ApoE-containing high density lipoproteins and phospholipid transfer protein activity increase in patients with a systemic inflammatory response

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Abstract **High density lipoproteins (HDL) mediate reverse cholesterol transport as well as the clearance of oxidation products or inflammatory mediators, thereby contributing to tissue integrity. The decrease in HDL in inflammation has been attributed to decreased lecithin:cholesterol acyltransferase activity, whereas the role of phospholipid transfer protein (PLTP) and cholesteryl ester transfer protein has not been analyzed in detail. We have studied the activities of HDL-modifying proteins and the heterogeneity of HDL in healthy control subjects and three groups of postsurgery patients: no bacterial infection (group 1), bacterial focus and systemic inflammatory response (group 2), and severe sepsis (group 3). For all patients, a decrease in total HDL could be demonstrated, with a loss of mainly large, apolipoprotein A-I (apoA-I) HDL particles, an almost total loss of apoC-I, and an increase in apoE HDL (200–500 kDa), which did not contain significant amounts of apoA-I, apoA-II, or apoC-I. PLTP activity was increased in patients of groups 2 and 3, paralleled by a redistribution of PLTP into a population of small (120- to 200-kDa) particles, probably representing PLTP homodimers or lipid-complexed PLTP. In summary, the increase in apoE HDL and PLTP activity may improve the delivery of energy substrates and phospholipids to tissues that must maintain cellular membrane homeostasis under conditions of inflammatory stress.**—Barlage, S., D. Fröhlich, A. Böttcher, M. Jauhiainen, H. P. Müller, F. Noetzel, G. Rothe, C. Schütt, R. P. Linke, K. J. Lackner, C. Ehnholm, and G. Schmitz. **ApoE-containing high density lipoproteins and phospholipid transfer protein activity increase in patients with a systemic inflammatory response.** ***J. Lipid Res.*** **2001.** 42: **281–290.**

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The host response in inflammation is accompanied by profound alterations in lipid metabolism and hence the distribution and composition of lipoprotein subclasses (1). Especially high density lipoproteins (HDL), which mainly consist of apolipoproteins and phospholipids and that represent the most frequent lipoproteins in human plasma, have been demonstrated to play an important physiological role in restricting the harmful effects of inflammation and infection. HDL have not only been shown to mediate reverse cholesterol transport but also the clearance of inflammatory mediators such as bacterial lipopolysaccharide (2) or the scavenging of oxidation products (3), thereby contributing to tissue integrity. HDL carries proteins such as paraoxonase, platelet-activating factor acetylhydrolase (4), protectin (CD59) (5), ceruloplasmin, transferrin (6), and clusterin (ApoJ, S40:40) (7), which may serve as membrane protectants, because they scavenge radicals and inhibit oxidation (3). HDL are also capable of binding mediators of the inflammatory response, such as lipopolysaccharide (LPS), a membrane lipid of gram-negative bacteria (2, 8). Elevated HDL concentrations protect against the lethal effects of endotoxic shock in animal models (2). In humans recombinant HDL reduces endotoxin-induced inflammatory responses to LPS (9). Alterations of HDL concentration and subclass composition, therefore, may be detrimental in systemic inflammation.

The decrease in HDL in inflammation has mainly been

Abbreviations: CETP, cholesteryl ester transfer protein; HDL, high density lipoprotein; ICU, intensive care unit; ITP, isotachophoresis; LBP, lipopolysaccharide-binding protein; LCAT, lecithin:cholesterol acyltransferase; LDL, low density lipoprotein; LPS, lipopolysaccharide; PLTP, phospholipid transfer protein; SAA, serum amyloid A; SIRS, systemic inflammatory response syndrome; SPSS, Statistical Package for the Social Sciences; VLDL, very low density lipoprotein.

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attributed to a decrease in lecithin:cholesterol acyltransferase (LCAT) activity. LCAT promotes the maturation of HDL particles via accumulation of cholesteryl esters and it thereby contributes to the process of reverse cholesterol transport (RCT) (10). The initial acceptor of peripheral cell cholesterol in the RCT process is pre-βHDL, which includes several HDL subpopulations that contain apolipoprotein A-I (apoA-I) but no apoA-II. During the acute-phase response, inflammatory mediators suppress LCAT expression and activity (11, 12) and, in addition, apoA-I, which is the principal activator of LCAT (13), is suggested to be displaced from the HDL particle by the acute-phase protein serum amyloid A (SAA) (14).

Although both the phospholipid transfer protein (PLTP) and the cholesteryl ester transfer protein (CETP) are known to have a major impact on the metabolism of HDL particles in healthy individuals, few data are available on their role during inflammation (15). Both proteins belong to a family of homologous lipid transfer/LPS-binding proteins, which also includes the LPS-binding protein (LBP) (16, 17), and, therefore, these proteins may link the metabolism of HDL to the clearance of lipid inflammatory mediators.

The purpose of this study was to characterize HDL metabolism during inflammation, with special emphasis on HDL particle composition and the analysis of lipid transfer/LPS-binding proteins as potent regulators of HDL maturation.

MATERIALS AND METHODS

Patients

The study population consisted of 91 intensive care patients. The study was approved by the ethics committee of the University of Regensburg (Regensburg, Germany), and samples were collected only after participants had given informed consent. All patients had had major surgery. They were assigned to three groups: group 1 (n = 20) included patients with no signs of local or systemic infection; group 2 (n = 43) included patients with a known bacterial focus, who met the criteria for systemic inflammatory response sydrome (SIRS), that is, had a white blood cell count >14,000 μl^{-1}, and a body temperature exceeding 38°C; and group 3 (n = 28) included patients meeting the same criteria as group 2, with additional circulatory and respiratory failure. Circulatory failure was assumed if the patient had a systolic blood pressure lower than 70 mm Hg or required more than 20 mg h^{-1} of dopamine or an equivalent dosage of epinephrine or norepinephrine to achieve adequate blood pressure. Respiratory failure was defined as a ratio of arterial oxygen tension (PaO_2) to the fraction of inspired oxygen (FiO_2) lower than 280. Patients with a history of recent irradiation, diabetes mellitus, or chronic liver dysfunction were excluded. The severity of septic organ dysfunction was assessed by the sepsis-related organ failure assessment (SOFA) scoring system (18). Demographic data, score values, and the localization of the bacterial focus are summarized in **Table 1**.

Blood was sampled from eligible patients between 24 and 48 h after the initial achievement of inclusion criteria. Blood samples were drawn from the arterial line in place to avoid contamination with administered infusions, processed to serum, and stored at −70°C.

Patients of all groups received the same parenteral nutrition regimen, including 0.25 g kg^{-1} h^{-1} of glucose and xylit (GX35%; Pharmacia, Erlangen, Germany), 0.15 g kg^{-1} h^{-1} of amino acids (Aminomel® 10 salvia; Clintec, Weinheim, Germany), and 0.03 g kg^{-1} h^{-1} of triglycerides (Lipofundin® MCT 20%; B. Braun Melsungen, Melsungen, Germany).

For control subjects (n = 156), blood was obtained with informed consent from the laboratory personnel and healthy blood donors.

Determination of lipids

Cholesterol and triglyceride concentrations were determined by enzymatic methods with reagents from Roche Diagnostics (Mannheim, Germany). HDL cholesterol and low density lipoprotein (LDL) cholesterol levels were determined after precipitation with reagents from Immuno (Vienna, Austria).

TABLE 1. Characterization of patients

Parameter	Group 1	Group 2	Group 3
Age (years, mean ± SD)	50 ± 20	51 ± 19	51 ± 19
Male/female	15/5	26/17	25/3
Bacterial focus			
Pneumonia	None	20	12
Peritonitis	None	14	8
Abscess	None	4	3
Mediastinitis	None	2	2
Pancreatitis	None	3	3
SOFA score[a] (mean ± SD)			
Cardiovascular	1.72 ± 1.4	1.25 ± 1.3	3.3 ± 0.7
Respiration	0.88 ± 0.9	1.93 ± 0.89	2.63 ± 0.85
Liver	0.52 ± 0.71	0.80 ± 1.5	0.92 ± 1.1
Renal	0.15 ± 0.36	0.21 ± 0.5	0.69 ± 0.86
Coagulation	0.46 ± 0.71	0.31 ± 0.68	1.1 ± 1.3
C-reactive protein (mg/l)	93.8 ± 65.0	178.7 ± 84.6	202.3 ± 99.5
Elastase-α_1-proteinase inhibitor complex (μg/l)	369.3 ± 176.6	455.8 ± 318.7	673.6 ± 517.9

Demographic data, score rating of patient groups according to the SOFA (sepsis-related organ failure assessment) score (18), and concentration of acute-phase proteins are presented.

[a] According to Vincent et al. (18).

Analytical capillary isotachophoresis of lipoproteins

Analytical capillary isotachophoresis (ITP) of samples was performed as described earlier (19), except for minor modifications. Lipoproteins were stained with the fluorescent lipophilic dye 7-nitro-benz-2-oxa-1,3-diazole-(NBD) ceramide (Molecular Probes, Eugene, OR). Plasma was diluted 1:3.5 (v/v) with leading buffer, which consisted of 10 mM HCl, 0.35% (w/v) hydroxypropylmethylcellulose (HPMC), adjusted with 2-amino-2-methyl-1,3-propanediol (ammediol) to pH 8.8. The diluted plasma (20 μl) was incubated for 1 min with 10 μl of NBD-ceramide solution [0.1 mg/ml in ethylene glycol–methanol 9:1 (v/v)]. Twenty microliters of this solution was mixed with 50 μl of spacer mixture [1:6 (v/v) with leading buffer]. The spacer mixture was prepared from stock solutions of 10 mg/ml of the following compounds in order of decreasing electrophoretic mobilities: 100 μl of *N*-2-acetamido-2-aminoethanesulfonic acid, 50 μl of glucuronic acid, 50 μl of octanesulfonic acid, 80 μl of 3-*N*-tris(hydroxymethyl)methylamino-2-hydroxy-propane sulfonic acid, 120 μl of *N*-tris(hydroxy-methyl)methyl-3-aminopropanesulfonic acid, 60 μl of serine, 80 μl of glutamine; 80 μl of methionine, and 50 μl of glycine. As internal standard 10 μl of 5-carboxy-fluorescein (10 μg/ml) was added. The terminating electrolyte contained 20 mM alanine and was adjusted to pH 9.4 with saturated barium hydroxide solution. Separations were performed on a P/ACE 5510 system (Beckman, Fullerton) equipped with a 27-cm (20-cm length to detector) dimethyl polysiloxane modified fused silica capillary, i.d. 180 μm (Restek Rtx-1; purchased from Alltech, Unterhaching, Germany). The capillary, samples, and buffers were thermostated at 20°C during separation. Samples were injected into the capillary using pressurized injection for 18 s at 3.44 kPa. Separation was performed at constant 25 μA. The separated zones were monitored with laser-induced fluorescence detection (excitation, 488 nm; emission, 520 nm).

Preparative isotachophoresis of lipoproteins

Preparative separation of lipoproteins was done as described (20). Briefly, the separation chamber was cooled to 4°C and filled with the chamber buffers. The sample was injected with a 1-ml syringe and the separation was started with constant current of 3 mA. The voltage remained constant for the first 10 min at 260 V and increased within 40 min to 1,200 V. The added marker dye (5-carboxyfluorescein) was focused to a sharp line and moved over half the length of the separation slit. The current was then reduced to 2 mA. After 90 min the dye line was 5 mm in front of the separation port and the voltage had reached a value of 1,900 V. This value was adjusted at the interface, as the signal to start the fraction collector. The dye line moved with an effective velocity of 2 mm/min. Thirty fractions of 300 μl were collected. After 110 min the separation was stopped. The voltage had reached a constant final value of 2,400 V. Serum was prepared by centrifugation at 1,700 *g* for 15 min. The obtained serum was stored at 4°C and used for separation within 1 day. Five hundred microliters of serum was diluted with 500 μl of leading chamber buffer containing 0.4% (w/v) HPMC and mixed with 500 μl of spacer mixture. As marker dye, 2 μl of a solution of 5-carboxyflourescein (concentration, 1 mg/ml) was added to the sample.

Determination of LCAT and lipid transfer protein activity

LCAT activity. LCAT activity was measured by a combination of the methods of Stokke and Norum (21) and Tato, Vega, and Grundy (22). According to Stokke and Norum, a cholesterol-albumin emulsion was prepared. According to the description by Tato, Vega, and Grundy, 250 μl of plasma or ITP fraction was preincubated with 62.5 μl of cholesterol-albumin emulsion for 1 h at 4°C, and the following incubation was for 4 h at 37°C. After incubation, 50 μl of sample was added to 200 μl of 0.15 M sodium chloride solution. The reaction was stopped after addition of 1 ml of 0.5% digitonin in 95% ethanol and 0.75% cholesterol in 95% ethanol. After centrifugation at 200 rpm for 15 min, free cholesterol was precipitated. A 500-μl aliquot of the supernatant was counted for radioactivity in a liquid scintillation counter. From radioactivity counts, the LCAT distribution in ITP fractions was calculated as percentage distribution.

CETP activity. CETP activity was determined by a fluorometric activity method (WAK-Chemie, Bad Homburg, Germany). Samples were incubated for 3 h in the presence of a donor particle, in which cholesteryl esters are linked to a fluorescent label (NBD-CE), and a suitable acceptor particle. The fluorescent cholesteryl ester is present in a self-quenched state when contained within the core of the donor. The CETP-mediated transfer is determined by the increase in fluorescence intensity (emission wavelength, 535 nm) as the fluorescent cholesteryl ester is removed from the self-quenched donor to the acceptor particle.

PLTP activity. PLTP activity was determined as described earlier (23). Briefly, the assay contained HDL (250 μg of protein), liposomes (150 nmol as total phosphatidylcholine [PC] with a [^{14}C]PC label), sample (5–25 μl), and 10 mM Tris-HCl buffer, pH 7.4, containing 150 mM NaCl and 1 mM ethylenediaminetetraacetic acid in a final volume of 400 μl. Tubes without sample and tubes with control plasma sample were included in each run. The assays were performed at 37°C in Eppendorf tubes, using 90-min incubations. The reaction was stopped by the addition of 0.3 ml of 536 mM NaCl and 363 mM $MnCl_2$ (containing 52 units of heparin), yielding final concentrations of 230 mM NaCl, 156 mM $MnCl_2$, and heparin at 74 units/ml. The tubes were vortexed for 10 s and centrifuged for 10 min at 15,000 rpm. Supernatant (0.5 ml) was used for radioactivity determinations. The results are expressed as nanomoles of phosphatidylcholine transferred from PC liposomes to HDL per milliliter per hour.

Determination of LBP

Antibodies against human LBP were raised in LBP knockout mice (17) immunized with recombinant human LBP. The LBP was produced as a histidine tag fusion protein, using transfected Chinese hamster ovary cells and was purified by Talon (Clontech, Palo Alto, CA) affinity chromatography. Mouse antibodies were purified from plasma by protein G-Sepharose chromatography. A peroxidase (POD) conjugate of the antibody was prepared essentially according to Wilson and Nakane (24).

Microtiter plates (Maxisorp; Nunc, Roskilde, Denmark) were coated overnight with anti-LBP IgG (5 μg/ml) and were subsequently blocked with a gelatin blocking solution (Boehringer, Mannheim, Germany). Serum fractions obtained from isotachophoretic separations were applied (50 μl/well) and incubated for 2 h. After washing, the POD-labeled anti-human LBP antibody was applied at a dilution of 1:1,000 and incubated for 2 h. Tetramethyl benzidine was used for detection and the plates were measured at 450 nm. For calibration of the plates a serial 2-fold dilution of purified human LBP was assayed on each plate. The working range of this enzyme-linked immunosorbent assay was between 0.002 and 2 μg/ml.

Two-dimensional nondenaturing gradient gel electrophoresis

For the first dimension, 5 μl of plasma or 20 μl of ITP fraction was separated in 0.7% (w/v) agarose (SeaKem® LE; FMC Bioproducts, Rockland, ME) gels in 50 mM barbital buffer (pH 8.6). Gels were free of bovine serum albumin. Samples were electrophoresed at 100-V constant voltage and 10°C for about 1 h, when the albumin stained with bromophenol blue had moved 4 cm.

For the second dimension, the agarose gel strips from the first dimension were transferred to a 4–15% polyacrylamide gradient gel (Ready gels; Bio-Rad, Munich, Germany). Separation in the second dimension was performed at 20 mA per gel for 4 h at 4°C. Molecular weight standards (Pharmacia, Freiburg, Germany) containing thyroglobulin, 669 kDa; ferritin, 440 kDa; catalase, 232 kDa; lactate dehydrogenase, 140 kDa; and albumin, 67 kDa were run simultaneously with the sample.

Immunoblotting and chemiluminescence detection

After electrophoresis, proteins were electrophoretically transferred to Fluorotrans transfer membranes of 0.2-μm pore size (Pall, Dreieich, Germany). Transfer was carried out for 18 h in 20 mM Tris and 150 mM glycine buffer, in a Trans-Blot cell (Bio-Rad) at a constant 30 V and 10°C. The lane with molecular weight standards was cut off and stained with Coomassie blue. Membranes were then blocked by incubation for 1 h in phosphate-buffered saline (PBS) containing 5% nonfat milk powder and 0.1% Tween 20. For the detection of the individual apolipoproteins, membranes were incubated for 1 h with the corresponding antibodies in a dilution of 1:5,000 in PBS with 1% nonfat milk powder and 0.1% Tween 20. The following antibodies were used: rabbit anti-human apoA-I antiserum (Calbiochem, La Jolla, CA), affinity-purified goat polyclonal anti-human apoE antibody (Calbiochem), goat anti-human apoC-I (Biodesign, Kennebunkport, ME), and anti-human $SAA_{1/2}$ (25, 26).

After antibody incubation, the membrane was washed three times (10 min each) in 50 ml of PBS containing 0.1% Tween 20 and then incubated for 1 h with anti-rabbit or anti-goat immunoglobulin-horseradish peroxidase conjugate (dilution, 1:10,000). Before chemiluminescent detection the washing step was repeated. The membrane was developed with the ECL-Plus Western blotting detection system (Amersham/Pharmacia, Freiburg, Germany) according to the manufacturer instructions and analyzed with a Lumi-Imager (Roche Diagnostics). For reprobing with another antibody, membranes were stripped of bound antibodies by incubation in 100 mM 2-mercaptoethanol, 2% sodium dodecyl sulfate, 62.5 mM Tris-HCl, pH 6.7, at 50°C for 30 min with gentle agitation. After washing twice (10 min each) with PBS containing 0.1% Tween 20, membranes were again blocked in 5% nonfat dry milk in PBS for 1 h at room temperature. The second immunodetection was performed as outlined above.

Statistics

Data are given as means ± SD. Rejection of the null hypothesis was tested by a two-sample rank test (Mann-Whitney test). Significance was accepted with P values < 0.05. In calculating the rank correlation coefficient, the Spearman method was used. All statistical analyses were performed with SPSS for Windows, version 6.0.1 (SPSS, Chicago, IL).

RESULTS

Characterization of patient groups

A description of all patient groups is given in Table 1. The mean age of the patients did not differ between study groups. Patients with pneumonia, peritonitis, abcesses, mediastinitis, or pancreatitis were distributed equally in groups 2 and 3. There was a predominance of male patients in all three groups.

The increase in score values from group 1 to group 3, based on the SOFA score system for the assessment of organ failure/dysfunction, reflects differences in terms of severity of infection. The most striking difference was seen in respiratory function. On the other hand, liver function was not significantly different between groups. All three groups presented with elevated levels of C-reactive protein (CRP) and elastase activity, indicating an inflammatory response independent of whether it was complicated by infection or not. Compared with group 1, group 3 also had a significant increase in serum elastase, creatinine, and bilirubin concentrations, whereas patients in groups 2 and 3 had significantly higher serum concentrations of the acute-phase protein CRP.

Analysis of plasma lipids and lipoproteins

Compared with healthy control subjects, total cholesterol, LDL cholesterol, and HDL cholesterol were reduced in all patient groups (**Fig. 1**). Triglycerides were slightly decreased in group 1 and increased in groups 2 and 3. Comparisons between patient groups showed no differences for total cholesterol and LDL cholesterol. Compared with group 1, triglyceride concentrations were significantly higher in groups 2 ($P < 0.001$) and 3 ($P < 0.005$), and HDL cholesterol was significantly lower in groups 2 ($P < 0.0005$) and 3 ($P < 0.0001$).

Analysis of HDL subclasses

Analysis of HDL subclasses by capillary ITP has been described in detail previously (20). α-HDL are separated into three fractions with fast, intermediate, and slow mobility (**Fig. 2A**). In healthy individuals, fast HDL and intermediate HDL contain the bulk of HDL and apoA-I. How-

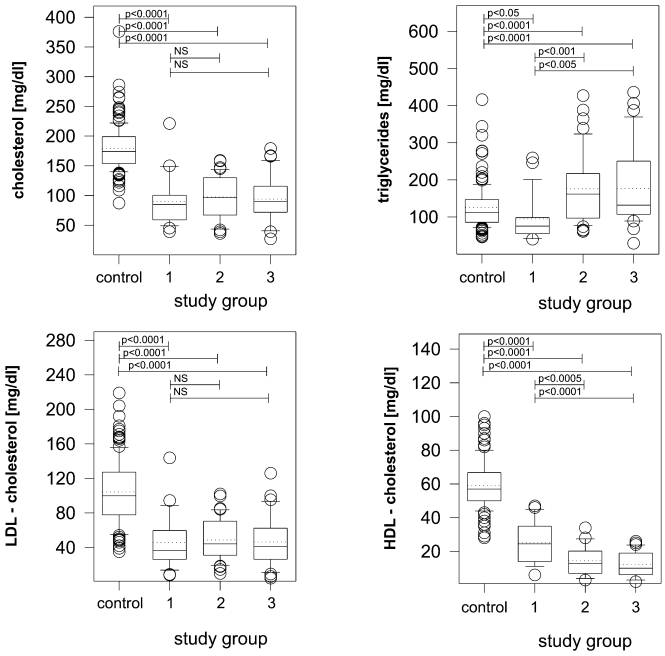

Fig. 1. Plasma concentrations of total cholesterol, triglycerides, LDL cholesterol, and HDL cholesterol. The horizontal lines mark the median 10th, 25th, 50th, 75th, and 90th percentile points of data. The dotted lines mark the mean values, and data points outside the 10th and 90th percentiles are shown.

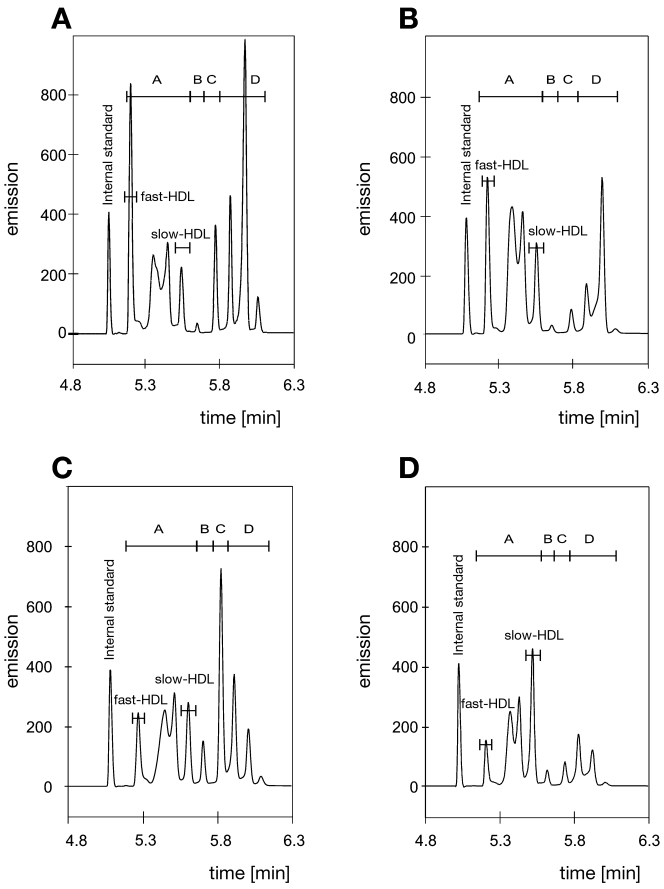

Fig. 2. Lipoprotein profiles from a healthy control (A) as well as a representative patient of group 1 (B), 2 (C), and 3 (D) as determined by analytical capillary ITP. The various lipoprotein subfractions known from gradient ultracentrifugation are depicted as follows: A: HDL, B: chylomicrons/remnants, C: VLDL/IDL, D: LDL. In sepsis, a marked change in the HDL subclass distribution occurs, with a decrease in fast HDL and an increase in slow HDL.

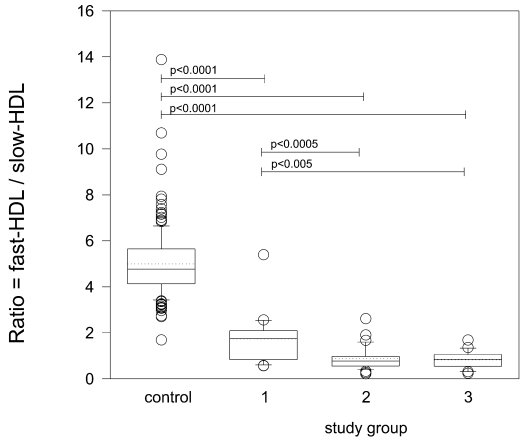

Fig. 3. The ratio of fast HDL to slow HDL within the study groups.

ever, compared with intermediate HDL, fast HDL have an increased ratio of apoA-I HDL to apoA-I:A-II HDL, and an increased proportion of cholesteryl esters compared with unesterified cholesterol. Slow HDL also have an increased ratio of apoA-I HDL to apoA-I:A-II HDL and contain several minor apolipoproteins, such as apoE. The mean ratio of fast HDL to slow HDL in healthy control subjects has been determined as 5.0 ± 1.5, because of the dominance of fast HDL. In all patient groups, fast HDL was reduced and slow HDL was slightly increased. These changes were most pronounced in patients with systemic inflammation (groups 2 and 3). Representative ITP patterns of sera from patients of groups 1, 2, and 3 are shown in Fig. 2B–D. The changes in HDL subfractions resulted in a reduction of the ratio of fast HDL to slow HDL to 1.7 ± 1.1 in patients from group 1 ($P < 0.0001$), to 0.9 ± 0.5 in patients from group 2 ($P < 0.0001$), and to 0.8 ± 0.4 in patients from group 3 ($P < 0.0001$). The ratio of fast HDL to slow HDL in both groups with systemic inflammation differed significantly from group 1 ($P < 0.0005$ and $P < 0.005$, respectively) (**Fig. 3**).

Determination of LCAT and lipid transfer protein (CETP, PLTP) activity

LCAT activity was reduced in all three patient groups, as compared with healthy control subjects (**Fig. 4A**). Furthermore, patients with bacterial infection (groups 2 and 3) presented with an even more pronounced decrease in median LCAT activity, although, because of variation, the comparison of mean LCAT activity between groups 1 and 3 did not reach significance.

In all patient groups, when compared with healthy control subjects, CETP activity was decreased (Fig. 4B). The reduction was not as profound as the reduction of LCAT activity. Again, patients from groups 2 and 3 showed a decreased CETP activity when compared with patients without systemic inflammation.

In contrast, the mean PLTP activity (Fig. 4C) was not significantly changed in postsurgery patients without infection (group 1), when compared with control subjects. In groups 2 and 3, that is, in patients with systemic inflammation, however, PLTP activity was significantly increased compared with group 1 (group 2, $P < 0.05$; group 3, $P < 0.005$). Furthermore, a positive correlation between PLTP activity and the concentration of the CRP could be demonstrated ($r_s = 0.29$; $P = 0.006$), although no significant difference in PLTP protein concentration could be detected, when compared between patient groups (data not shown).

Analysis of HDL-associated proteins in sepsis

The distribution of PLTP and CETP in serum was analyzed by preparative ITP. Both have been described to be associated with HDL. In healthy individuals, both transfer proteins were found in the slow HDL and pre-βHDL fractions. The increase in PLTP activity seen in patient plasma samples could also be demonstrated within the ITP fractions. The reduction of CETP was seen mostly in the slow HDL, while the reduction in the pre-βHDL was not as prominent (**Fig. 5A** and **B**).

The distribution of LBP was also analyzed in ITP fractions. Only a little LBP was found in control plasma, whereas in patients with systemic inflammation a massive

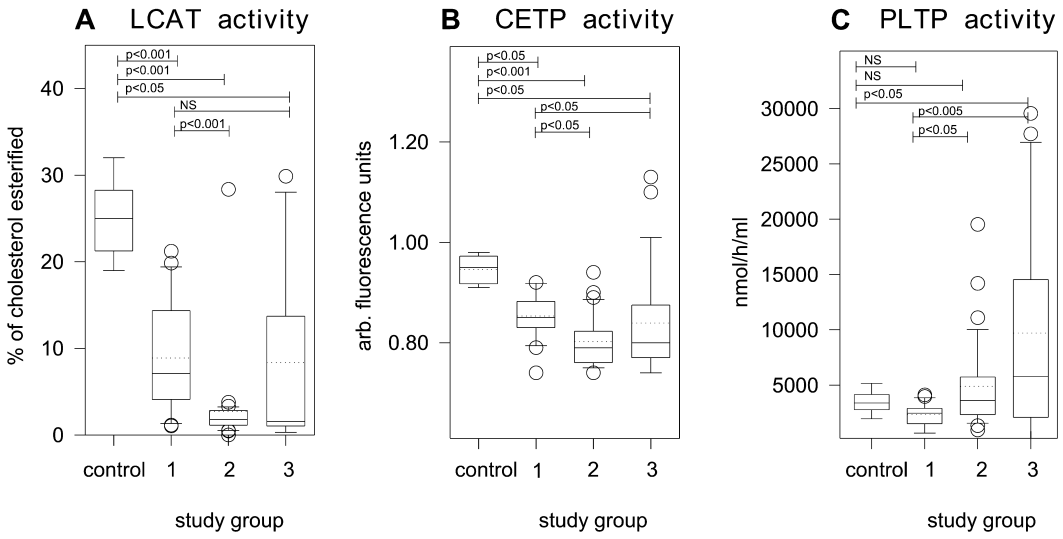

Fig. 4. LCAT (A), CETP (B), and PLTP(C) activities for the study groups. The horizontal lines mark the median 10th, 25th, 50th, 75th, and 90th percentile points of data. The dotted lines mark the mean values.

increase in LBP was observed. The majority of LBP was found in apoB-containing lipoproteins free of apoA-I (Fig. 5C), a result further supported by data obtained by two-dimensional nondenaturing gradient gel electrophoresis (2D-GGE), which demonstrated almost all of the LBP immunoreactivity to be separated from apoA-I (Fig. 5, inset D).

2D-GGE of HDL subpopulations

2D-GGE of plasma samples obtained from healthy individuals and septic patients was performed in order to demonstrate differences in the distribution of apoA-I-containing HDL particles as well as of apoE and lipid transfer proteins.

When compared with healthy control subjects, the immunoreactivity for apoA-I markedly decreased in patients with inflammatory disease, mainly because of a loss of large apoA-I particles. In parallel with the decrease in apoA-I, apoC-I immunoreactivity also decreased, with some activity remaining in the intermediate and slow HDL range. As SAA is known to inhibit LCAT activity during inflammation by its association with HDL, we also analyzed the distribution of SAA protein. The inflammation-associated isoforms of serum amyloid A protein ($SAA_{1/2}$) could be detected to comigrate nearly exclusively with the remaining apoA-I. Contrary to this, apoE immunoreactivity increased especially within the slow HDL range and at a molecular mass of >300 kDa. The increase in HDL particles containing apoE accounted for the net increase of slow HDL, while apoA-I HDL was reduced. Within this region the reactivity for apoA-I or apoC-I was nearly absent (**Fig. 6**).

In healthy probands, PLTP could be detected with slow HDL mobility (300–400 kDa) located between the α- and pre-β_2 region, with only minor overlap between the immunoreactivity of PLTP and apoA-I. In septic patients, a loss of large apoA-I-containing HDL particles could be observed, clearly demonstrating now that nearly all the PLTP immunoreactivity at 300–400 kDa did not colocalize with apoA-I but only with apoE. Whereas in healthy individuals most of the apoE immunoreactivity overlapped with that of apoC-I, in septic patients, apoC-I reactivity markedly decreased and could no longer be detected to colocalize with apoE (Fig. 6). In addition, in healthy subjects, PLTP particles with pre-β mobility could be demonstrated in a region between pre-β_1- and pre-β_2HDL (140–200 kDa). Because, within this region, PLTP did not comigrate with any of the major apolipoproteins, these particles may thus represent PLTP homodimers or lipid-complexed PLTP. In septic patients, a redistribution of PLTP could be observed, with an increase in small PLTP particles and a decrease in large PLTP particles.

CETP immunoreactivity with pre-β mobility could be demonstrated only within a region between pre-β_1- and pre-β_2HDL (140–200 kDa). Again, these particles did not colocalize with apoA, apoE, or apoC-I, suggesting the presence of free, potentially lipid-complexed CETP. The localization of PLTP and CETP differed slightly, with PLTP having a higher molecular weight and faster electrophoretic mobility. In septic patients, a decrease in CETP immunoreactivity was observed (Fig. 6).

DISCUSSION

On the basis of the current knowledge of the protective effects of HDL during inflammation, for example, as scavengers of activated complement factors (27) or oxidation products (3) and as acceptors of bacterial lipopolysaccharide (2), it seems reasonable to assume that alterations in HDL metabolism might have clinical relevance with regard to the frequency and/or severity of inflammatory diseases. To further elucidate the mechanisms that contribute to the decrease in HDL seen during the acute-phase

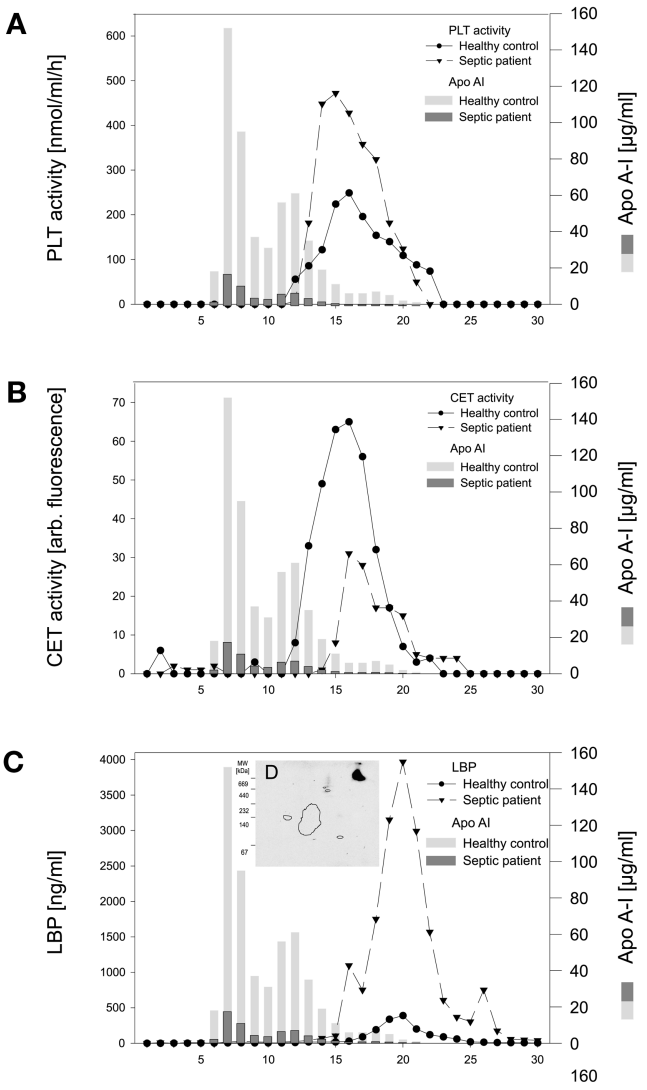

Fig. 5. The distribution of PLT activity (A), CET activity (B), and LBP (C) within serum subfractions isolated by preparative isotachophoresis (representative data for n = 5 independent separations for each group) is illustrated in relation to the distribution of apolipoprotein (apo)A-I. Moreover, the association of LBP with lipoproteins was analyzed by 2D-GGE (insert D).

response, we determined, whether intensive care patients who met the criteria of SIRS and bacterial infection (group 2) or severe sepsis (group 3) differed regarding HDL cholesterol concentration, distribution of HDL subpopulations, as well as the activity of HDL-remodeling enzymes and lipid transfer proteins, when compared with intensive care patients without infection (group 1) or healthy control subjects.

In accordance with previously published data, we found reduced total cholesterol, LDL cholesterol, and HDL cholesterol levels in all patient groups (28). In patients with systemic inflammation (groups 2 and 3) the reduction in HDL cholesterol was more pronounced than in postsurgery patients without signs of infection or systemic inflammation (group 1), although these differences were not as profound as when compared between healthy control sub-

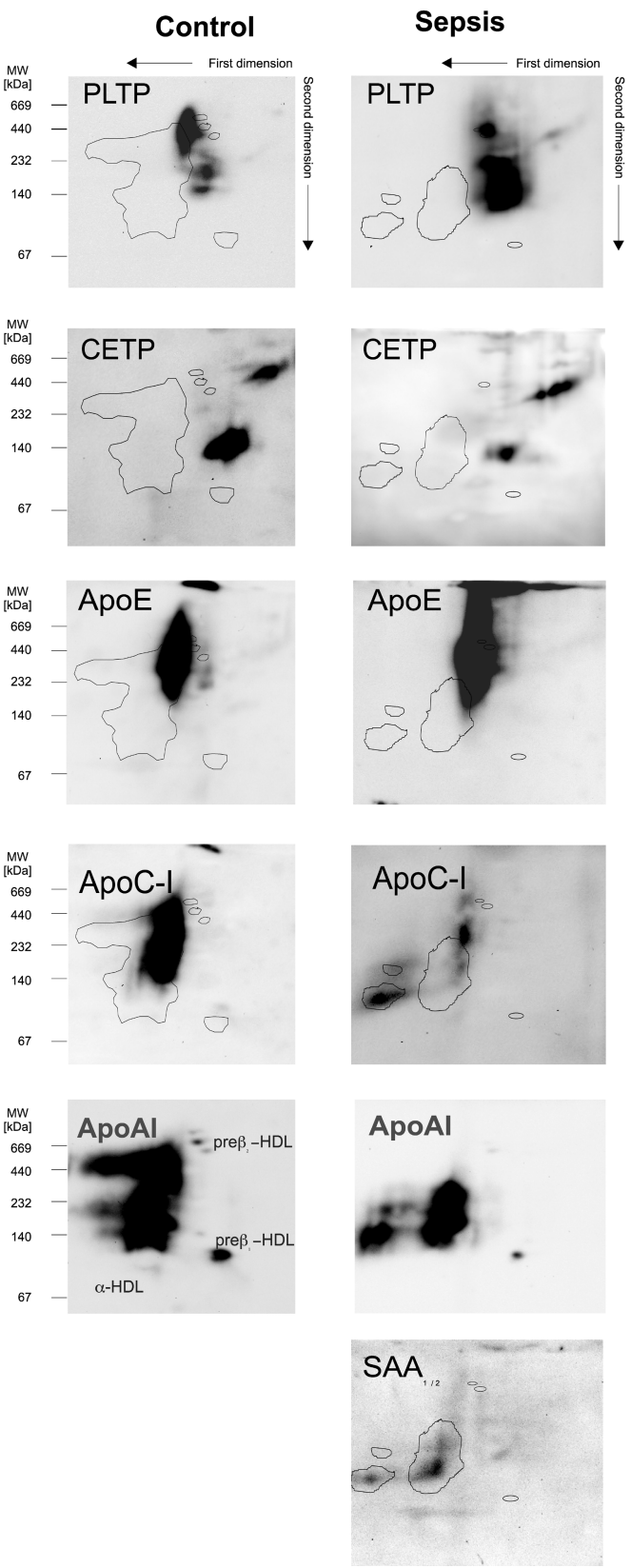

Fig. 6. Immunoblots of 2D-GGE for PLTP, CETP, apolipoprotein (apo)E, apoC-I, apoA-I, and $SAA_{1/2}$ (for sepsis only) of a healthy individual (left row) and an individual with sepsis (group 3). The circled areas represent areas of immunoreactivity for apoA-I. 2D-GGE has been performed from five representative patients from each group.

jects and group 1. By 2D-GGE separation of lipoproteins, we could confirm that mainly large, mature apoA-I-rich HDL particles are reduced or even absent during inflammation. According to our data, the decrease in HDL cholesterol during inflammation can be attributed to a decreased LCAT activity leading to impaired cholesteryl ester formation and HDL maturation. Several potential mechanisms for the decrease in LCAT activity in inflammation have been reported, such as regulatory effects of cytokines such as interleukin 2 (IL-2) (11), a reduced association of LCAT with HDL due to an altered lipid composition of the HDL particle (29), or due to the displacement of the LCAT activator apoA-I by $SAA_{1/2}$ (12). However, because we could detect $SAA_{1/2}$ reactivity almost only colocalizing with the remaining apoA-I immunoreactivity, SAA may remain with the residual HDL particles, probably substituting for only one or two of the apoA-I molecules associated with an HDL particle. We could also demonstrate a decrease in the ratio of fast HDL to slow HDL during inflammation and the decrease correlated with the severity of inflammation. Fast HDL, which mainly consists of cholesteryl ester-rich particles, is nearly absent in subjects suffering from LCAT deficiency, confirming the importance of LCAT activity for the formation of fast HDL. Thus the decrease in fast HDL is probably due to the low LCAT activity seen in inflammation. As the difference between group 1 and healthy control subjects is much bigger than the difference between groups 1 and 2, this indicates that the acute inflammatory response after surgery, as indicated by an increase in the CRP concentration, triggers major changes in the metabolism of lipoproteins, independent of whether it is accompanied by infection or not.

As we could not attribute the increase in slow HDL during inflammation to an increase in apoA-I HDL particles, we further characterized the lipoprotein particles contributing to slow HDL during inflammation. An apoE particle not containing apoA-I could be identified within the slow HDL range, and it increased in concentration during inflammation. While in control sera from healthy individuals apoE also comigrated in part with apoC-I, an almost total loss also of apoC-I could be observed during inflammation. Solely apoE-containing HDL particles have been demonstrated to be present in the slow HDL plasma fraction from healthy individuals (20). Krimbou et al. (30) could demonstrate a significant proportion of HDL-sized lipoproteins, containing apoE, that did not comigrate with apoA-I, apoA-II, apoC-III, or apoB-100 in normal human plasma samples, suggesting that these lipoproteins contain apoE as their major apolipoprotein component. Moreover, apoE HDL have been found to accumulate in abetalipoproteinemia (31) and human cord blood plasma (32) and, therefore, it has been suggested that these particles may functionally substitute for cholesterol delivery to cells (33). Furthermore, apoE-rich HDL has also been found in LCAT or CETP deficiency. As in these inherited LCAT and CETP deficiency states, the low LCAT and CETP activities observed during inflammation may favor the production of apoE HDL.

ApoC-I is known to inhibit the binding of lipoprotein particles to several lipoprotein receptors such as the apoB/E receptor, the LDL receptor-related protein and the very low density lipoprotein (VLDL) receptor (34). The low levels of apoC-I seen in septic patients may improve the uptake of lipoproteins into peripheral cells. As HDL are mainly surface lipoproteins, with phospholipids and apolipoproteins contributing to more than 80% of the total mass, we suggest that a compensatory change in lipoprotein targeting during inflammation toward peripheral cells may help to satisfy an increased demand for phospholipids, for example, for lung surfactant production or the regeneration of damaged cellular membranes of epithelia (e.g., intestinal mucosa) or endothelial (e.g., vessel wall) cells.

Because changes in the activities of HDL-modifying proteins have been reported to result in an increase in apoE HDL, we also analyzed the lipoprotein association and the activities of PLTP and CETP in plasma samples from healthy control subjects and patients with inflammatory disease. On 2D-GGE of normal human plasma, we found PLTP to migrate with slow HDL mobility, associated with large particles (>300 kDa), which comigrated with apoE rather than with apoA-I. In addition, CETP and PLTP could both be demonstrated to localize within a region between pre-β_1- and pre-β_2HDL (140–200 kDa). These smaller particles did not colocalize with apoA-I or with any other major apolipoprotein, or with each other. Our data are in agreement with those described by Speijer et al. (35), who could demonstrate CETP and PLTP to coelute with different HDL fractions of nearly the same size as demonstrated by our group with 2D-GGE. However, contrary to our results, they could not detect PLTP associated with smaller particles. As the localization of PLTP and CETP in the lower molecular weight range differed in such a way that two different particles could be separated by 2D-GGE, with the PLTP spot having higher molecular mass and slow HDL mobility, these particles may represent either lipid-complexed proteins or homodimers. Oka et al. (36) have demonstrated the presence of large (12- to 14-nm) particles with PLTP immunoreactivity. These particles carry an inactive form of PLTP, whereas the active form of PLTP was associated with smaller, 9- to 11-nm particles. This study is further supported by our observation that, in septic patients, the increase in PLTP activity was paralleled by a redistribution of PLTP toward small particles, whereas no significant increase in PLTP protein concentration could be found. These data suggest that PLTP activity may increase at least in part due to a redistribution of the PLTP protein between a more active PLTP population of smaller size, for example, representing free or dimerized protein, and PLTP associated together with larger, apoE-containing particles. As we could detect an increase in PLTP activity only in patients with infection, it is tempting to speculate that a change in the lipid composition may modulate the interaction of PLTP with lipoproteins, as already demonstrated for the interaction of LCAT and sphingomyelin-rich lipoproteins (29), for example, found in endotoxemia (37).

CETP was not found to be associated with any of the major apolipoproteins, indicating an active CETP particle which, similar to PLTP, consists of homodimers or lipid-complexed CETP. Also, the immunoreactivity for CETP decreased in parallel with CETP activity in samples obtained during inflammation. On the basis of the analysis of ITP fractions from human plasma, which allows the distinction of different lipoprotein entities with high sensitivity, we cannot confirm previously published data, suggesting the existence of a functional lipoprotein unit containing apoA-I, apoD, LCAT, and CETP (38), as we could not detect an overlap between the staining patterns for CETP and apoA-I and apoD.

It has been demonstrated previously that PLTP is able to increase the in vivo turnover of labeled phosphatidylcholine and to promote the net mass transfer of α-tocopherol into the vessel wall, suggesting that an increase in PLTP activity might facilitate the transfer of phospholipids and α-tocopherol from HDL to tissues (39, 40). In inflammation, this may support lung surfactant formation in type II pneumocytes, which is critically dependent on exogeneous phospholipids. It may also support the protection and regeneration of cellular membranes, such as of vascular endothelial cells, which may be a primary target of activated components of the coagulation and complement system. In addition, PLTP has been shown to bind LPS and to transport this lipid-like inflammatory mediator to HDL, which results in LPS inactivation. As we could detect the majority of LBP comigrating with apoB-containing lipoproteins, it might be suggested that LBP mainly transfers LPS to these lipoproteins, whereas PLTP mainly mediates the transfer of LPS to HDL-sized particles. The increase in PLTP activity in inflammation therefore might also represent a protective mechanism against deleterious LPS effects in endotoxemia.

In conclusion, our data have shown *1*) that CETP cannot be found associated with any of the major apolipoproteins; *2*) that PLTP can be found as a small particle that does not colocalize with any of the major apolipoproteins, as well as associated with large lipoprotein particles that fully colocalize with apoE; and *3*) that in inflammation the activity of PLTP is increased, probably because of a relative increase in more active, small PLTP particles. Furthermore, *4*) solely apoE-containing HDL were found to accumulate during inflammation, which may contribute, together with an increase in PLTP activity, to an improved supply of fatty acid molecules for energy supply and phospholipids to various peripheral tissues that must maintain cellular membrane homeostasis under conditions of inflammatory stress.

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