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Altered lipid, apolipoprotein, and lipoprotein profiles in inflammatory bowel disease: consequences on the cholesterol efflux capacity of serum using Fu5AH cell system

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Abstract

Epidemiological data suggest a link between chronic inflammation condition and atherosclerosis. Infection and inflammation can also impair lipoprotein metabolism and produce a wide variety of changes in plasma concentrations of lipids and lipoproteins. Twenty-one patients with inflammatory bowel diseases (IBDs) and 28 healthy subjects were recruited. Serum concentrations of lipids, lipoproteins, apolipoproteins, leptin, ghrelin, and inflammation markers (C-reactive protein and serum amyloid A) were measured, and subjects' lipoproteins were characterized. The ability of patients with serum IBD to efflux free cell cholesterol was measured. Serum cholesterol, high-density lipoprotein cholesterol, apolipoprotein (apo) A-I, apoC-II, apoC-III bound to apoB, phospholipid, and phospholipids not bound to apoB levels were significantly lower, whereas serum triglyceride, serum amyloid A, and C-reactive protein levels were significantly higher in patients with active IBD. Apolipoprotein A-I immunoreactivity (pre-β small particles and small α–high-density lipoprotein particles) is decreased in patients with IBD. In contrast, apoE immunoreactivity (slow/small apoE containing lipoprotein particles [LpE particle]) increased in these patients. The efflux capacity of serum from patients with IBD using [^{3}H]-cholesterol–labeled Fu5AH cells was reduced ($P < .005$). Our results demonstrate that, in subjects with active IBD, inflammation leads to alterations in lipid, apolipoprotein, and lipoprotein profiles and reduced cholesterol efflux. These changes are similar to those proposed to promote atherogenesis and may contribute to the development of cardiovascular events.

1. Introduction

Epidemiological data suggest a link between chronic inflammation and atherosclerosis. During infection and inflammation, a wide range of alterations in metabolism occurs. These are part of the body reaction known as the acute phase response (APR). Levels of positive acute phase proteins, C-reactive protein (CRP), and serum amyloid A (SAA) increase during the APR. Infection and inflammation can also impair lipoprotein metabolism and produce a wide variety of changes in plasma concentrations of lipids and lipoproteins [1]. Alterations in plasma lipids and lipoproteins have been described in inflammatory bowel diseases (IBDs) [2]. These alterations can be summarized by an increase in triglycerides (TGs) due to an accumulation of very low density lipoprotein (VLDL) and a decrease in high-density lipoprotein (HDL) and apolipoprotein (apo) A-I levels [1]. Although low-density lipoprotein (LDL) cholesterol levels decrease during infection and inflammation in humans, there is an appearance of small dense LDL [3], particles believed to be more atherogenic than those of a larger size [4]. Infection and inflammation are also associated with a decrease in HDL cholesterol (HDL-C) levels [5]. A persistently low level of HDL-C in chronic infection and inflammation may be undesirable because data from epidemiological studies have shown a greater risk of coronary heart disease in subjects with low HDL-C levels. The function of HDL is explained by its role in reverse

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cholesterol transport (RCT) [6]. The cholesterol efflux is the first step of RCT, a process mediated by a small-sized HDL particle named "pre-β HDL" and containing apoA-I, which is able to interact with ATP-binding cassette A1 protein and accepts excess cholesterol from peripheral tissues. Cholesterol efflux can also be explained by passive diffusion of cholesterol from cells to mature HDL that constitutes the bulk of plasma HDL. Several studies have demonstrated that apoA-I, the prominent HDL apolipoprotein mediating cholesterol efflux from peripheral tissues, is displaced by acute phase SAA protein [7]. Lecithin cholesterol acyltransferase (LCAT) is another component of HDL, which efficiently esterifies free cholesterol in pre-β HDL, leading to the appearance of α-HDL particles. This process, activated by apoA-I, may influence cholesterol efflux by keeping the indispensable cholesterol gradient between cells and HDL. Impaired activity of LCAT might be responsible for altered cholesterol homeostasis of HDL during the APR [8], thus leading to a decrease in α-HDL particles.

The aim of this study was to examine the lipid and lipoprotein profile in subjects with IBD. Because little is known about the consequences of lipid and lipoprotein alterations on cholesterol efflux, we also investigated the ability of IBD serum to modulate cellular cholesterol efflux, which is considered as the first step of RCT, using Fu5AH cells as cholesterol donors.

2. Materials and methods

2.1. Sample population

Twenty-one patients with IBD were studied. The diagnosis of Crohn's disease or ulcerative colitis was based on standard clinical symptoms. Disease activity in patients with Crohn's disease at a given visit was quantified using the Crohn's disease Activity Index score [9]. Disease activity for patients with ulcerative colitis was assessed using the simple clinical colitis activity index described by Walmsley et al [10]. Apart from IBDs, no subjects had any cardiovascular disease. There was no special family history. They were not taking any medication known to affect plasma lipid levels. There was no special diet advice except the usual recommendation done to subjects with IBD in a period of disease activity. The clinical profile of our IBD population is summarized in Table 1.

Table 1
Clinical profile of the patient population

	Total
No.	21
Men/women	11/10
Active/quiescent	16/5
Treatment[a]	
Azathioprine/6-mercaptopurine	14
Methotrexate	5
Infliximab	6
Antibiotics	2
Hydrocortisone	2
Aminosalicylate	1
Thalidomide	1
None	1

[a] Some patients received more than 1 drug.

2.2. Blood samples

Blood samples were collected after subjects had fasted for 12 hours overnight. EDTA plasma and serum were separated immediately by centrifugation (3000 rpm for 15 minutes) at 4°C, divided into aliquots, and kept at −80°C until needed for analyses.

2.3. Analytical methods

Blood cholesterol, TGs, phospholipids (PLs), and HDL-C were assayed enzymatically in EDTA plasma using a multiparametric analyzer (Hitachi 917, Roche Molecular Biochemicals, Meylan, France). Low-density lipoprotein cholesterol was calculated with the Friedewald formula [11]. In any case, the TGs were high enough to invalidate the use of this formula. Phospholipids and PLs not bound to apoB (PL Lp–non-B) were measured with Wako reagent (Wako Chemicals GmbH, Neuss, Germany) made available in France through DiaSys (DiaSys, Bouffémont, France). Serum apoA-I, apoB, high-sensitivity CRP (hs-CRP), and SAA were assessed by immunonephelometry on Behring Nephelometer 2 (Dade-Behring, La défense, France). Plasma, leptin, and ghrelin were measured using a commercial enzyme immunoassay kit (Cayman Chemical, Ann Arbor, MI, Peninsula Laboratories, San Carlos, CA, and Mercodia, Uppsala, Sweden, respectively). Total serum apoC-II, apoC-III, and apoE were measured by immunoturbidimetric assay with Kamiya reagents (Kamiya Biomedical, Seattle, WA) made available in France through DiaSys (DiaSys, Bouffémont, France). Apolipoprotein E bound to apoB (apoE Lp-B) and apoE not bound to apoB (apoE Lp–non-B) were obtained by the same method after precipitation of LDL, VLDL, and chylomicrons by adding anti-apoB antibody (SEBIA, Issy-les-Moulineaux, France). Apolipoprotein C-III bound to apoB (apoC-III Lp-B) and apoC-III not bound to apoB (apoC-III Lp–non-B) were obtained by the same method after precipitation of LDL, VLDL, and chylomicrons by adding phosphotungstic acid and magnesium ions (DiaSys). The ratio between apoE Lp-B and apoC-III Lp-B (apoE/apoC-III Lp-B) was used to estimate the relative importance of apoE and apoC-III Lp-B. Similarly, the ratio between apoE Lp–non-B and apoC-III Lp–non-B (apoE/apoC-III Lp–non-B) was used to estimate the relative importance of apoE and apoC-III Lp–non-B.

2.4. Enzyme-linked immunosorbent assay

Apolipoprotein A-IV concentration was determined by a highly sensitive and accurate enzyme-linked immunosorbent assay [12]. Briefly, microtiter plates were coated with 100 μL per well of anti–apoA-IV antibody (Institut Pasteur, Lille, France) that was diluted in phosphate-buffered saline

Table 2
Clinical and biological characteristics of the patients with IBD and healthy controls

	Healthy controls (n = 28)	Quiescent IBD (n = 6)	Active IBD (n = 15)
Age (y)	30.9 ± 9.2	32.7 ± 10.7	28.2 ± 9.0
BMI (kg/m^2)	21.6 ± 2.03	24.8 ± 8.2	21.53 ± 4.42
hs-CRP[a] (mg/L)	0.9 ± 1.09	9.04 ± 8.55[b]	22.03 ± 35.38[b]
SAA[a] (g/L)	3.33 ± 4.02	35.00 ± 43.43[b]	74.79 ± 196.13[b]
Leptin (ng/mL)	9.2 ± 7.4	38.7 ± 46.3[b,c]	9.32 ± 11.24
Ghrelin (ng/mL)	1.01 ± 0.5	0.67 ± 0.16	1.03 ± 0.72

Data are presented as mean ± SD.
[a] Log-transformed variables.
[b] Significantly different from healthy controls ($P < .05$).
[c] Significantly different from active IBD ($P < .05$).

(PBS) (GibcoTM, Invitrogen, Cergy Pontoise, France) at a final concentration of 20 μg/mL. After incubation at 4°C overnight, the plates were washed 4 times with washing solution (PBS) and treated with blocking reagent (PBS containing bovine serum albumin [BSA] 10 mg/mL; Sigma, Saint Quentin Fallavier, France) for 1 hour at 37°C. The plates were then washed again 4 times. One hundred microliters of each sample and of appropriately diluted standards was added to each well, and the plates were incubated for 2 hours at 37°C. The plates were then washed 4 times with PBS and incubated with 100 μL per well of horseradish peroxidase–labeled secondary antibody solution (0.5 μg/mL) for 2 hours at 37°C. The horseradish peroxidase activity was measured using 200 μL of ABTS (Boehringer Mannheim, Roche, Meylan, France) solution as substrate. The reaction was analyzed on a Spectra Max 190 (Molecular Devices, Saint Gregoire, France) at 405 nm and at 37°C.

2.5. Cholesterol efflux experiments

The ability of patients with serum IBD to efflux free cell cholesterol was measured by a procedure previously described [13] using [^{3}H]-cholesterol–labeled Fu5AH. Briefly, Fu5AH was cultured in minimum essential medium eagle (EMEM) (Sigma) containing 10% fetal calf serum (FCS) (Sigma). Penicillin, streptomycin, and glutamine were present in all media.

For efflux experiments, cells were plated in costar 24-well plates and grown in appropriate medium containing 10% FCS at 37°C in a humidified 5% CO_2 atmosphere. When they were nearly confluent, cells were incubated for 24 hours at 37°C with 1 μCi/mL of [1,2-^{3}H]cholesterol (10% FCS in EMEM). To ensure the label was evenly distributed among cellular pools, the labeling medium was replaced with EMEM containing 1% BSA (Sigma), and cells were incubated in albumin for 18 to 20 hours before measuring cholesterol efflux. The cells were then washed and incubated with the indicated serum prepared in EMEM (5% [vol/vol]), and efflux was performed for 4 hours. Radioactivity was then measured in the medium by liquid scintillation counting.

2.6. Two-dimensional nondenaturing gradient gel electrophoresis of lipoproteins

For the first dimension, 10 μL of serum from patients with IBD or healthy subjects' serum was separated by electrophoresis in 0.7% (wt/vol) agarose (Sigma) gel with a Tris-Tricine (Sigma) buffer (25 mmol/L, pH 8.6) and 3 mmol/L calcium lactate (Sigma), as described by Asztalos et al [14]. Samples were electrophoresed at 250 V constant voltage for about 40 minutes. For the second dimension, the agarose gel strips from the first dimension containing the preseparated lipoproteins were transferred to a 2% to 36% polyacrylamide gradient gel and sealed with the same agarose. Electrophoresis was performed using a buffer containing 90 mmol/L Tris (Sigma), 80 mmol/L boric acid (Euromedex, Mundolsheim, France), and 2.5 mmol/L EDTA (Prolabo, Paris, France) (pH 8.3). Separation in the second dimension was performed at 220 V constant voltage overnight. Molecular weight standards (Amersham Pharmacia Biotech, Orsay, France) containing thyroglobulin (669 kd, 17 nm), ferritin (440 kd, 12.2 nm), catalase (232 kd, 9.51 nm), lactate dehydrogenase (140 kd, 8.16 nm), and BSA (67 kd, 7.10 nm) were run simultaneously with the samples. After electrophoresis, proteins were electrophoretically transferred to nitrocellulose membranes of 0.2-μm pore (Protran Schleicher & Schuell, VWR, Fontenay sous Bois, France). Transfer was carried out for 18 hours in 20 mmol/L Tris and 150 mmol/L glycine buffer (pH 8.4) in a Transfer Power-LID Model TE 50 (Hoefer Scientific Instruments, San Francisco, CA) at a constant voltage (30 V) and at 10°C.

Table 3
Lipid parameters in patients with IBD and healthy controls

	Healthy controls (n = 28)	Quiescent IBD (n = 6)	Active IBD (n = 15)
Cholesterol (g/L)	2.03 ± 0.41	1.71 ± 0.37	1.62 ± 0.51[b]
HDL-C (g/L)	0.69 ± 0.18	0.535 ± 0.146	0.478 ± 0.088[b]
LDL cholesterol (g/L)	1.20 ± 0.34	0.96 ± 0.25	0.93 ± 0.46
Triglyceride[a] (g/L)	0.4 ± 0.41	1.08 ± 0.35	1.07 ± 0.53[b]
Phospholipids (g/L)	2.28 ± 0.45	2.01 ± 0.38	1.87 ± 0.36[b]
PL Lp–non-B (g/L)	1.42 ± 0.36	1.2 ± 0.29	1.04 ± 0.15[b]
PL Lp-B (g/L)	0.86 ± 0.29	0.81 ± 0.13	0.82 ± 0.33
ApoA-I (g/L)	1.76 ± 0.33	1.51 ± 0.31	1.38 ± 0.20[b]
ApoB (g/L)	0.87 ± 0.21	0.80 ± 0.18	0.79 ± 0.33
ApoA-IV (mg/L)	159.46 ± 49.2	164.96 ± 52.76	144.87 ± 51.20
ApoE (mg/L)	38.04 ± 8.8	38.67 ± 9.73	33.6 ± 10.03
ApoE Lp–non-B (mg/L)	29.15 ± 9.34	10.04 ± 1.38	26.53 ± 9.42
ApoE Lp-B (mg/L)	8.85 ± 5.6	8.67 ± 2.25	7.07 ± 5.05
ApoC-II (mg/L)	27 ± 9.34	24.67 ± 7.37	27.47 ± 22.12
ApoC-III (mg/L)	113.07 ± 28.93	102.17 ± 30.82	78.07 ± 33.43[b]
ApoC-III Lp–non-B (mg/L)	38.50 ± 22.66	24.75 ± 0.79	28.81 ± 11.35
ApoC-III Lp-B (mg/L)	74.57 ± 11.34	60.17 ± 22.80	52.35 ± 27.33[b]
ApoC-II/TG[a]	43.26 ± 18.67	23.28 ± 5.33[b]	27.42 ± 15.07[b]

Data are presented as mean ± SD.
[a] Log-transformed variable.
[b] Significantly different from healthy controls ($P < .05$).

Apolipoprotein A-I and apoE in serum from patients with IBD and from healthy subjects were identified by the use of a polyclonal antihuman apoA-I and monoclonal antihuman apoE antibodies (Institut Pasteur).

2.7. Statistical analyses

Results were analyzed using SAS statistical software, version 8.2 (SAS Institute, Cary, NC). For non–normally distributed variables, values were log-transformed before analysis of variance, linear correlation, and multiple regression analysis. Significance levels are shown for all results where P is less than .05. A logistic multiple regression analysis was run stepwise to assess which parameters were significantly and independently associated with the presence and severity of the disease. In this model, the severity of the disease, coded 0 for healthy controls, 1 for quiescent IBD, and 2 for active IBD, was the explained variable, and all biological parameters, as well as age and body mass index (BMI), were introduced as explaining variables. Finally, to assess which parameters were the best contributors to cholesterol efflux, a multiple regression analysis was run stepwise. In this analysis, efflux was the explained variable, and all biological parameters, as well as age, BMI, and disease activity (0 for healthy controls, 1 for quiescent IBD, and 2 for active IBD), were introduced as explaining variables.

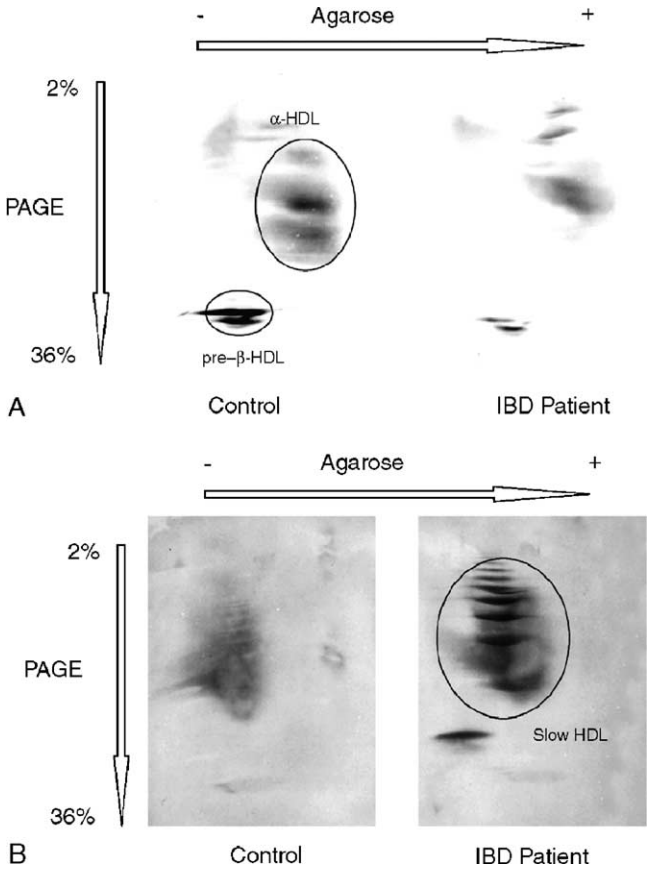

Fig. 1. Immunoblots of 2D-PAGE for apoA-I (A) and apoE (B). Serum samples from patients with IBD and healthy subjects were separately electrophoresed in the first dimension in 0.7% agarose, followed by application of agarose strip to the top of a 2% to 36% nondenaturing concave polyacrylamide gel and subsequently electrophoresed. The circles indicate the position of human apoA-I and apoE obtained after immunolocalization by a polyclonal antihuman apoA-I and a monoclonal antihuman apoE antibody. 2D-PAGE has been performed from 5 representative patients from each group. The patient with IBD in this figure is quiescent; however, there is no apparent difference in figures obtained for quiescent or active patients.

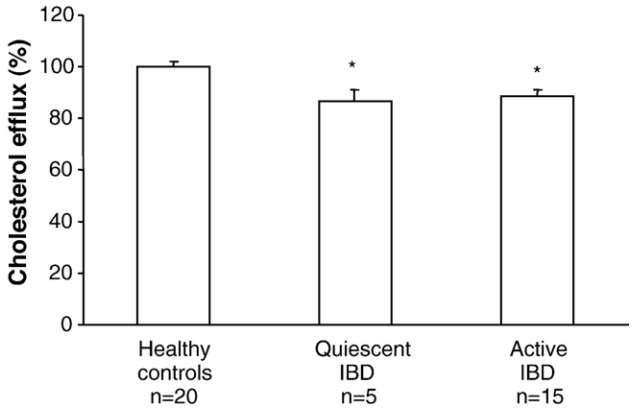

Fig. 2. [^{3}H]-Cholesterol efflux from Fu5AH. [^{3}H]-Cholesterol–labeled Fu5AH cells were incubated with serum from patients with IBD and healthy subjects, and efflux was performed for 4 hours. Radioactivity was then measured in the medium by liquid scintillation counting (for details, see Materials and Methods). Data are presented as mean ± SEM. *Significantly different from healthy controls ($P < .05$).

3. Results

Clinical and biological characteristics of the subjects studied are presented in Table 2. The concentrations of serum SAA and hs-CRP were significantly higher in samples from all patients, as compared with healthy controls ($P < .05$), as expected for inflammatory state. Leptin levels were significantly higher in patients with quiescent IBD than in healthy controls and patients with active IBD ($P < .05$). Insulin had similar tendency as leptin, but the difference did not reach significance. No difference in ghrelin concentrations was observed between the 3 groups of subjects.

Table 4
Linear correlations between cholesterol efflux and various lipid and lipoprotein parameters

	Healthy controls (n = 20)		IBD (n = 20)	
	r	*P*	*r*	*P*
Cholesterol	0.48	<.05	0.58	<.01
HDL-C	0.79	<.0001	0.38	.097
LDL-C	0.107	.65	0.50	<.03
ApoA-I	0.69	<.0005	0.49	<.026
ApoB	0.096	.69	0.47	<.05
ApoA-IV	0.28	.24	0.57	<.008
Phospholipids	0.76	<.0005	0.72	<.0005
PL Lp–non-B	0.63	<.005	0.53	<.02
PL Lp-B	0.226	.34	0.53	<.02
ApoC-II/TG[a]	0.24	.31	−0.67	<.002

[a] Log-transformed variable.

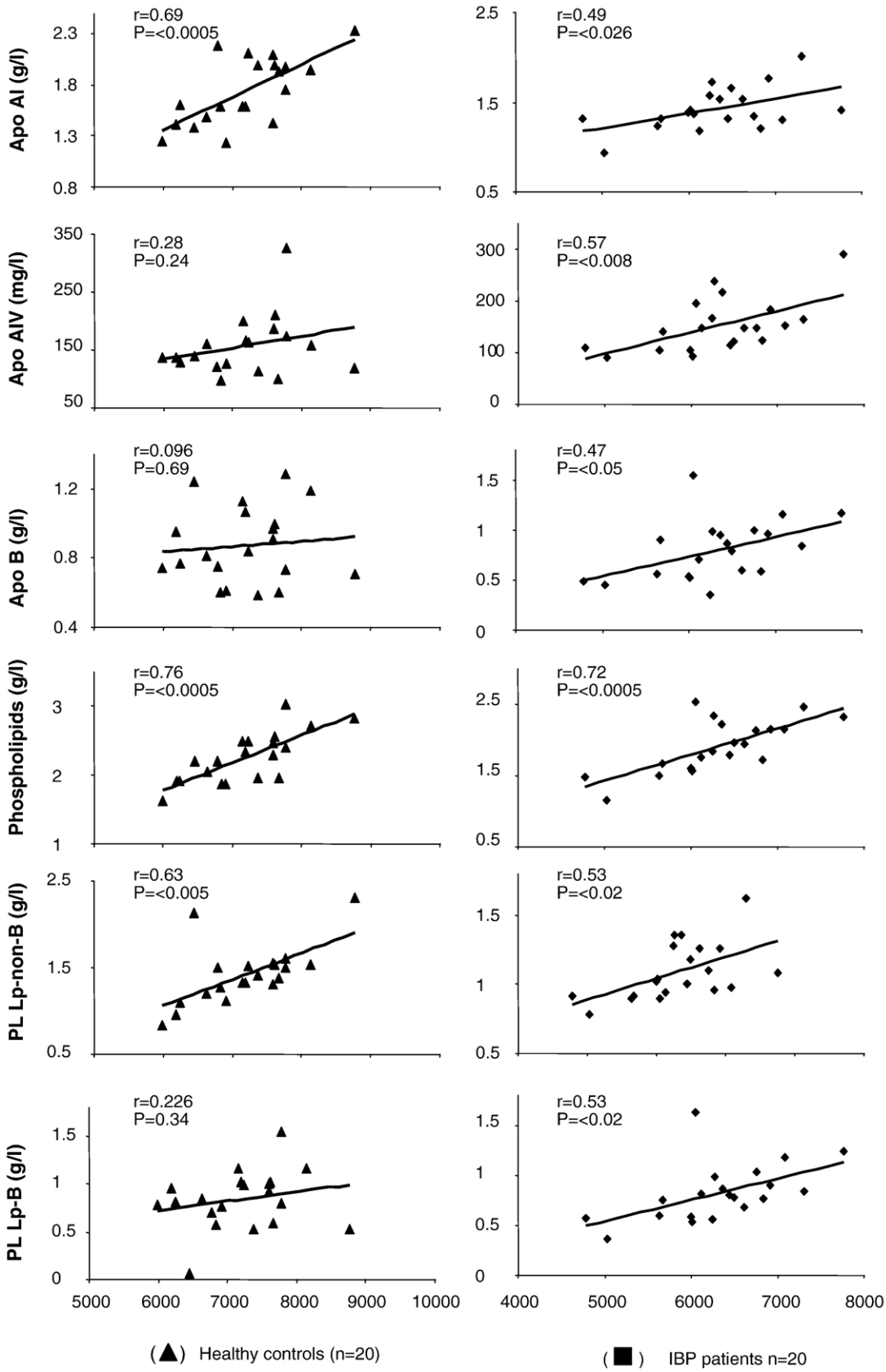

Fig. 3. Linear correlations between cholesterol efflux and apoA-I, apoB, apoA-IV, PL, PL Lp–non-B, and PL Lp-B.

Lipids, apolipoproteins, and lipoproteins of the subjects studied are presented in Table 3. Patients with active IBD had significantly lower concentrations of total cholesterol ($P < .05$), HDL-C ($P < .05$), and apoA-I ($P < .05$) than healthy controls, whereas no significant difference was found between quiescent IBD and controls, although a tendency toward a decreased concentration was observed.

The levels of TGs were higher in patients with active IBD than in healthy subjects ($P < .05$). The same was true for quiescent IBD, although the significance was not reached probably because of the low number of subjects in this group. Patients with active IBD also displayed lower total PLs ($P < .05$) and PL Lp–non-B ($P < .05$). Apolipoprotein C-III and apoC-III Lp-B levels were also decreased in patients with active IBD when compared with controls ($P < .05$). The levels of total apoE, apoE Lp–non-B, apoE Lp-B, apoA-IV, and apoC-II did not differ between patients with active and quiescent IBD and healthy subjects. However, the apoC-II/TG ratio was decreased in both groups of patients with IBD ($P < .05$) when compared with controls, and apoE Lp–non-B tended to be lower in quiescent IBD, although the significance was not reached.

Two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) of plasma samples obtained from patients with IBD and healthy individuals was performed to demonstrate differences in the distribution of apoA-I– and apoE-containing lipoprotein particles (Fig. 1). When compared with healthy subjects, the reduced immunoreactivity for apoA-I (Fig. 1A) was mainly due to a loss of pre-β small particles and small α-HDL particles. In contrast, apoE immunoreactivity increased in inflammation especially within the slow/large HDL range and a slow/small LpE particle (Fig. 1B).

To examine the properties of serum from the patients with IBD in promoting cholesterol efflux in vitro, we measured the efflux of labeled cholesterol from Fu5AH cells after 4 hours of incubation. Compared with that from control subjects, the efflux of cell-derived [^{3}H]-cholesterol from patients with active and quiescent IBD was reduced by 11.53% and 13.64%, respectively ($P < .05$) (Fig. 2).

Table 4 presents the relationship between major lipid and lipoprotein parameters and cholesterol efflux, as determined by simple correlation. Better correlations listed in Table 4 are presented in Fig. 3 as x-y plots. In healthy control serum, HDL-C best correlated with [^{3}H]-cholesterol efflux ($r = 0.79$, $P < .0001$), followed by PL ($r = 0.76$, $P < .0005$), apoA-I ($r = 0.69$, $P < .0005$), and PL Lp–non-B ($r = 0.63$, $P < .005$). Positive correlations were also found between PL ($r = 0.72$, $P < .004$) and PL Lp–non-B ($r = 0.53$, $P < .05$) and cholesterol efflux using sera from subjects with IBD. However, these correlations were apparently not as strong as for sera from control subjects. Surprisingly, no significant correlation was found in IBD between HDL-C or apoA-I and cholesterol efflux, whereas in the difference with control subjects, apoA-IV correlated positively with cholesterol efflux ($P = .71$, $P = .005$).

Table 5
Logistic multiple regression analysis on the variables linked to the clinical state of the disease

Variable	$\beta \pm$ SD	P
Cholesterol	−2.82 ± 1.02	.0057
SAA[a]	1.16 ± 0.36	.0011

Model includes age, BMI, cholesterol, HDL-C, LDL-C, apoA-I, apoB, apoA-IV, apoC-II, apoC-III Lp-B, leptin, ghrelin, insulin, apoE Lp–non-B, apoE Lp-B, PL Lp–non-B, PL Lp-B, apoE/apoC-III Lp-B, apoE/apoC-III Lp–non-B, efflux, CRP[a], SAA[a], TG[a], and apoC-III Lp–non-B[a].

[a] Log-transformed variable.

Table 6
Multiple stepwise regression of the parameters linked to efflux

Variable	$\beta \pm$ SD	P
ApoB	−1434.75 ± 498.74	.0075
PL Lp–non-B	2856.91 ± 472.71	<.0001
PL Lp-B	3235.91 ± 516.40	<.0001
TG[a]	−506.59 ± 171.94	.0063

Model includes age, BMI, cholesterol, HDL-C, LDL-C, apoA-I, apoB, apoA-IV, apoC-II, apoC-III Lp-B, leptin, ghrelin, insulin, apoE Lp–non-B, apoE Lp-B, PL Lp–non-B, PL Lp-B, apoE/apoC-III Lp-B, apoE/apoC-III Lp–non-B, CRP[a], SAA[a], TG[a], apoC-III Lp–non-B[a], and disease activity.

[a] Log-transformed variable.

To check what parameters were related to the clinical state of the disease (no disease, quiescent disease, active disease), a logistic multiple regression was run stepwise. The model included age, BMI, and all the biological variables measured. Results are presented in Table 5. This analysis shows clearly that 2 parameters are independently related to the clinical state of the disease: cholesterol, which contributes negatively to the model, and SAA, which contributes positively to the model. This indicates clearly that SAA discriminates between various degrees of IBD.

A stepwise multiple regression model including parameters related to efflux of cell-derived [^{3}H]-cholesterol with a P value less than .15 was run to assess their independent contribution to efflux. As shown in Table 6, which summarizes the results of this analysis, PL Lp–non-B and PLs bound to apoB (PL Lp-B) appeared to be the best positive contributor to efflux, whereas TGs and apoB were independently and negatively related to this parameter.

4. Discussion

Epidemiological studies suggest a link between chronic inflammatory diseases and atherosclerosis [15]. Although IBDs affect an organ directly involved in lipid metabolism, a few studies have been focused on lipid and lipoprotein profile as well as cholesterol efflux, the first step of RCT. The present study was undertaken to describe these lipoprotein abnormalities in 21 subjects with IBD compared with 28 healthy controls. Among the 21 subjects with IBD, 18 had Crohn's disease, and 3 had ulcerative colitis. No difference between these 2 subgroups was observed (data not shown).

In our study, we have shown that hs-CRP and SAA, 2 positive acute phase proteins, are highly increased during

IBD. In addition, SAA was the only variable which was positively and independently linked to the severity of the disease, indicating that SAA may help to discriminate patients at various states of the disease. As suggested by several works, this may contribute to the risk of future coronary events in this population [16].

Leptin is synthesized and secreted predominantly by adipose tissue, and it has been associated with obesity [17]. Our results show that leptin levels were significantly higher in quiescent IBD than in control subjects and active IBD. Controls and active IBD have similar BMI, whereas subjects with quiescent IBD have higher BMI (24.8 kg/m^2). We may speculate from this observation that leptin is associated with BMI and not with disease activity. In fact, it is well known that during remission period, patients with IBD gain weight, consistent with improved nutritional status [18]. Thus, these results suggest that changes in body weight explain the elevation in leptin levels during the remission period and that active disease is not associated with increases in leptin levels.

Ghrelin is an orexigenic peptide that antagonizes leptin action and causes weight gain by increasing food intake and reducing fat use in rodents and humans [19,20]. Considering that ghrelin is secreted by the neuroendocrine tissue located in the stomach [21], it would have been expected that its level would be affected by IBD. Surprisingly, we did not find any difference between our 3 groups of subjects. However, ghrelin concentrations tended to be higher in healthy controls and patients with active IBD than in patients with quiescent IBD, although it did not reach statistical significance. This may reflect the competitive interaction between ghrelin and leptin in feeding regulation, which was suggested by the study of Vendrell et al [22] demonstrating, in morbid obesity, an inverse relationship between ghrelin and leptin.

Our data also documented abnormally low plasma cholesterol, HDL-C, and apoA-I concentrations in patients with active IBD compared with healthy controls of the same age and sex. In a recent study of pediatric Crohn's disease, levels of plasma apoA-I and apoB-100 were also found to be lower in patients with Crohn's disease than in controls [2]. At this time, it is uncertain whether hypocholesterolemia found in subjects with IBD results from intestinal malabsorption, malnutrition, or other mechanisms.

The decrease in HDL and apoA-I noted in subjects with active IBD may be related to the increase in SAA concentrations, as has been demonstrated by in vitro and in vivo studies. In fact, it has been shown that when HDL3 was incubated with SAA, apoA-I and apoA-II, 2 HDL apolipoproteins, were displaced up to 52% by SAA [7]. This assumption is also supported by metabolic studies indicating that SAA-containing HDL is cleared more rapidly from the circulation than normal HDL [23]. Serum amyloid A also enhances HDL/SAA binding and uptake preferentially to macrophages rather than to hepatocytes [24]. Consequently, more cholesterol in HDL may be directed to macrophages during inflammation, which can result in foam cell formation in the arterial wall. These proatherogenic changes in HDL may contribute to the link between inflammation and atherosclerosis.

Our data show an increase in TG levels in subjects with active IBD. This situation has been widely described during inflammation [25]. The hypertriglyceridemia associated with infection and inflammation has been attributed to both an increase in hepatic lipoprotein production and a decrease in lipoprotein clearance. These changes may be due to a decreased activity of lipoprotein lipase (LPL) [26]. However, results of the literature are conflicting in this point of view, and a few studies failed to demonstrate any change in LPL activity.

Apolipoprotein C-II and apoC-III are involved in the metabolic conversion of different TG-rich lipoprotein classes. Apolipoprotein C-II has an essential role in activating LPL, whereas apoC-III inhibits the lipolytic process [27]. In our study, no change in apoC-II concentration was found, whereas the apoC-II/TG ratio was lower in both groups of subjects with IBD than in controls. It may be that this low apoC-II/TG ratio contributes to a lower clearance of TG-rich lipoproteins in IBD. However, it was surprising to find a lower concentration of apoC-III Lp-B in active IBD when compared with controls, whereas TGs are increased. This intriguing finding suggests that besides a lower clearance of VLDL, subjects with IBD may synthesize VLDL with an abnormal composition in apolipoproteins.

By 2D-PAGE separation, we were able to show that mainly small pre-β and α-HDL particles are reduced in subjects with IBDs. We further characterized the lipoprotein particles by 2D-PAGE using an anti-apoE antibody. The apoE immunoreactivity did not colocalize with apoA-I–containing particles, as has already been demonstrated by Krimbou et al [28], suggesting that some of these lipoproteins contain apoE as their major apolipoprotein component. This apoE-rich HDL has already been found in LCAT or cholesteryl ester transfer protein deficiency [29]. Taken together, these observations suggest that HDL may decrease in our patients for 2 reasons: the presence of SAA in the particle and a probable decreased LCAT activity, a situation leading to a loss of mature HDL [8].

As inflammation induces functional and compositional changes in HDL lipoproteins, we have investigated the capability of IBD serum to promote cholesterol efflux, the first step in RCT. The cell line selected for the efflux was Fu5AH. This cell line has been used in our study because it has provided highly reproducible data on cholesterol efflux in numerous studies [13,30-32]. This cell line demonstrates binding of HDL [33], and, importantly, Fu5AH cells exhibited the most rapid efflux of any cell line studied for the efflux [34]. The results of the present work show that the cholesterol efflux is decreased in the presence of sera from a patient with IBD compared with sera from a matched healthy subject. The small-sized pre-β and small α-HDL have been demonstrated to be the most efficient cholesterol

acceptors in human serum [35]. Therefore, the loss of these HDL particles in subjects with IBD may probably contribute to the low cholesterol efflux observed in these patients.

Cholesterol efflux depends on HDL and its major components (apoA-I and apoA-IV), PLs, and LCAT, the key enzyme that influences cholesterol efflux in which it keeps the indispensable cholesterol gradient between cells and HDL. Previous studies with apoA-IV have provided considerable evidence that apoA-IV contributes to RCT. In in vitro studies, apoA-IV has been shown to promote cholesterol efflux [36,37]. Our observation that efflux is not correlated to HDL-C but is correlated to apoA-IV in subjects with IBD suggests that apoA-IV represents a backup system for RCT in these subjects. Another factor related to efflux is the PL content of HDL, which may increase the affinity of apoA-I for the cell surface, either by protein-protein interaction [38] or by modifying the distribution of charged lipids on the cell surface [39], as has been previously suggested [38,39]. Although subjects with IBD have lower PL and PL Lp–non-B concentrations, this component seems to play a role in cholesterol efflux in these subjects. As suggested by our multiple stepwise regression analysis, this parameter remained a contributor to cholesterol efflux, independently of the severity of the disease.

In summary, our results have demonstrated that, in subjects with active IBD, inflammation leads to profound changes in circulating lipoproteins, probably related to the presence of high concentrations of SAA. Many of these changes are similar to those proposed to promote atherogenesis. In these patients, it is not unlikely that impairment in lipoprotein metabolism and reduced cholesterol efflux that we described would contribute to the development of cardiovascular events.

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