lipoproteins (%)	21 ± 5	17 ± 6	NS
Apo A1 (g/L)	1.41 ± 0.33	1.47 ± 0.16	NS
Apo B (g/L)	0.87 ± 0.13	0.79 ± 0.21	NS
Apo B/Apo A1	0.595 ± 0.08	0.548 ± 0.197	NS
TC/apo B (mmol/g)	4.36 ± 0.47	5.74 ± 0.64	0.003
Apo A1/HDL-C (g/mmol)	1.41 ± 0.13	1.05 ± 0.13	0.003

* Values are mean ± SD.

Table 3. P_f value of DPH and cholesterol:protein ratio in erythrocyte membranes of controls and psoriatic patients

	Controls (n = 14)	Patients (n = 15)	p
P _f	0.316 ± 0.009	0.304 ± 0.007	0.01
Cholesterol:protein ratio (nmol/mg)	503 ± 117	422 ± 127	NS

Table 4. Erythrocyte membrane fatty acid composition in psoriatic children and controls

Fatty acids	Controls (n = 14)	Patients (n = 15)
16:0	30.98 ± 1.06	30.44 ± 1.37
16:1	1.51 ± 0.45	1.74 ± 0.43
18:0	14.11 ± 2.01	14.38 ± 1.71
18:1	17.78 ± 1.33	18.11 ± 0.98
18:2	21.35 ± 1.37	20.51 ± 1.10
20:0	0.32 ± 0.19	0.30 ± 0.20
20:1	0.31 ± 0.20	0.45 ± 0.25
20:2	1.34 ± 0.32	1.28 ± 0.32
20:3	2.04 ± 0.46	1.96 ± 0.56
20:4	6.90 ± 1.32	6.90 ± 1.33
22:5	1.18 ± 0.48	1.38 ± 0.36
22:6	2.11 ± 0.87	2.51 ± 0.70
Polyunsaturated	34.97 ± 1.99	34.56 ± 2.36
Saturated	45.42 ± 2.18	45.12 ± 2.28
P/S*	0.77 ± 0.75	0.76 ± 0.83

* P/S, polyunsaturated/saturated.

in erythrocytes of psoriatic children in absence of significant changes of phospholipid fatty acid composition; moreover, erythrocyte membrane showed a slight but not significant decrease in the cholesterol:protein ratio. Studies of Srinivsan *et al.* (26) have demonstrated that erythrocyte membrane cholesterol content is sensitive even to slight modifications of the level of plasma lipoproteins involved in plasma cholesterol transport. In various human diseases characterized by hormonal and/or metabolic alterations associated with modifications in the composition of circulating lipoproteins, concomitant changes in the lipids of the erythrocyte membrane have been shown (27). Mature erythrocyte does not contain lipoprotein receptors; moreover, the membrane cholesterol content depends on exchange, very active both

in vitro and *in vivo*, of free cholesterol with plasma lipoproteins because the lipid biosynthetic pathways are absent (8, 9). LDL particles are the primary carriers of cholesterol from plasma to peripheral cells; in contrast, HDL play an important role in the reverse transport of cholesterol from tissue. The level of LDL in relation to HDL may influence the direction of lipid transfer between cells and plasma. The relative proportions of LDL-C and HDL-C (LDL-C:HDL-C ratio) particularly appear to be better related to membrane cholesterol content than their absolute levels as demonstrated by the positive correlation between LDL-C:HDL-C ratio and erythrocyte membrane cholesterol:protein ratio or cholesterol:phospholipid ratio (26). Therefore, we hypothesize that the lower values of the cholesterol:protein ratio in erythrocytes of psoriatic children could be related to the significant decrease of the LDL-C:HDL-C ratio in plasma.

The absence of significant changes of phospholipid fatty acid composition in erythrocytes of psoriatic children does not agree with previous results in adult patients (5). In fact, a significant increase in arachidonic acid and an increased susceptibility to lipid peroxidation have been shown in middle-aged psoriatic patients (5). These compositional changes have been related to the decrease of membrane fluidity in erythrocytes of adult patients (28). The absence of phospholipid fatty acid alterations suggests the existence of compensatory mechanisms maintaining phospholipid composition relatively constant in psoriatic children in spite of modifications of TC and HDL-C levels.

Psoriatic children included in our study showed a mild-to-moderate severity of the disease. Conversely, the relevant changes of phospholipid fatty acid composition in adult patients concerned subjects with a severe form of psoriasis involving more than 30% of skin (5). No data are available concerning erythrocyte membrane composition in patients with a mild-to-moderate form of psoriasis.

No significant correlation between biochemical findings and duration of the disease, ranging from 2 to 9 y, has been observed in the psoriatic children considered in our study. It is likely that the severity more than the duration of the disease could be of interest in metabolic alterations.

The absence of significant changes of fatty acids in psoriatic children does not justify a dietary treatment with fish oil as is prescribed for adult patients. Also of consideration is the fact that only a modest clinical improvement has been obtained in adult age (29). Furthermore, it has to be taken into account that the large increase of polyunsaturated fatty acids in plasma lipoproteins and in biologic membranes increases the risk for lipid peroxidation (30).

In conclusion, our results support the relation between childhood psoriasis and plasma lipid modifications that are likely related to the slight compositional changes in erythrocytes. These abnormalities appear to be expressed differently in pediatric patients than in adult patients. The increase in TC suggests, for psoriatic children, an increased risk for atherosclerosis; however, it must be considered that the increase in TC is associated mainly with a significant increase in HDL-C, which is considered a protective factor. Inasmuch as it has been suggested that the protective effect of HDL resides mainly in the more floating fraction HDL₂ (31), further studies are necessary to fully elucidate whether or not the risk for psoriatic children to develop athero-

sclerosis in the future is increased and to establish whether the plasma lipid alterations are primary events or a consequence of an abnormal lipid metabolism of inflamed skin.

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