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Abnormalities of Plasma Lipoprotein Composition and Fluidity in Psoriasis

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We have studied the lipoprotein chemical composition and fluidity, using the fluorescence polarization of the probe 1,6-diphenyl-1,3,5-hexatriene (DPH) in two age- and sex-matched groups of subjects (14 healthy normolipidemic children and 15 psoriatic children). The compositional study has shown a significant increase of the percentage content in triacylglycerol and a significant decrease of the apo-protein content in all lipoprotein fractions of psoriatic children compared to the controls. A significant increase of the percentage content in total cholesterol and of the cholesterol/protein ratio has also been observed in low-density lipoproteins (LDL) and in high-density lipoproteins (HDL) of psoriatic children. The compositional changes were associated with alterations of fluidity in LDL and HDL of psoriatic patients. The modifications of lipoprotein composition and fluidity observed in psoriatic patients could be of physiopathological and clinical relevance in relation to the pathogenesis of the disease. ***Key word: fluorescence polarization.***

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An increased incidence of cardiovascular diseases associated with alterations of plasma lipoprotein pattern has been observed in adult patients affected by psoriasis (1). This chronic inflammatory disease of the skin, with a multifactorial inheritance, is characterized by keratinocyte proliferation and incomplete differentiation (1). A tendency towards a decrease of cholesterol associated with high-density lipoproteins (HDL-C) and an increase of triacylglycerol (TG) in very low-density lipoproteins (VLDL) and in low-density lipoproteins (LDL) have been shown in adult patients compared with controls (2). Modifications of fatty acid composition of TG, phospholipids (PL) and cholesterol esters of lipoproteins have also been observed in adult patients (3).

Plasma lipoproteins, beside the important role in the bulk transport of lipids, are also involved in maintaining the proper lipid composition of cell membranes. Changes of lipid composition in cell membranes in consequence of modification of lipid exchange processes between the lipoprotein surface and the counterparts in cell plasma membranes have been observed in clinical disorders characterized by an abnormal plasma lipoprotein profile, such as hypercholesterolemia, diabetes, hypothyroidism and abetalipoproteinemia (4–6).

In psoriasis, at membrane level, structural and functional alterations are not limited to epidermal plasma membranes. Changes of membrane lipid composition and/or properties have also been observed in lymphocytes, polymorphonuclear leukocytes (PMN) and platelets of psoriatic patients (7–9). Moreover, significant changes of PL fatty acid composition and a decrease of membrane fluidity have been observed in erythrocytes of adult patients (10). Conversely to these results we have demonstrated an increase of erythrocyte membrane fluidity in the absence of PL fatty acid composition in psoriatic prepubertal patients (11). Erythrocytes are particularly sensitive to abnormalities of plasma lipoprotein composition because lipid biosynthetic processes are lacking in these cells and their membrane composition and stability depend on lipid exchange processes with circulating lipoproteins (12).

In order to further investigate abnormalities of plasma lipoprotein in childhood, we studied the composition of the three main lipoprotein classes (VLDL, LDL and HDL) in 15 prepubertal psoriatic children, with the duration of the disease ranging from 2 to 9 years, compared to 14 prepubertal sex- and age-matched controls.

Since lipoprotein fluidity, strictly related to lipoprotein lipid composition (13, 14), has been considered an important factor which influences lipid exchange processes between lipoproteins and membranes and interactions between lipoproteins and cell receptors, the application of fluorescence polarization of lipophilic probes to the study of lipoprotein fluidity has received an increasing interest in the recent years (15–18). Therefore the compositional analysis of lipoproteins of psoriatic children has been associated to the study of lipoprotein fluidity using the polarization of the fluorescent molecule 1,6-diphenyl-1,3,5-hexatriene (DPH), which has been widely used to investigate fluidity in natural membranes, as well as in human lipoproteins in normal and pathological conditions (11, 15–18).

METHODS

We studied, with parental consent, 15 prepubertal children with psoriasis (6 females and 9 males, mean age 9.4 ± 2 years). In all the patients the disease was mild–moderate, 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 weeks preceding the study. The duration of disease ranged from 2 to 9 years. None of the patients had a history of cardiovascular disease or familial hyperlipidemia, or had a known diabetes mellitus, and all had normal laboratory tests for liver and renal function.

The control group consisted of 14 healthy, prepubertal children (6 females and 8 males, mean age 9 ± 2.3 years) who were checked at the Dermatological Institute. They were not taking any drugs. Patients and controls came from the same region of Italy and had similar nutritional habits evaluated by a questionnaire concerning their dietary habits.

The body mass index (BMI) (i.e. weight in kg divided by the square of height in metres), taken as obesity index, was similar in both groups (mean value 16.6 ± 3 kg/m^2 in controls and 18.7 ± 2.7 kg/m^2 in psoriatic patients).

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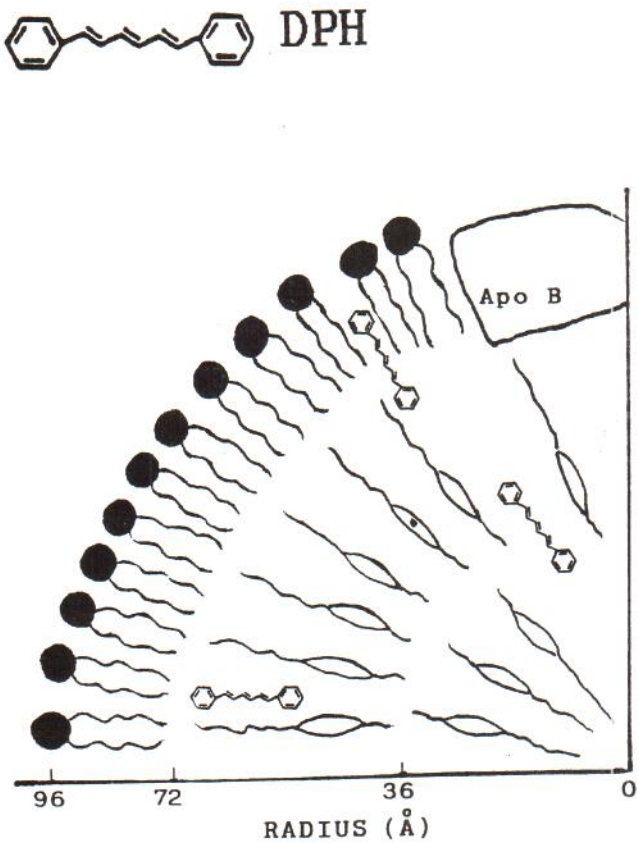

Fig. 1. Schematic diagram of LDL showing the localization of the fluorescent probe 1,6-diphenyl-1,3,5-hexatriene (DPH). LDL is depicted as comprised of a monolayer of phospholipid and apoprotein (cholesterol is omitted) surrounding a core of cholesterol esters (triglyceride is omitted).

Preparation of lipoproteins

Blood samples were drawn after 12 h overnight fast and were put in tubes containing 1 mmol/l disodium EDTA as anticoagulant. Lipoproteins were isolated by ultracentrifugation using a density gradient of NaBr as described by Kelly & Kruski (19). Ultracentrifugation was performed at 15°C for 24 h at 38.000 rpm using a TFT 80.31 rotor in a Centrikon T 2070 ultracentrifuge (Kontron Instrument). The density range for the lipoproteins was as follows: VLDL ($d < 1.006$ g/ml); LDL ($d = 1.019$–1.063 g/ml); HDL ($d = 1.063$–1.21 g/ml).

Analytical methods

Lipoprotein analysis in plasma was performed using electrophoresis on agarose-gel (Sebia, France). Total cholesterol (TC), PL and TG in plasma and in each lipoprotein fraction were determined using enzymatic methods (2022) on a analyzer (Synchron Cx5/Beckman, Germany). Apoprotein (P) concentration of lipoproteins was determined as described by Lowry et al. using serum albumin as standard (23).

Fluorescence polarization measurements

Lipoprotein fluidity was studied by determining the fluorescence polarization of the probe DPH (Aldrich Chemical Co., Germany), whose chemical structure and localization in plasma lipoproteins are shown in Fig. 1. The fluorescence polarization values (P_f) depend on probe rotational mobility and on the degree of order of lipid molecules in the microenviroment of the probe (24). The parameter P_f is considered to be inversely related to the bulk lipoprotein fluidity (25). Lipoproteins were labelled with DPH, following the procedure of Dachet et al. (15). Briefly, a fresh dispersion of DPH was incubated in a buffer containing 0.15 M NaCl, 20 mM Tris-HCl, pH 7.4 with 100 to 300 µl of lipoprotein (HDL, LDL, VLDL) corresponding to about 50–100 µg of apoprotein.

After labelling the fluorescence polarization measurements of DPH were performed using a spectrofluorimeter Perkin Elmer MPF66 equipped with fluorescence polarization accessory and a controlled temperature cell holder. The excitation and emission wavelengths were 365 nm and 430 nm, respectively. The fluorescence polarization value (P_f) was obtained from the fluorescence intensities parallel ($I\|$) and perpendicular ($I\perp$) 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 used to correct the unequal transmission of differently polarized light. Scattered intensity from unlabelled lipoprotein was less than 3% of that emitted from DPH-labelled lipoprotein.

Statistics

All the results are expressed as mean ± SD. Statistical differences between data from psoriatic patients and controls were determined in accordance with the Student's *t*-test.

Table I. *Plasma lipids and lipoproteins in controls and patients*

Values are expressed as means ± SD.

		Controls (14)	Patients (15)
Total cholesterol	(mg/dl)	158±26	174±23
VLDL-cholesterol	(mg/dl)	13± 5	15± 5
LDL-cholesterol	(mg/dl)	98±22	107±25
HDL-cholesterol	(mg/dl)	46± 9	50±10
Total triacylglycerol	(mg/dl)	67±16	80±17
VLDL-triacylglycerol	(mg/dl)	15± 8	24± 9
LDL-triacylglycerol	(mg/dl)	18±10	25±10
HDL-triacylglycerol	(mg/dl)	15± 5	22± 5
Lipoproteins			
HDL	(%)	32± 4	33± 6
LDL	(%)	47± 5	48± 6
VLDL	(%)	21± 5	19± 5

Table II. *Chemical composition of plasma lipoprotein in controls and psoriatic patients*

	Composition weight %			
	Triacyl-glycerol (TG)	Total cholesterol (TC)	Phospho-lipids (PL)	Apoprotein (P)
VLDL				
Controls	33±17	16±8	16±4	34±18
Patients	45±9*	11±2	17±4	27±1**
LDL				
Controls	6±4	25±6	21±3	48±7
Patients	10±2**	34±3*	26±3*	30±6°
HDL				
Controls	3±1	9±3	18±5	70±9
Patients	4±1§	16±3§§	32±7§§	46±8§§

* $p < 0.01$; ** $p < 0.02$; § $p < 0.006$; ° $p < 0.002$; §§ p 0.001.

Table III. *Fluorescence polarization values (P_f) of DPH in lipoproteins of 14 controls and 15 patients*

Measurements were performed at 25°C. Values are expressed as mean ± SD.

Lipoproteins	Controls (P_f)	Patients (P_f)
VLDL	0.205 ± 0.013	0.200 ± 0.010
LDL	0.333 ± 0.012	0.326 ± 0.010*
HDL	0.295 ± 0.013	0.311 ± 0.020*

* $p < 0.001$.

RESULTS

Plasma lipids and lipoproteins

Plasma lipid and lipoprotein levels of psoriatic children and controls are summarized in Table I. We have not observed any sex-related changes in plasma lipid and lipoprotein levels between controls and patients (data not shown); therefore the effect of sex was not further taken into account in the study.

In psoriatic children the levels of TC and TG were increased, although not significantly (Table I). The percentage composition of lipoprotein of controls is in good agreement with previous studies (15) (Table II). No modifications of plasma lipoprotein levels were observed in psoriatic patients; however, the compositional analysis showed significant modifications with respect to controls (Tables I and II).

A significant decrease of the apoprotein content (Table II), with a statistically significant increase of the TG/P ratio in LDL (0.32 ± 0.12 vs 0.10 ± 0.04; $p < 0.001$) was observed in psoriatic patients. A significant increase of the percentage content of TC ($p < 0.01$ and $p < 0.001$) and of the TC/P ratio (1.14 ± 0.33 vs 0.49 ± 0.18; $p < 0.001$) was shown in LDL and in HDL (0.35 ± 0.013 vs 0.14 ± 0.06; $p < 0.002$) of psoriatic children. PL were significantly higher in HDL ($p < 0.0001$) and in LDL ($p < 0.01$). No relevant changes of PL content were observed in VLDL; however, the ratio TG/PL was significantly increased in this lipoprotein class (2.69 ± 0.58 vs 1.95 ± 1.12; $p < 0.04$).

Lipoprotein fluidity

The fluorescence polarization (P_f) values of DPH are in the order LDL > HDL > VLDL, in agreement with previous studies (15, 18) and indicate that DPH is located in a less ordered environment in VLDL ($P_f = 0.205 \pm 0.013$ at 25°C) compared to LDL and HDL ($P_f = 0.333 \pm 0.012$ and $P_f = 0.295 \pm 0.013$ at 25°C, respectively, Table III). The study of P_f values in lipoproteins of psoriatic patients showed a significant increase of P_f in HDL ($p < 0.001$) and a significant decrease in LDL with respect to the controls ($p < 0.001$). No significant changes were observed in VLDL (Table III).

Composition-fluidity correlations in lipoproteins

In order to investigate if the changes of lipoprotein fluidity are related to the modifications of lipoprotein composition, we studied the correlation between P_f and lipoprotein lipids by linear regression analysis. A negative correlation between P_f and TG/P ratio, which indicates that the higher values of the TG/P ratio are associated with lower values of P_f, was observed in LDL of controls ($r = -0.53$), in psoriatic patients ($r = -0.60$, $p < 0.01$) and in the two groups ($r = -0.69$, $p < 0.001$). The P_f values of LDL were also negatively correlated with the PL/P ratio of patients ($r = -0.48$) and in the two groups ($r = -0.48$, $p < 0.01$). The TG/PL ratio was negatively correlated with P_f of LDL in the whole population examined ($r = -0.55$, $p < 0.001$), but not in the separated groups. Moreover, the TG/PL ratio was negatively correlated with P_f in VLDL of control subjects ($r = -0.76$, $p < 0.01$). No significant correlation was observed for HDL.

DISCUSSION

Psoriasis has been associated in middle-aged patients with alterations of lipid metabolism, as evidenced by changes of plasmatic parameters with respect to controls (2, 3). In our previous studies we observed modifications of plasma lipid levels also in pediatric patients with a significant increase in TC and in cholesterol associated with HDL (11, 26). The significant increase of the ratio TC/apoB and the decrease of the ratio apoA1/HDL-C suggested abnormalities of plasma lipid-apoprotein relationships in lipoproteins of psoriatic children. However, a detailed biochemical compositional study of plasma lipoproteins has not been carried out in psoriasis. Moreover the content in PL and apoprotein that, at the lipoprotein surface, constitutes the interphase between water and non-polar lipid core and which contributes to stabilize the proper structure of all lipoprotein particles has not yet been investigated. The interphase is important for lipoprotein metabolism since it is directly involved in the exchange processes between lipoproteins and cells (27). In the present study, the compositional analysis comparatively evaluated in the single lipoprotein classes has shown, in the absence of quantitative modifications of plasma lipoprotein levels, significant modifications in psoriatic children affected by a mild degree of the disease. A significant increase of TG content and a significant decrease of apoprotein in all lipoprotein fractions in the psoriatic children compared to controls have been observed.

Fluorescence polarization (P_f) of DPH has been previously used to characterize the fluidity of human lipoproteins in normal and pathological conditions (15–18), and it has been demonstrated that the P_f of the probe is sensitive to modifications of lipoprotein lipid composition and can be a useful tool to study lipoprotein structural organization and fluidity in human diseases involving altered lipoprotein metabolism (15, 18). In our study P_f values were lower in VLDL than in other lipoproteins, as observed previously (15, 18). These results have to be related to the high content of TG in VLDL core, and to the potent fluidizing effect exerted by TG on lipoprotein fluidity (28, 18). In lipoproteins of psoriatic patients the compositional changes are associated with a significant increase of fluidity in LDL and a significant decrease of fluidity in HDL; no modifications have been observed in VLDL of pediatric patients. Lipoproteins have two distinct regions: the surface and the core, which differ in their composition, structure and physical properties. The hydrophobic probe DPH prefers to partition into the core, and its presence in the envelope is essentially dependent on envelope to core volume ratio (18). Therefore, in lipoproteins, with a rela-

tively small core, such as HDL, it is found also in the interface between the two regions (18). The P_f of DPH in lipoproteins depends on many biochemical factors such as content in TG, the TG/P or TG/PL and TG/C ratio. The DPH P_f can be directly influenced either by a major factor or the result of a combination of several ones. Moreover, since DPH localizes in different microenvironments in the three lipoprotein classes (15, 18), it could be influenced by compositional factors to different extent in the three lipoprotein fractions and could explain the opposite changes of fluidity in LDL and HDL of psoriatic children despite the similar lipid compositional changes compared with lipoproteins of controls.

The modifications of apoprotein content observed in all lipoprotein fractions of psoriatic patients could also induce abnormalities of the surface lipid-apoprotein interactions and in turn modulate lipoprotein fluidity, in particular in HDL where the marked curvature of the outlayer leads to a stronger interaction between the apoprotein moiety and the phospholipid fatty acids with respect to other lipoprotein classes.

Some hypotheses can be put forward about the mechanism(s) that result in the changes observed in lipoproteins of psoriatic children. The regulation of cholesterol content in lipoproteins involves complex mechanisms; crucial components are lipoprotein receptors in the liver and extra hepatic tissues that mediate the uptake and degradation of cholesterol-carrying lipoproteins (29). Changes in the transport of LDL receptors to the cell surface have been shown in psoriatic skin compared to normal skin (30), and a lowered LDL-receptor activity has been observed in cultured dermal fibroblasts isolated from the skin of psoriatic patients (31). Therefore the LDL receptor abnormalities, if confirmed also in other psoriatic cells, could be involved in the changes of low density lipoprotein composition.

Several experimental findings also suggest an abnormal lipid metabolism in epidermal cells of psoriatic patients with an increase of total lipids and phospholipids (32). The modifications of lipid metabolism in the peripheral cells of psoriatic patients can be translated into changes of lipoprotein composition and fluidity. On the other hand the lipoprotein alterations can induce further changes in the cells.

Studies are in progress to better elucidate the sequence of the events involved in the pathogenesis of the disease and to establish if lipoprotein alterations are primary events or the consequences of an abnormal metabolism of inflamed skin. However, the modifications shown in the present study in prepubertal children affected by a mild form of psoriasis support the hypothesis of a strong precocious linkage between psoriasis and plasma lipoprotein abnormalities.

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