A single intravenous dose of endotoxin rapidly alters serum lipoproteins and lipid transfer proteins in normal volunteers

Lisa C. Hudgins,[1,*,†] Thomas S. Parker,[*,†] Daniel M. Levine,[*,†] Bruce R. Gordon,[*,†] Stuart D. Saal,[*,†] Xian-cheng Jiang,[§] Cindy E. Seidman,[*] Jolanta D. Tremaroli,[*] Julie Lai,[*] and Albert L. Rubin[†,*]

The Rockefeller University,* 1230 York Avenue, New York, NY 10021; The Rogosin Institute,† 505 East 70th Street, New York, NY 10021; and SUNY Downstate Health Sciences Center,§ 450 Clarkson Avenue, Brooklyn, NY 11203

Abstract Endotoxemia is associated with rapid and marked declines in serum levels of LDL and HDL by unknown mechanisms. Six normal volunteers received a single, small intravenous (iv) dose of endotoxin (*Escherichia coli* 0113, 2 ng/kg) or saline in a random order, cross-over design. After endotoxin treatment, volunteers had mild, transient flu-like symptoms and markedly increased serum levels of tumor necrosis factor and its soluble receptors, interleukin-6, cortisol, serum amyloid A, and C-reactive protein. Triglyceride (TG), VLDL-TG, and nonesterified fatty acid increased (peak at 3–4 h), then TG declined (nadir at 9 h), and then cholesterol, LDL cholesterol, apolipoprotein B (apoB), and phospholipid declined (nadirs at 12–24 h). HDL cholesterol and apoA-I levels were not affected, but half of the decrease in phospholipid was HDL phospholipid. Lipopolysaccharide binding protein (LBP) rose 3-fold (peak at 12 h), with smaller and later decreases in the activities of phospholipid transfer protein and cholesteryl ester transfer protein. In conclusion, a decline in LDL was rapidly induced in normal volunteers with a single iv dose of endotoxin. The selective loss of phospholipid from HDL may have been mediated by LBP and, after more intense or prolonged inflammation, could result in increased HDL clearance and reduced HDL levels.— Hudgins, L. C., T. S. Parker, D. M. Levine, B. R. Gordon, S. D. Saal, X-c. Jiang, C. E. Seidman, J. D. Tremaroli, J. Lai, and A. L. Rubin. **A single intravenous dose of endotoxin rapidly alters serum lipoproteins and lipid transfer proteins in normal volunteers. *J. Lipid Res.* 2003.** 44: **1489–1498.**

Supplementary key words lipopolysaccharide • sepsis • phospholipid • cholesterol • triglyceride • lipopolysaccharide binding protein • phospholipid transfer protein • cholesteryl ester transfer protein • high density lipoprotein • low density lipoprotein

The release of endotoxin or lipopolysaccharide (LPS) from the cell walls of gram-negative bacteria into the circulation precipitates an acute inflammatory response that often leads to shock and death (1, 2). A less-recognized component of the acute-phase response to endotoxemia and a variety of inflammatory states in humans is a rapid and marked decline in serum levels of cholesterol, phospholipid, apolipoprotein B (apoB), and apoA-I carried in LDL and HDL (3–5). Low levels of cholesterol, in turn, predict a poor prognosis in hospitalized (3) and chronically ill patients (6, 7). It has been proposed that the lipoproteins protect against the lethal effects of endotoxin by binding it and reducing the secretion of proinflammatory cytokines by monocytes and macrophages (8–11). Thus, a better understanding of the mechanism(s) of the decrease in plasma lipoproteins by circulating endotoxin may result in new therapeutic approaches to increase lipoproteins in patients who have, or are at high risk for, endotoxemia and inflammation. This may include treatment of low HDL levels that may result from and contribute to the inflammation of atherosclerotic cardiovascular disease (12).

The injection of a single dose of endotoxin into experimental animals or humans has been used for decades as a model to study the interrelationships between infection and the host response (13–15). In humans, there is a well-characterized dose-related response to intravenous (iv) endotoxin consisting of mild fever, flu-like symptoms, marked increases in tumor necrosis factor α (TNFα), interleukin-6 (IL-6), cortisol, epinephrine, serum amyloid A (SAA), and C-reactive protein (CRP), and changes in other cytokines, hormones, and inflammatory markers. However, there are no detailed studies in humans of the response of plasma lipoproteins to iv endotoxin. In animals, the time course and nature of the lipoprotein response has varied depending on the host species, the source and dose of endotoxin, and the preceding diet. In rodents, rabbits, and hamsters with lipoprotein profiles and metabolism distinctly different from those of humans, endotoxin raised cholesterol and LDL cholesterol (15). In contrast, a single dose of endotoxin given to nonhuman primates with lipoprotein levels and metabolism more similar to those of humans rapidly decreased LDL and HDL cholesterol ester, apoA-I,

Manuscript received 13 November 2002 and in revised form 2 May 2003.

Published, JLR Papers in Press, May 16, 2003.
DOI 10.1194/jlr.M200440-JLR200

[1] To whom correspondence should be addressed.
e-mail: hudgins@mail.rockefeller.edu

and HDL particle size by 24 h (16, 17). In addition, endotoxin increased triglyceride (TG) and VLDL, which has consistently occurred in other animal models due to increased fatty acid synthesis, increased VLDL production, and/or decreased clearance (15). However, in humans, TG levels are variably affected by infection (3). In an uncontrolled study of normal volunteers, TG (and cholesterol) levels did not change from baseline at 2 h, 6 h, or 24 h after a single iv dose of endotoxin (18).

Using this established model of endotoxemia in normal volunteers after a controlled diet, we compared the effects of a single iv dose of endotoxin to saline on serum lipids, lipoproteins, and lipid transfer proteins. By matching the fatty acid composition of the diet to each subject's adipose tissue and comparing it to the composition of VLDL-TG, we also determined whether endotoxin increased fatty acid synthesis and newly formed palmitate in VLDL (19).

METHODS

Subjects

Six normal volunteers (three males, three females, ages 23–35) were admitted to The Rockefeller University Clinical Research Center in a random-order cross-over design twice for 8 days with a 6 week washout period between admissions. After a diet stabilization period, they received either a single iv dose of endotoxin or saline. Healthy volunteers, aged 21 to 45, who did not smoke or take prescription medications were eligible for the study. Body weight was at least 110 pounds, within 10% of current weight for at least 6 months, and within 80–120% desirable body weight by the 1959 Metropolitan Life Insurance Table. The fasting lipid profiles of participants met the following criteria: LDL cholesterol $<$ 160 mg/dl, TG $<$ 200 mg/dl, and HDL cholesterol between 35 and 70 mg/dl. A physical exam and screening tests performed within 2 months of admission, including a complete blood count, biochemical screen, HIV, hepatitis B and C, thyroid stimulating hormone, pregnancy test, urinalysis, chest X-ray, and EKG, were all normal. Aspirin, nonsteroidal anti-inflammatory drugs, or vitamins were discontinued at least 1 week prior to admission. Patients with histories of alcohol or substance abuse, psychiatric disease, asthma, severe allergic reactions, or a febrile illness within the month prior to admission were excluded. All participating subjects gave informed written consent, and the study was approved by The Rockefeller University Institutional Review Board.

Diet

During both admissions, each subject received identical single-menu solid food diets as three meals/day with 35% of calories as fat, 50% as carbohydrate, and a starch/sugar ratio of 70:30. The distribution of calories was achieved with food items used in previous studies that tested the effects of dietary carbohydrates on fatty acid synthesis and was chosen to ensure that fatty acid synthesis was not stimulated in the basal state (19). As required by the linoleate dilution method to measure fatty acid synthesis, a mixture of corn oil, olive oil, and lard was added to a 2% fat solid food diet. The fatty acid composition of each diet was matched to the composition of each subject's adipose tissue and compared with the composition of VLDL-TG (19, 20). By chance, the volunteers had similar percentages of fatty acids in adipose tissue (range for linoleate, 17–20%; palmitate, 18–20%; and oleate, 40–43%). Cholesterol was added to total 200 mg/day in all diets. The total calories to maintain constant weight were estimated from the surface area as 1,360 kcal/meter^2. On Day 5, after the endotoxin or saline bolus (see below), the diet (except water) was withheld for 24 h and then given again for the next 2 days until discharge on Day 8.

IV endotoxin or saline administration

On Day 5 at 9 AM after a 12 h overnight fast, either endotoxin (US Standard Reference Endotoxin from *Escherichia coli* 0113, 2 ng/kg, 200 ng/ml sterile water) or saline in a plastic syringe was slowly administered intravenously over 1 min, close to the 18 gauge catheter insertion site in the antecubital fossa. This was followed by a slow flush of 10 ml sterile saline and then a saline infusion at 20 ml/h. The endotoxin was stored as a lyophilized powder in single-use vials provided by the Pharmacy Department of The National Institutes of Health, Bethesda, MD. For hydration, additional saline was infused at 100 ml/h through an 18 gauge catheter in the other arm to total 120 ml/h for 24 h. Water by mouth was also encouraged.

Baseline vital signs were obtained twice, 5 min before the injection and then approximately every 15 min for the first 3 h, every 1/2 h for the next 3 h, and then every 3 h for the remainder of the 24 h period. Blood pressure, pulse, respirations, EKG, and O_2 saturation were monitored using a Datascope (first two subjects) or Hewlett Packard Veridia. Symptoms of headache, chills, muscle aches, backache, nausea, and vomiting were recorded by the nurse as: absent, 0; mild, 1; moderate, 2; or severe, 3. The principal investigator and two nurses were continuously present for the first 4 h after each endotoxin infusion.

Lipid and lipoprotein analysis

For the fasting specimens on Days 1, 4–8, and, on Day 5, at 1.5 h, 3 h, and then every 3 h until the following morning, 7 ml of blood was drawn in EDTA on ice. The plasma was separated by low-speed centrifugation at 4°C for 20 min, and VLDL was isolated by density-gradient ultracentrifugation. The fatty acid composition of VLDL-TG was analyzed by capillary gas chromatography after chloroform-methanol extraction of lipids; separation of TG by thin-layer chromatography (TLC) with silica gel G and a solvent system of hexane, diethyl ether, and glacial acetic acid, 60:40:1; and transmethylation with 5% methanolic HCL to form fatty acid methyl esters (20). The fatty acid compositions of the total lipid extracts from the diets and subcutaneous adipose tissue sampled from the gluteal site prior to admission were similarly analyzed by gas chromatography.

The phospholipid subclasses phosphatidyl choline (PC), lysophosphatidyl choline (LPC), sphingomyelin (SM), phosphatidyl ethanolamine (PE), phosphatidyl inositol (PI), and phosphatidyl serine (PS), in total lipid extracts from serum obtained at the time of maximum difference in serum total phospholipid 12–24 h after iv endotoxin or saline, were separated by TLC using silica gel H and a solvent system of chloroform, methanol, acetic acid, and H_20 (25:15:4:2) (21). The minor amounts of PI and PS migrated as a single band and are presented as a single value. The fatty acid compositions of each phospholipid class were determined as above after methylation with 5% methanolic HCL. C17:0 PC and LPC were added as internal recovery standards at the start of lipid extraction to quantitate the total amount of each respective lipid; C 17:0 PC was added at the TLC step to quantitate PE and PS-PI. Due to lower recovery and the lack of an internal recovery marker, SM was measured in triplicate using an enzymatic kit with bacterial sphingomyelinase (Cardiovascular Target, Inc., New York) and an interassay coefficient of variation of 3%.

At the above time points, and, on Day 5, at 1 h, 2 h, and 4.5 h, other lipid assays were performed. Total serum cholesterol and

TG were measured using enzymatic methods on a COBAS Integra (Roche Diagnostic Systems, Indianapolis, IN). The concentration of HDL cholesterol was measured after precipitation of β-lipoproteins with dextran sulfate and magnesium chloride (22). LDL cholesterol was calculated using the Friedewald equation. Immunoturbidimetric methods were used to measure apoA-I and apoB (Roche Diagnostic Systems). SAA was measured by a double antibody sandwich ELISA method (BioSource International, Camarillo, CA). Serum phospholipid and nonesterified fatty acids (NEFAs) were measured by enzymatic methods (Wako Chemical USA, Inc., Richmond, VA) in blood samples placed immediately on ice and spun in a refrigerated centrifuge to prevent ex vivo hydrolysis. The phospholipid assay measured the amount of choline-containing phospholipids (PC, LPC, and SM).

Fast protein liquid chromatography (FPLC) was used to measure the amounts of cholesterol, TG phospholipid, apoA-I, apoB, and SAA in VLDL, LDL, and HDL at the time of maximum difference in the concentration of phospholipid (12–24 h after endotoxin injection). Serum samples of 200 μl were chromatographed in phosphate-buffered saline (0.5 ml/min) from two Superose 6 columns (Pharmacia/LKB) connected in series. Fractions (650 μl) were collected and analyzed by methods described above after slight modification (23). For all assays, the mean recoveries were greater than 85%.

To measure HDL size, serum lipoproteins were separated by flotation from 1.24 g/ml NaBr in a Beckman TL 100 rotor at 100,000 RPM for 3 h at 20°C. Native gradient gel electrophoresis was done on 4–20% Tris-Glycine polyacrylamide gel gradient gels from Invitrogen (Grand Island, NY) stained with Coomassie blue (Bio Rad G250), as described by Nichols (24) and scanned by laser densitometry.

Lipid transfer proteins

Serum obtained at 0 h, 12 h, 24 h, 48 h, and 72 h after endotoxin or saline was assayed for the activities of phospholipid transfer protein (PLTP) and cholesteryl ester transfer protein (CETP) in triplicate using assay kits (Cardiovascular Target, Inc., and Roar Biomedical, Inc., New York). Incubation of donor and acceptor particles with 3 μl of human plasma results in the PLTP- or CETP-mediated transfer of fluorescent phospholipid or neutral lipid, which is quenched when associated with the donor. The transfer is determined by the increase in fluorescence intensity as the fluorescent lipid is removed from the donor and transferred to the acceptor. The interassay coefficient of variation for both assays was 3%. The serum concentration of LPS binding protein (LBP) was measured by an enzyme-amplified chemiluminescent method on an Immulite Analyzer (Diagnostic Products Corp., Los Angeles, CA).

Cytokines

Blood samples were obtained at the above time points and placed immediately on ice, and the serum was separated in a refrigerated centrifuge. TNFα was measured by ELISA (RPN 2148 and 2755, Amersham Life Science, Little Chalfont, Buckinghamshire, England). IL-6 and the soluble receptors for TNFα (p55, p75) were measured by enzyme immunoassay (BioSource International) on the Roche COBAS Core II (Roche Diagnostics Systems, Basel, Switzerland).

Endotoxin assay

At 0 and 5 min from the start of the injection of endotoxin or saline, 4 ml blood was drawn into endotoxin-free tubes with heparin, spun within 1/2 h at 2,500 rpm, 4°C, for 5 min, and the platelet-rich plasma was stored at −80°C until assayed. Concentrations of endotoxin were measured in triplicate by a kinetic limulus amebocyte lysate method (Coamatic Endotoxin, Associates of Cape Cod, Falmouth, MA). A Molecular Devices Thermomax Microplate Reader and SoftMax Pro software were used to collect kinetic data for 20 min.

Other tests

High sensitivity CRP was measured by a latex-enhanced immunoturbidimetric method on the Roche COBAS Integra. White blood cells were measured on a Bayer Advia 120 (Bayer Diagnostics, Tarrytown, NY). Cortisol was measured by enzyme-amplified chemiluminescence on an Immulite Analyzer by Diagnostic Products Corp. Catecholamines (epinephrine, norepinephrine, and dopamine) were measured by HPLC in acidified urine collected from 6 AM–6 PM and 6 PM–6 AM on the day of the endotoxin or saline injection.

Statistics

Means and SD or SE (figures) were calculated for six subjects at each time point. Two-tailed paired Student's *t*-tests were performed to compare values after endotoxin or saline. Simple linear regression was used to assess the relationships among the lipids, lipid transfer proteins, and inflammatory markers. Statistical significance was defined as $P < 0.05$. Excel and SigmaStat statistical softwares were used.

RESULTS

Clinical response to iv endotoxin

The incidence of clinical effects and average clinical scores after iv endotoxin are shown in **Table 1**. All six volunteers manifested typical flu-like symptoms: headache, chills, muscle ache, backache, and/or nausea. These symptoms peaked at 1–2 h and were resolved 6–9 h after the injection. One subject vomited and had a vasovagal reaction with a brief drop in blood pressure and pulse. Another subject received supplemental O_2 by nasal canula for peripheral cyanosis. The mean maximum oral temperature ± SD was 38.2 ± 0.4°C (range, 37.8–38.7°C) and peaked 3–4 h after endotoxin. The mean pulse increased from baseline (64 ± 12 vs. 92 ± 18), but there was no significant change in systolic or diastolic blood pressure. There were no complaints or significant changes in vital signs from subjects after the saline injection. Baseline levels of endotoxin were low, but were 10-fold higher 5 min after injection of endotoxin compared with saline (7.7 ± 11.3 vs. 0.7 ± 0.5 U/ml).

Cytokines, cortisol, SAA, and CRP

For all measurements, baseline values were not significantly different before endotoxin and saline injections. The top three panels of **Fig. 1** show that, as expected after

TABLE 1. Clinical response to iv endotoxin

		Score	
	Incidence	Mean	Range
Headache	6	1.2	1–2
Chills	6	1.7	1–3
Myalgia	4	1.0	0–2
Backache	4	0.8	0–2
Nausea	2	0.7	0–3

iv, intravenous. Symptoms in six volunteers were rated as: 0, absent; 1, mild; 2, moderate; 3, severe.

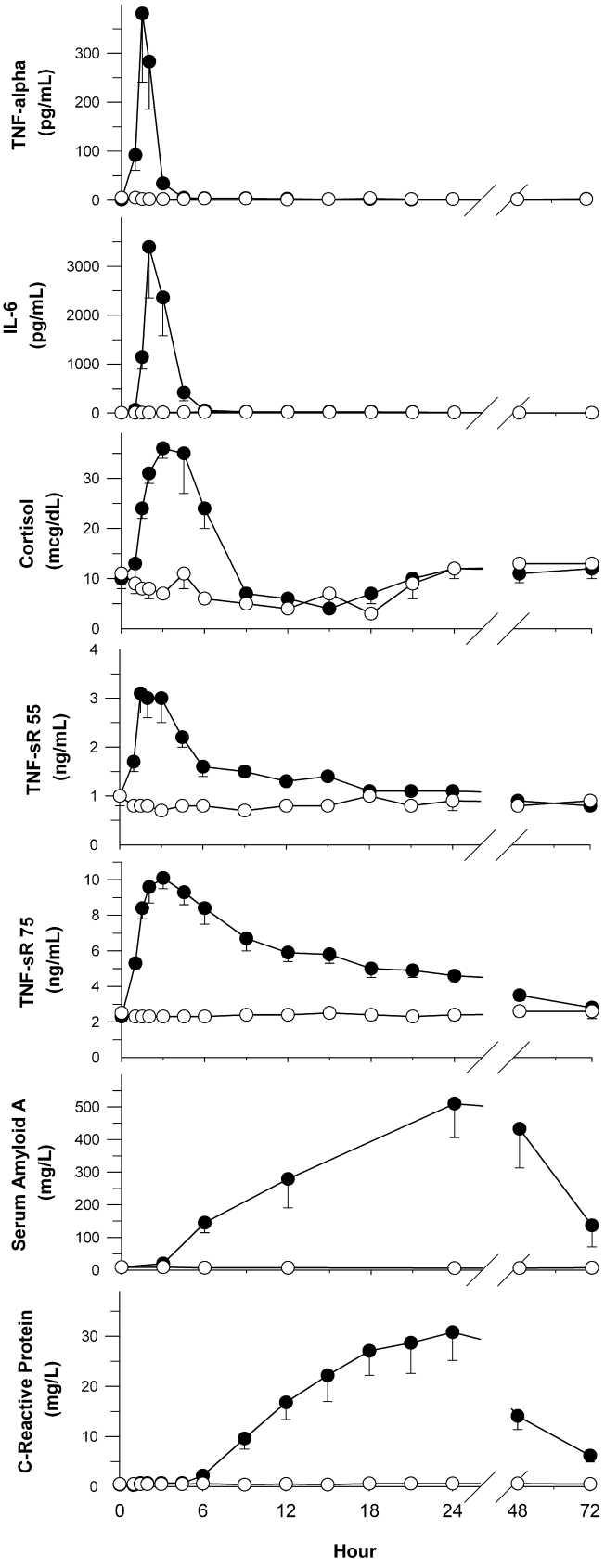

Fig. 1. Serum concentrations of tumor necrosis factor α (TNFα), interleukin-6, cortisol, TNF-sR 55, TNF-sR 75, serum amyloid A, and C-reactive protein (mean ± SE) for 72 h after an intravenous (iv) dose of endotoxin (closed circles) or saline (open circles). Peak differences between endotoxin and saline values are all significantly different ($P < 0.05$).

iv endotoxin, serum TNFα, IL-6, and cortisol rapidly and markedly increased (14) and then returned to control levels by 9 h. TNFα first peaked at 1.5 h (381 ± 343 vs. 2.3 ± 3.2 pg/ml, $P = 0.02$), then IL-6 at 2 h (3,395 ± 2,542 vs. 4.7 ± 6.4 pg/ml, $P = 0.02$), and then cortisol at 3 h (36 ± 4.3 vs. 7 ± 3.5 μg/dl, $P < 0.001$). The peak levels of TNFα and IL-6 were positively correlated with each other ($P = 0.02$). During the saline infusion, IL-6, but not TNFα or cortisol, slightly increased above baseline (peak at 12 h of 19.2 ± 25 pg/ml).

The next two panels in Fig. 1 show that the soluble receptors of TNFα, p55, and p75 also sharply increased after endotoxin and peaked at 2–3 h, but slowly declined to baseline levels over the next 48–72 h (peak p55-3.1 ± 1.1 vs. 0.8 ± 0.2 ng/ml; p75-10.1 ± 1.6 vs. 2.3 ± 0.6 ng/ml, $P < 0.01$). The bottom two panels show that, compared with the cytokines, cortisol, and cytokine receptors, SAA and CRP more sluggishly increased after endotoxin, peaking at 24 h and then slowly declining (peak SAA 510 ± 259 vs. 6 ± 5 mg/l; CRP 30.8 ± 13.8 vs. 0.6 ± 1.1 mg/l, $P < 0.01$). Both were still elevated compared with the saline period at discharge 72 h later (SAA 137 ± 166 vs. 7 ± 5 mg/l; CRP 6.2 ± 3.1 vs. 0.5 ± 0.8 mg/l). Despite their parallel time course, SAA and CRP were not significantly correlated with one another or with the cytokines.

These concentrations of the cytokines and acute-phase proteins after endotoxin were within the ranges reported in acute inflammatory disease (3, 4, 25).

Other markers of inflammation

The expected changes in white blood cells occurred after endotoxin: an initial, sharp decrease at 1 h (3.0 ± 0.6 vs. 5.6 ± 1.8 thousand cells/μl, $P = 0.02$), then a marked increase between 6 and 9 h (14.9 ± 4.4 vs. 6.0 ± 2.0, $P = 0.03$), 90% as neutrophils, then a slow return to baseline by 48 h (data not shown). Two hours after endotoxin, the platelet count transiently decreased 17% from baseline values. In four subjects with complete urine collections, total urinary epinephrine in the first 12 h after endotoxin was twice that after saline (10 ± 5 vs. 5 ± 4 μg, $P = 0.05$). The mean values for norepinephrine and dopamine in the first 12 h urine collection were also higher after endotoxin than after saline, but the differences did not reach statistical significance.

Changes in lipids and lipoproteins

Figure 2 shows the sequential changes in serum lipids and lipoproteins following iv endotoxin and saline. After saline injection, all lipid and lipoprotein levels except NEFAs slightly declined from baseline levels, as would be expected after a 36 h fast and dilution from iv fluids, and returned to baseline by 48–72 h. NEFA levels plateaued above baseline levels at 9–15 h of fasting and were back to baseline at 48 h.

After endotoxin at 3–4.5 h, shortly after the sharp peaks in TNFα, IL-6 and cortisol, NEFA, and TG peaked 106% and 26% above control values. The percent increase in NEFA and TG were inversely correlated with each other ($r = -0.85$, $P = 0.03$), and positively correlated with maximum

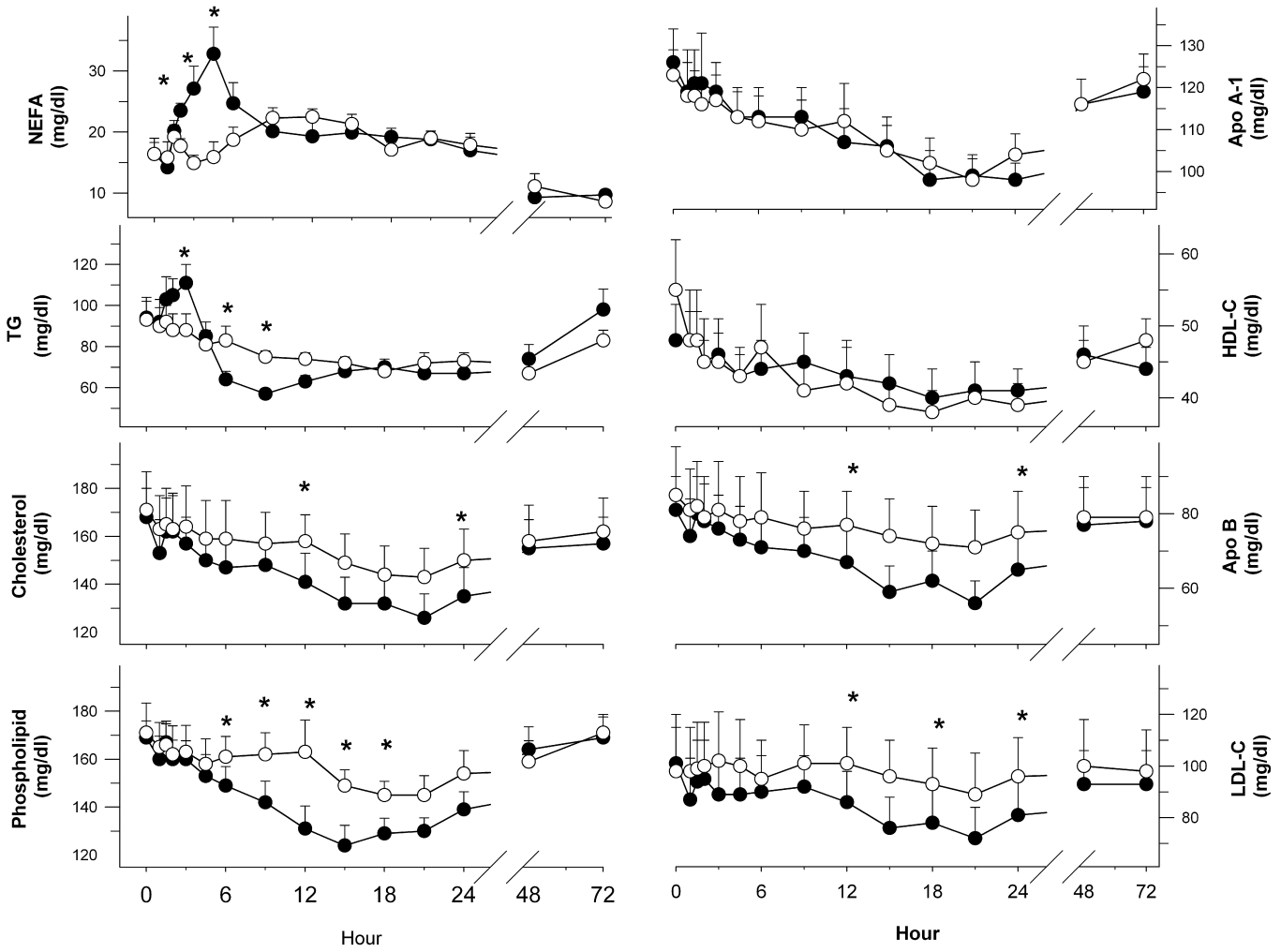

Fig. 2. Serum concentrations of nonesterified fatty acid, triglyceride (TG), cholesterol, phospholipid, apolipoprotein A-I (apoA-I), HDL cholesterol, apoB, and LDL cholesterol (mean ± SE) for 72 h after an iv dose of endotoxin (closed circles) or saline (open circles). Significant differences are shown with an asterisk ($P < 0.05$). There were no significant differences for apoA-I and HDL cholesterol.

IL-6 and CRP ($P = 0.03$ for both). NEFA and TG then abruptly declined so that at 9 h, NEFA was at control values but TG was 24% below control levels, returning to control values by 18 h. VLDL-TG changed in parallel with the total TG (data not shown).

Following the decline in TG were small (11–20%) but significant declines in total cholesterol, phospholipid, LDL cholesterol, and apoB below control values, reaching nadirs 12–24 h after injection (Fig. 2). Unexpectedly, HDL cholesterol and the primary apolipoprotein in HDL, apoA-I, did not significantly differ from control values. Note, however, the 20% decline in the concentration of phospholipid, a lipid carried largely by HDL. The decline in phospholipid was larger and slightly earlier than the declines in cholesterol, LDL cholesterol, and apoB. The statistical evaluation of the relationships among the lipids and acute-phase proteins revealed a highly significant linear relationship between the percent decrease in phospholipid and peak value for CRP ($r^2 = 0.97$, $P = 0.001$).

The amount of cholesterol, TG, and phospholipid in VLDL, LDL, and HDL at the nadir of phospholipid for each subject 12–24 h after endotoxin was determined with FPLC. As shown in **Fig. 3**, compared with the saline control sample at the same time point, phospholipid decreased mainly in LDL and HDL (−10 and −8 mg/dl), cholesterol mainly in LDL cholesterol (−16 mg/dl), and TG in VLDL-TG (−8 mg/dl). The percent decline in phospholipid, cholesterol, and TGs in LDL (7–15%) and VLDL (30–40%) were proportional, consistent with declines in the numbers of LDL and VLDL particles. FPLC performed on a subset of samples from two subjects with the greatest difference in total phospholipid confirmed that >90% of the total apoB was in LDL and that the decrease in total apoB was almost entirely accounted for by a decrease in LDL apoB. For HDL, however, phospholipids significantly declined 11% ($P = 0.004$) without changes in cholesterol or TG. Since serum total apoA-I also did not differ, this suggested a selective decrease in HDL phospholipid without a decrease in the number of HDL particles. FPLC in the same subset confirmed that similar amounts of apoA-I migrated only with HDL lipids and SAA. Furthermore, in this subset, there was no effect of

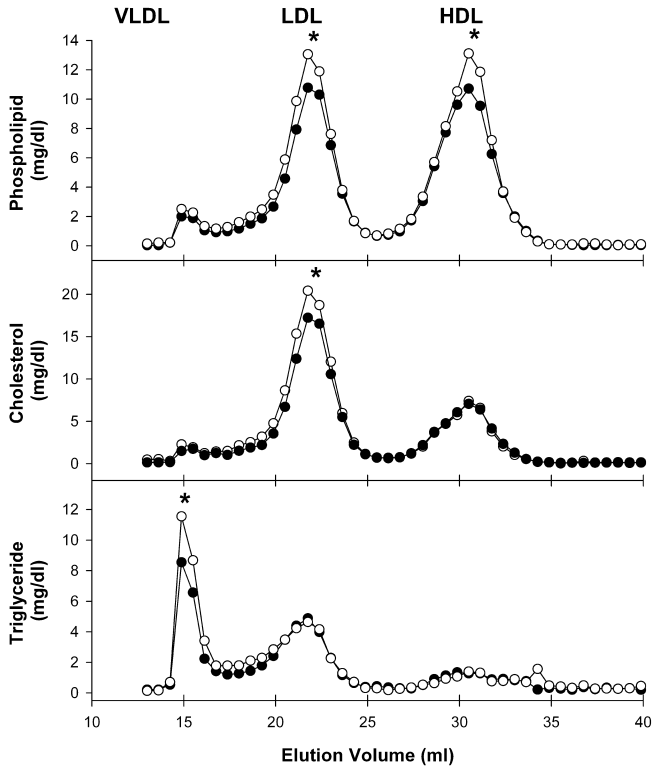

Fig. 3. Fast protein liquid chromatography (FPLC) of serum obtained at the time of maximum difference in phospholipid, 12–24 h after endotoxin (closed circles) or saline (open circles). Results are expressed as the mean concentration/dl of phospholipid (top panel), cholesterol (middle panel), and TG (bottom panel) measured in multiple fractions eluted in 40 ml from the FPLC column. The sum of the fraction concentrations for each lipid equals the total concentration in injected serum. Significant differences occurred for LDL and HDL phospholipid, LDL cholesterol, and VLDL TG ($*P < 0.05$).

endotoxin on HDL particle size measured by nondenaturing gradient gel electrophoresis (data not shown).

Composition of phospholipid subclasses

Table 2 shows that the decline in phospholipid after endotoxin treatments was due to declines in all phospholipid subclasses (PC, LPC, SM, PE, and PI-PS). A reciprocal decrease in PC and increase in LPC, as would be expected from increased activity of secretory phospholipase A2 (26, 27), or a selective decrease in SM from increased activity of secretory sphingomyelinase (28), were not observed. The fatty acid compositions of PC (Table 2) and other phospholipid subclasses (not shown) showed no meaningful differences between endotoxin and saline treatments. Thus, there was no evidence for an effect on the esterification or hydrolysis of a specific phospholipid fatty acid.

Lipid transfer proteins

The decrease in all classes of serum phospholipids after endotoxin could be due to a change in the activity of one or more phospholipid transfer proteins, with increased transfer of phospholipid out of the plasma into tissues or decreased transfer from tissues into plasma. The three main lipid transfer proteins reported to shuttle phospholipids in serum, LBP, PLTP, and CETP, were differentially affected by endotoxin. As shown in the top panel of **Fig. 4**, the concentration of LBP markedly increased 3-fold, peaked 12 h after endotoxin treatment compared with saline, and was still slightly elevated at 72 h. Of note is that the time of the peak increase in LBP coincided with the peak decrease in phospholipid. There was also a positive linear relationship between the maximum level of LBP and the maximum decrease in phospholipid (but not HDL or LDL phospholipid) of borderline statistical significance ($P = 0.047$).

In contrast, shown in the lower panels of Fig. 4, the activities of PLTP and CETP were reduced only 23% and 10% at later times after endotoxin treatment and with more variable time courses. The slight effect of the endotoxin on CETP, which exchanges HDL cholesterol ester for VLDL and LDL TG, was consistent with the absence of change in HDL cholesterol. Thus, LBP, rather than PLTP or CETP, showed the greatest alteration in response to iv endotoxin treatment. A predominant effect on LBP has also been reported in surgical patients 24–48 h after the onset of acute systemic inflammation; LBP was increased

TABLE 2. Phospholipid composition after iv endotoxin or saline

	Post iv Endotoxin[a]		Post iv Saline[a]			
	Mean	SD	Mean	SD	Difference	*P*
	mg/dl		*mg/dl*		%	
Phosphatidyl choline	99.3	22.2	117.4	19.5	−15	0.005
Lysophosphatidyl choline	6.1	1.6	8.0	1.7	−24	0.003
Sphingomyelin	37.1	5.6	41.6	9.0	−12	0.155
Phosphatidyl ethanolamine	3.0	0.6	3.1	1.2	−3	0.864
Phosphatidyl serine+inositol	3.3	1.0	5.0	2.0	−33	0.069
Phosphatidyl choline fatty acids (weight %)						
16:0	31.3	1.6	30.5	0.9	3	0.087
18:0	13.1	0.6	13.8	0.9	−5	0.047
9c-18:1	7.5	0.6	7.5	0.5	0	0.784
18:2	21.6	3.0	22.1	2.8	−2	0.031
20:4 n-6	12.7	3.5	12.2	3.4	4	0.117
22:6 n-3	3.7	0.8	4.0	1.0	−8	0.626

[a] At time point of maximum difference in phospholipid, 12–24 h postendotoxin.

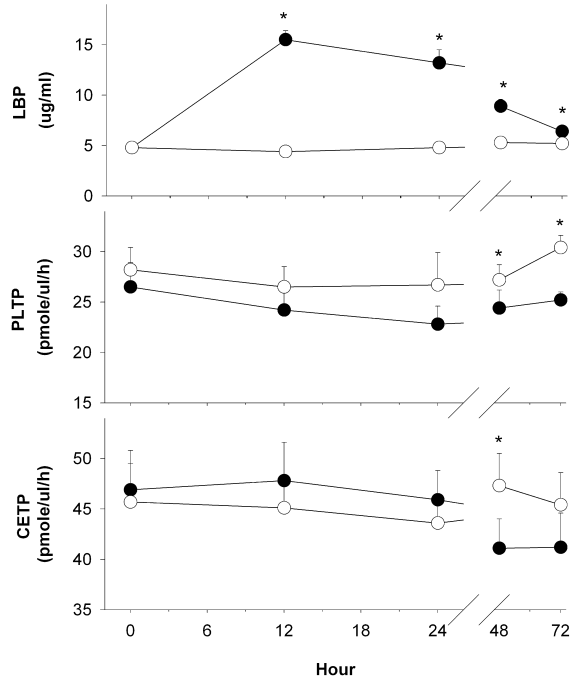

Fig. 4. Serum concentrations of lipopolysaccharide binding protein and the activities of phospholipid transfer protein and cholesteryl ester transfer protein (mean ± SE) for 72 h after an iv dose of endotoxin (closed circles) or saline (open circles). Significant differences between endotoxin and saline values are marked with an asterisk ($P < 0.05$).

10-fold, CETP activity was slightly decreased, and PLTP activity was slightly increased compared with healthy controls (4).

Fatty acid composition of VLDL-TG

Since the composition of the dietary fat was matched to each subject's adipose tissue, an increase in fatty acid synthesis would have been manifested by an increase in the saturated fatty acid palmitate (16:0), and a decrease in the essential fatty acid linoleate (18:2) in VLDL-TG compared with TG in the diet and adipose tissue (19). **Table 3** illustrates the lack of difference in the percentages of the major fatty acids in VLDL-TG for 72 h following the injection of endotoxin compared with saline. Therefore, the increase in TG after iv endotoxin treatment did not appear to be due to an increase in TG synthesis from newly made fatty acids.

TABLE 3. Fatty acid composition of VLDL-TG after iv endotoxin or saline

	VLDL-TG					
	0 h	3 h	max 24 h	48 h	72 h	Diet and AT
	% weight of total fatty acids					
18:2						
Endotoxin	23.8	24.6	22.6	22.1	22.4	18.7
Saline	23.3	24.2	22.9	23.0	23.0	18.7
16:0						
Endotoxin	24.2	24.8	27.2	24.8	24.1	19.0
Saline	23.9	25.1	27.4	24.4	23.0	19.0
9c-18:1						
Endotoxin	31.9	30.9	28.8	32.1	32.7	40.2
Saline	32.2	30.5	29.0	31.8	32.6	40.2

TG, triglyceride. There were no significant differences between endotoxin and saline treated groups ($P > 0.05$).

DISCUSSION

We demonstrate for the first time sequential changes in serum lipoproteins and lipid transfer proteins after a single iv dose of endotoxin in normal volunteers. As expected, participants developed mild flu-like symptoms and the typical, rapid, and marked increase in serum TNFα, IL-6, cortisol, SAA, CRP, epinephrine, neutrophils, and other markers of inflammation (14). The earliest serum lipid response was a transient, sharp increase in NEFA and TG peaking at 3–4 h, followed by a decrease below control values at 9–24 h in cholesterol, LDL cholesterol, TG, phospholipid, and apoB, which slowly returned to baseline levels by 48–72 h after endotoxin injection. Thus, many of the lipid and lipoprotein changes observed in septic humans with endotoxemia were reproduced in normal volunteers after a single iv dose of endotoxin. The results illustrate the utility of this model to study the mechanisms of lipid lowering in human endotoxemia and inflammation.

Early increases in serum NEFA and TG were previously observed in humans, nonhuman primates, and rodents given iv endotoxin (17, 29–31). The mobilization of NEFA from adipose tissue stores and the increase in plasma TG has long been known to occur as part of the acute-phase response to injury (32–34). The increase in TG may be due to increased hepatic synthesis and secretion of VLDL-TG from fatty acids mobilized from adipose tissue in response to increased cytokines, cortisol, and catecholamines. In a previous study in normal volunteers with catheters placed in the hepatic artery and vein, 1–2 h after a single iv dose of endotoxin, coincident with large increases in the levels of TNFα and IL-6, there was a 6-fold increase in the hepatic uptake of NEFA and a 3-fold increase in the hepatic output of TG (29). At a higher exposure to endotoxin, the increase in TG may be mediated by decreased lipoprotein lipase in adipose tissue and reduced VLDL clearance (31, 35). As still another mechanism, endotoxin rapidly increased fatty acid synthesis and hepatic TG secretion in rodents, but only when the preceding diet was fat free and high in sucrose (30, 31). In our study, iv endotoxin treatments did not increase fatty acid synthesis, as manifested by the lack of increase in palmitate and decrease in linoleate in VLDL-TG relative to the diet and adipose tissue for 3 days following endotoxin administration (19). However, different results may be obtained with endotoxin administered after a low-fat, high-sugar diet or an iv infusion of glucose.

The subsequent declines in serum cholesterol, TG, phospholipid, and apoB in LDL and VLDL were compatible with declines in the numbers of LDL and VLDL particles. It is unknown whether a decrease in synthesis, an increase in clearance, or both are responsible for the decrease in apoB-containing lipoproteins during human endotox-

emia. In vitro, HepG2 cells exposed to IL-6 and/or TNFα showed both decreased apoB synthesis (36) and increased LDL receptor expression (37).

The most consistent lipid-lowering effect by a variety of types of inflammation has been a decrease in serum HDL, HDL cholesterol, and its major protein, apoA-I (3, 4, 11, 38). Thus, it was unexpected that HDL phospholipid declined after iv endotoxin without a change in HDL cholesterol or apoA-I. The reduction in total plasma phospholipid and selective decrease of phospholipid in HDL suggests that an early effect of human endoxemia is an alteration in the plasma input or clearance of HDL phospholipid. Since the decrease in HDL phospholipid occurred in all subclasses, mechanisms that shift the relative amounts of phospholipid subclasses, such as a decrease in the synthesis of PC (39), a decrease in PC and increase in LPC by secretory phospholipase A_2 (26, 27, 40, 41), a decrease in SM by sphingomyelinase (28), or an increase in the biliary excretion of PC via the ABC membrane transporter, MDR3 P-glycoprotein (42), are less likely. Mechanisms that affect both cholesterol and phospholipid in HDL, such as an increase in the scavenger receptor class B type I scavenger receptor (43) or the ABC1 membrane transporter (44), are also less likely. Instead, our results point to an activation of a pathway that increases the transfer of HDL phospholipid of several subclasses from the plasma to the tissue compartment.

One possible pathway is the transfer of HDL phospholipid from the plasma to the tissues by LBP. Our data show that LBP, an acute-phase protein that binds a variety of phospholipids in addition to the phospholipid-like lipid A segment of endotoxin (4, 45, 46), was greatly increased after a single small dose of endotoxin to normal volunteers. Furthermore, unlike the other lipid transfer proteins, the time of the peak increase in LBP corresponded to the nadir in phospholipid. During the inflammatory response, LBP may transport both phospholipid and endotoxin from the plasma to tissues. A larger and/or more persistent depletion of phospholipid from HDL than produced in this study, in turn, may lead to an increase in HDL clearance and the reductions in HDL cholesterol and apoA-I typically observed during the acute-phase response. Other recent observations indicate that HDL phospholipid is an important determinant of HDL levels and clearance rates (40, 41, 47).

In contrast to the marked stimulatory effect of endotoxin on the level of LBP, the activities of two other major lipid transfer proteins, PLTP and CETP, were only slightly suppressed, with more variable time courses. This difference from LBP was interesting, given the similar organization of the LBP, PLTP, and CETP genes (48). Like LBP, PLTP binds endotoxin and exchanges phospholipids among lipoproteins (46, 49). After endotoxin administration to wild-type mice, PLTP activity was also reduced, but HDL phospholipid, cholesterol, and particle size were slightly increased (50). In the PLTP knock-out mouse, the catabolism of HDL, depleted of phospholipid, was markedly increased (47). CETP, which transfers cholesterol ester from HDL to VLDL and LDL, also decreased in mice transgenic for the human CETP gene and treated with endotoxin (51). The small, late changes in CETP in our human volunteers treated with endotoxin are consistent with the lack of change in HDL cholesterol. The results show that of the three related lipid transfer proteins in humans, LBP is the most sensitive to endotoxin and the most likely candidate to play a role in the decline in lipoproteins during the early acute-phase response.

The decrease in serum phospholipid and HDL phospholipid after a single dose of endotoxin is particularly intriguing in light of evidence for an important role of phospholipid in the defense against endotoxemia. We previously demonstrated that, although all lipoproteins bind endotoxin, HDL is the most protective because it is rich in surface phospholipids (52). The phospholipid-like lipid A domain of endotoxin is neutralized by inserting into the surface phospholipid monolayer of lipoproteins instead of or after binding to LBP. In support for the protective role of plasma phospholipid in reducing the inflammatory response, the prophylactic iv administration of a protein-free phospholipid emulsion improved survival in a porcine model of sepsis (53). Furthermore, healthy volunteers showed fewer flu-like symptoms and much lower plasma inflammatory cytokines when reconstituted HDL made with HDL apolipoproteins and PC instead of saline was intravenously administered 3.5 h before a single iv dose of endotoxin (54). In contrast, volunteers who were infused with 20% Intralipid (20% soybean TG, 1.2% phospholipid, 2.3% glycerol) instead of saline for 2 h before an iv dose of endotoxin showed no reduction in clinical symptoms or plasma cytokines (18). In addition to phospholipid, other studies indicate that apoA-I (55) and apoE (56) may also facilitate the binding of endotoxin by HDL. Although a reduction of HDL during inflammation may provide less capacity for neutralization of endotoxin in plasma, it is possible that an increased plasma clearance of HDL may be beneficial to the host. An increased transfer of HDL phospholipids to tissues may help regenerate damaged cellular membranes and lung surfactant, and endotoxin bound to the HDL phospholipid may be excreted into bile (57).

In conclusion, after a single iv dose of endotoxin in normal volunteers, a burst of cytokines, cortisol, and epinephrine was followed by rapid changes in lipids, lipoproteins, and lipid transfer proteins. VLDL-TG increased first, probably due to an increase in the hepatic synthesis and secretion of TG from preformed fatty acids mobilized from adipose tissue. ApoB-containing lipoproteins (VLDL and LDL) then decreased due to decreased synthesis or increased clearance. A selective loss of phospholipid from HDL may have been mediated by LBP and could result in increased HDL clearance during more-intense or prolonged inflammation. Additional studies of lipid and lipoprotein turnover are clearly needed to better define the effects of endotoxin on biosynthetic and catabolic pathways that rapidly alter lipid and lipoprotein levels in humans.

The excellent technical assistance of Kaun Mao, Aline Baday, Yaoxin Chen, Suizhen Qiu, and The Rogosin Institute Clinical

Laboratory is much appreciated. The authors also are thankful for the skill and guidance of Dr. Joseph Parrillo and the nursing and dietary staff of The Rockefeller GCRC. This work was supported by the National Institutes of Health GCRC Grant MO1-RR00102.

REFERENCES

1. Parrillo, J. E. 1993. Pathogenetic mechanisms of septic shock. *N. Engl. J. Med.* **328:** 1471–1477.
2. Gabay, C., and I. Kushner. 1999. Acute-phase proteins and other systemic responses to inflammation. *N. Engl. J. Med.* **340:** 448–454.
3. Gordon, B. R., T. S. Parker, D. M. Levine, S. D. Saal, J. C. L. Wang, B. Sloan, P. S. Barie, and A. L. Rubin. 2001. Relationship of hyperlipidemia to cytokine concentrations and outcomes in critically ill surgical patients. *Crit. Care Med.* **29:** 1563–1568.
4. Barlage, S., D. Frohlich, A. Bottcher, M. Jauhiainen, H. P. Muller, F. Noetzel, G. Rothe, C. Schutt, R. P. Linke, K. L. Lackner, C. Ehnholm, and G. Schmitz. 2001. ApoE-containing high density lipoproteins and phospholipid transfer protein activity increase in patients with a systemic inflammatory response. *J. Lipid Res.* **42:** 281–290.
5. Sammalkorpi, K., V. Valtonen, Y. Kerttula, E. Nikkila, and M. Taskinen. 1988. Changes in serum lipoprotein pattern induced by acute infections. *Metabolism.* **37:** 859–865.
6. Jacobs, D., H. Blackburn, M. Higgins, D. Reed, H. Iso, G. McMillan, J. Neaton, J. Nelson, J. Potter, B. Rifkind, J. Rossouw, R. Shekelle, and S. Yusuf. 1992. Report of the conference on low blood cholesterol: mortality associations. *Circulation.* **86:** 1046–1060.
7. Bologa, R. M., D. M. Levine, T. S. Parker, J. S. Cheigh, D. Serur, K. H. Stenzel, and A. L. Rubin. 1998. Interleukin-6 predicts hypoalbuminemia, hypocholesterolemia, and mortality in hemodialysis patients. *Am. J. Kidney Dis.* **32:** 107–114.
8. Ulevitch, R. J., A. R. Johnston, and D. B. Weinstein. 1981. New function for high density lipoproteins. Isolation and characterization of a bacterial lipopolysaccharide-high density lipoprotein complex formed in rabbit plasma. *J. Clin. Invest.* **67:** 827–837.
9. Levine, D. L., T. S. Parker, T. M. Donnelly, A. Walsh, and A. L. Rubin. 1993. In vivo protection against endotoxin by plasma high density lipoprotein. *Proc. Natl. Acad. Sci. USA.* **90:** 12040–12044.
10. Rauchhaus, M., A. J. S. Coats, and S. D. Anker. 2000. The endotoxin-lipid hypothesis. *Lancet.* **356:** 930–933.
11. Khovidhunkit, W., R. A. Memon, K. R. Feingold, and C. Grunfeld. 2000. Infection and inflammation-induced protatherogenic changes of lipoproteins. *J. Infect. Dis.* **181(Suppl 3):** S462–S472.
12. Ross, R. 1999. Atherosclerosis—an inflammatory disease. *N. Engl. J. Med.* **340:** 115–126.
13. Martich, G. D., A. J. Boujoukos, and A. F. Suffredini. 1993. Response of man to endotoxin. *Immunobiology.* **187:** 403–416.
14. Suffredini, A. F., H. D. Hochstein, and F. G. McMahon. 1999. Dose-related inflammatory effects of intravenous endotoxin in humans: evaluation of a new clinical lot of *Escherichia coli* O:113 endotoxin. *J. Infect. Dis.* **179:** 1278–1282.
15. Hardardottir, I., C. Grunfeld, and K. R. Feingold. 1994. Effects of endotoxin and cytokines on lipid metabolism. *Curr. Opin. Lipidol.* **5:** 207–215.
16. Auerbach, B. J., and J. S. Parks. 1989. Lipoprotein abnormalities associated with lipopolysaccharide-induced lecithin:cholesterol acyltransferase and lipase deficiency. *J. Biol. Chem.* **264:** 10264–10270.
17. Ettinger, W. H., L. D. Miller, J. J. Albers, T. K. Smith, and J. S. Parks. 1990. Lipopolysaccharide and tumor necrosis factor cause a fall in plasma concentration of lecithin:cholesterol acyltransferase in cynomolgus monkeys. *J. Lipid Res.* **31:** 1099–1107.
18. Van der Poll, T., C. C. Braxton, S. M. Coyle, M. A. Boermeester, J. C. L. Wang, P. M. Jansen, W. J. Montegut, S. E. Calvano, C. E. Hack, and S. F. Lowry. 1995. Effect of hypertriglyceridemia on endotoxin responsiveness in humans. *Infect. Immun.* **63:** 3396–3400.
19. Hudgins, L. C., M. K. Hellerstein, C. E. Seidman, R. A. Neese, J. D. Tremaroli, and J. Hirsch. 2000. Relationship between carbohydrate-induced hypertriglyceridemia and fatty acid synthesis in lean and obese subjects. *J. Lipid Res.* **41:** 595–604.
20. Hudgins, L. C., M. Hellerstein, C. Seidman, R. Neese, J. Diakun, and J. Hirsch. 1996. Human fatty acid synthesis is stimulated by a eucaloric low fat, high carbohydrate diet. *J. Clin. Invest.* **97:** 2081–2091.
21. Christie, W. W. 1989. Gas Chromatography and Lipids. The Oily Press, Alloway, United Kingdom. 40.
22. Warnick, G., J. Benderson, and J. Albers. 1982. Dextran sulfate-Mg2+ precipitation procedure for quantitation of high-density-lipoprotein cholesterol. *Clin. Chem.* **28:** 1379–1388.
23. Li, J., B. Fang, R. C. Eisensmith, X. H. C. Li, I. Nasonkin, Y-C. Lin-Lee, M. P. Mims, A. Hughs, C. Montgomery, J. Roberts, T. S. Parker, D. M. Levine, and S. L. C. Woo. 1995. In vivo therapy for hyperlipidemia: phenotypic correction in Watanabe rabbits by hepatic delivery of the rabbit LDL receptor gene. *J. Clin. Invest.* **95:** 768–773.
24. Nichols, A. V., R. M. Krauss, and T. A. Musliner. 1986. Nondenaturing polyacrylamide gradient gel electrophoresis. *Methods Enzymol.* **128:** 417–431.
25. Buttenschoen, K., D. C. Buttenschoen, D. Berger, C. Vasilescu, S. Schafheutle, B. Goeltenboth, M. Seidelmann, and H. G. Beger. 2001. Endotoxemia and acute-phase proteins in major abdominal surgery. *Am. J. Surgery*. **181:** 36–43.
26. Pruzanski, W., D. W. Wilmore, A. Suffredini, G. D. Martich, A. G. D. Hoffman, J. L. Browning, E. Stefanski, B. Sternby, and P. Vadas. 1992. Hyperphospholipasemia A2 in human volunteers challenged with intravenous endotoxin. *Inflammation.* **16:** 561–570.
27. Pruzanski, W., E. Stefanski, F. C. de Beer, M. C. de Beer, P. Vadas, A. Ravandi, and A. Kuksis. 1998. Lipoproteins are substrates for human secretory group IIA phospholipase A2: preferential hydrolysis of acute phase HDL. *J. Lipid Res.* **39:** 2150–2160.
28. Wong, M., B. Xie, N. Beatini, P. Phu, S. Marathe, A. Johns, P. W. Gold, E. Hirsch, K. J. Williams, J. Licinio, and I. Tabas. 2000. Acute systemic inflammation up-regulates secretory sphingomyelinase in vivo: a possible link between inflammatory cytokines and atherogenesis. *Proc. Natl. Acad. Sci. USA.* **97:** 8681–8686.
29. Fong, Y., M. A. Marano, L. L. Moldawer, H. Wei, S. E. Calvano, J. S. Kenney, A. C. Allison, A. Cerami, G. T. Shires, and S. F. Lowry. 1990. The acute splanchnic and peripheral tissue metabolic response to endotoxin in humans. *J. Clin. Invest.* **85:** 1896–1904.
30. Feingold, K. R., M. Soued, M. K. Serio, S. Adi, A. H. Moser, and C. Grunfeld. 1990. The effect of diet on tumor necrosis factor stimulation of hepatic lipogenesis. *Metabolism.* **39:** 623–632.
31. Feingold, K. R., I. Staprans, R. A. Memon, A. H. Moser, J. K. Shigenaga, W. Doerrler, C. A. Dinarello, and C. Grunfeld. 1992. Endotoxin rapidly induces changes in lipid metabolism that produce hypertriglyceridemia: low doses stimulate hepatic triglyceride production while high doses inhibit clearance. *J. Lipid Res.* **33:** 1765–1776.
32. Samra, J. S., L. K. M. Summers, and K. N. Frayn. 1996. Sepsis and fat metabolism. *Br. J. Surg.* **83:** 1186–1196.
33. Carpentier, Y. A., M. Jeevanandam, A. P. Robin, J. Nordenstrom, R. E. Burr, R. L. Liebel, J. Hirsch, D. H. Elwyn, and J. M. Kinney. 1984. Measurement of glycerol turnover by infusion of isotopic glycerol in normal and injured subjects. *Am. J. Physiol.* **247:** E405–E411.
34. Gallin, J. I., D. Kaye, and W. M. O'Leary. 1969. Serum lipids in infection. *N. Engl. J. Med.* **281:** 1081–1086.
35. Fried, S. K., and R. Zechner. 1989. Cachectin/tumor necrosis factor decreases human adipose tissue lipoprotein lipase mRNA levels, synthesis and activity. *J. Lipid Res.* **30:** 1917–1923.
36. Ettinger, W. H., V. K. Varma, M. Sorci-Thomas, J. S. Parks, R. C. Sigmon, T. K. Smith, and R. B. Verdery. 1994. Cytokines decrease apolipoprotein accumulation in medium from Hep G2 cells. *Arterioscler. Thromb.* **14:** 8–13.
37. Stopeck, A. T., A. C. Nicholson, F. P. Mancini, and D. P. Hajjar. 1993. Cytokine regulation of low density lipoprotein receptor gene transcription in Hep G2 cells. *J. Biol. Chem.* **268:** 17489–17494.
38. Pruzanski, W., E. Stefanski, F. C. de Beer, M. C. de Beer, A. Ravandi, and A. Kuksis. 2000. Comparative analysis of lipid composition of normal and acute-phase high density lipoproteins. *J. Lipid Res.* **41:** 1035–1047.
39. Mallampalli, R. K., A. J. Ryan, R. G. Salome, and S. Jackowski. 2000. Tumor necrosis factor-alpha inhibits expression of CTP: phosphocholine cytidyltransferase. *J. Biol. Chem.* **275:** 9699–9708.
40. Tietge, U. J. F., C. Maugeais, W. Cain, D. Grass, J. M. Glick, F. C. de Beer, and D. J. Rader. 2000. Overexpression of secretory phospholipase A2 causes rapid catabolism and altered tissue uptake of HDL cholesteryl ester and apolipoprotein A-I. *J. Biol. Chem.* **275:** 10077–10084.
41. Tietge, U. J. F., C. Maugeais, S. Lund-Katz, D. Grass, F. C. deBeer,

and D. J. Rader. 2002. Human secretory phospholipase A2 mediates decreased plasma levels of HDL cholesterol and Apo-A1 in response to inflammation in human ApoA-1 transgenic mice. *Arterioscler. Thromb. Vasc. Biol.* **22:** 1213–1218.
42. Crawford, A. R., A. J. Smith, V. C. Hatch, R. P. J. O. Elferink, P. Borst, and J. M. Crawford. 1997. Hepatic secretion of phospholipid vesicles in the mouse critically depends on mdr2 or MDR3 P-glycoprotein expression. *J. Clin. Invest.* **100:** 2562–2567.
43. Ueda, Y., L. Royer, E. Gong, J. Zhang, P. N. Cooper, O. Francone, and E. M. Rubin. 1999. Lower plasma levels and accelerated clearance of high density lipoprotein (HDL) and non-HDL cholesterol in scavenger receptor class B type I transgenic mice. *J. Biol. Chem.* **274:** 7165–7171.
44. Wang, N., D. L. Silver, P. Costet, and A. R. Tall. 2000. Specific binding of apoA-1, enhanced cholesterol efflux, and altered plasma membrane morphology in cells expressing ABC1. *J. Biol. Chem.* **275:** 33053–33058.
45. Yu, B., E. Hailman, and S. D. Wright. 1997. Lipopolysaccharide binding protein and soluble CD14 catalyze exchange of phospholipids. *J. Clin. Invest.* **99:** 315–324.
46. Vesy, C. J., R. L. Kitchens, G. Wolfbauer, J. J. Albers, and R. S. Munford. 2000. Lipopolysaccharide-binding protein and phospholipid transfer protein release lipopolysaccharides from Gram-negative bacterial membranes. *Infect. Immun.* **68:** 2410–2417.
47. Qin, S., K. Kawano, C. Bruce, M. Lin, C. Bisgaier, A. R. Tall, and X. Jiang. 2000. Phospholipid transfer protein gene knock-out mice have low high density lipoprotein levels, due to hypercatabolism, and accumulate apoA-IV-rich lamellar lipoproteins. *J. Lipid Res.* **41:** 269–276.
48. Kirschning, C. J., J. Au-Young, N. Lamping, D. Reuter, D. Pfeil, J. J. Seilhamer, and R. R. Schumann. 1997. Similar organization of the lipopolysaccharide-binding protein (LBP) and phospholipid transfer protein (PLTP) genes suggests a common gene family of lipid-binding proteins. *Genomics.* **46:** 416–425.
49. Hailman, E., J. J. Albers, G. Wolfbauer, Y. Tu, and S. D. Wright. 1996. Neutralization and transfer of lipopolysaccharide by phospholipid transfer protein. *J. Biol. Chem.* **271:** 12172–12178.
50. Jiang, X. C., and C. Bruce. 1995. Regulation of murine plasma phospholipid transfer protein activity and mRNA levels by lipopolysaccharide and high cholesterol diet. *J. Biol. Chem.* **270:** 17133–17138.
51. Tall, A., D. Sharp, S. Zhong, T. Hayek, L. Masucci-Magoulas, E. M. Rubin, and J. L. Breslow. 1997. Cholesteryl ester transfer protein and atherogenesis. *Ann. N. Y. Acad. Sci.* **811:** 178–182.
52. Parker, T. S., D. M. Levine, J. C. C. Chang, J. Laxer, C. C. Coffin, and A. L. Rubin. 1995. Reconstituted high-density lipoprotein neutralizes gram-negative bacterial lipopolysaccharides in human whole blood. *Infect. Immun.* **63:** 253–258.
53. Goldfarb, R. D., T. S. Parker, D. M. Levine, D. Glock, I. Akhter, A. Alkhudari, R. J. McCarthy, E. M. David, B. R. Gordon, S. D. Saal, A. L. Rubin, G. M. Trenholme, and J. E. Parrillo. 2003. Protein free phospholipid emulsion treatment improved cardiopulmonary function and survival in porcine sepsis. *Am. J. Physiol.* **284:** R550–R557.
54. Pajkrt, D., J. E. Doran, F. Koster, P. G. Lerch, B. Arnet, T. Van der Poll, J. W. ten Cate, and S. J. H. van Deventer. 1996. Antiinflammatory effects of reconstituted high-density lipoprotein during human endotoxemia. *J. Exp. Med.* **184:** 1601–1608.
55. Massamiri, T., P. S. Tobias, and L. K. Curtiss. 1997. Structural determinants for the interaction of lipopolysaccharide binding protein with purified high density lipoproteins: the role of apolipoprotein A-1. *J. Lipid Res.* **38:** 516–525.
56. Van Oosten, M., P. C. N. Rensen, E. S. Van Amersfoort, M. Van Eck, A. Van Dam, J. J. P. Breve, T. Vogel, A. Panet, T. J. C. Van Berkel, and J. Kuiper. 2001. Apolipoprotein E protects against bacterial lipopolysaccharide-induced lethality. *J. Biol. Chem.* **276:** 8820–8824.
57. Mimura, Y., S. Sakisaka, M. Harada, M. Sata, and K. Tanikawa. 1995. Role of hepatocytes in direct clearance of lipopolysaccharide in rats. *Gastroenterology.* **109:** 1969–1976.