The role of apolipoprotein CI in lipid metabolism and bacterial sepsis

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Chapter 7

Plasma Apolipoprotein CI Correlates with Increased Survival in Patients with Severe Sepsis

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Submitted for publication

Abstract

Objective: We recently reported that apolipoprotein CI (apoCI) protects against the development of murine bacterial sepsis. Therefore, we now examined the time course of plasma apoCI levels in survivors and non-survivors of severe sepsis.

Design: Prospective study in patients meeting predefined criteria for severe sepsis.

Setting: University hospital intensive care unit.

Patients: Seventeen patients with severe sepsis.

Interventions: In each patient, serial blood samples for total cholesterol, LDL-cholesterol, HDL-cholesterol, triglycerides, apoCI, apoAI, apoB, and apoCIII protein determination as well as clinical outcome data were collected over 30 days. Control values were obtained in healthy subjects (n=18).

Results: At day 0, plasma apoCI levels were 3-fold lower in septic patients as compared to healthy volunteers (2.0 ± 0.5 versus 6.0 ± 0.6 mg/dL, respectively; $P<0.0001$). After a nadir on day 2 (1.7 ± 0.3 mg/dL), apoCI gradually increased to 5.8 ± 1.1 mg/dL on day 28. At day 0, apoCI levels tended to be lower in non-survivors as compared to survivors. Remarkably, apoCI levels remained low in non-survivors, whereas apoCI levels gradually increased in survivors, reaching normal levels within 4 weeks. Such a difference between survivors and non-survivors could not be found for plasma levels of triglycerides, total cholesterol, and LDL-cholesterol, or HDL-cholesterol. Interestingly, the difference in apoCI levels between survivors and non-survivors remained significant after adjustment for lipoprotein lipids, whereas no such effect between survivors and non-survivors could be found for other apolipoproteins (*i.e.* apoAI, apoB, and apoCIII) after lipid adjustment.

Conclusions: Plasma apoCI levels are markedly decreased in patients with severe sepsis. ApoCI levels were higher in survivors, even after adjustment for lipid levels, and recovered progressively to normal levels. In contrast, apoCI levels remained low in non-survivors. Therefore, a high plasma apoCI level predicts survival in patients with severe sepsis.

Introduction

Sepsis is the leading cause of death among critically ill patients with overall mortality rates ranging from 15 to 80%[1,2]. Septic patients show profound alterations in plasma lipid levels. They have decreased plasma cholesterol levels, in both low-density lipoprotein (LDL)[3-5] and high-density lipoprotein (HDL)[3-6], whereas triglycerides are increased[3-5]. Lipoproteins, and in particular HDL, have been demonstrated to play an important role in modulating inflammation and the

response to infection[7-9].

On their surface, HDL particles expose high amounts of apolipoproteins[10,11], which are the proposed determinants of both the anti-inflammatory[9,12-14] and pro-inflammatory[8,15,16] properties of HDL. Apolipoprotein CI (apoCI) circulates predominantly on HDL[17,18], and is the third most abundant apolipoprotein on HDL particles[17,18]. A single report has shown that HDL is virtually depleted from apoCI during human sepsis[3]. Interestingly, we recently revealed that apoCI is directly involved in the protection against bacterial sepsis. ApoCI dose-dependently increased the survival rate in a fatal murine sepsis model by enhancing the eradication of invading microorganisms[19]. However, at present, there are no data on plasma apoCI levels during the time course of sepsis.

Therefore, the aim of the current study was to examine the time course of plasma apoCI levels in patients with severe sepsis, and to determine the correlation between plasma apoCI levels and survival in these patients.

Materials and Methods

Patients − The study design and baseline characteristics of the original patient population have been described elsewhere[5]. Briefly, a total of 17 critically ill patients aged ≥ 18 years were studied as part of the KyberSept study, a multinational, double-blind, randomized, placebo-controlled phase III study of antithrombin (AT) III (Kybernin P, Aventis Behring GmbH, Marburg, Germany) in patients with severe sepsis[20]. Patients admitted to the medical intensive care unit (ICU) between November 1997 and January 2000 were enrolled in the study as soon as they fulfilled the criteria for severe sepsis, as described in the American College of Chest Physicians/Society of Critical Care Medicine consensus conference definition[21]. The local Institutional Ethics Committee approved the study.

Study Protocol − Patients who entered the study fulfilled the criteria for severe sepsis within a 6-hour period exactly as previously described[5]. As soon as the patients met the criteria of severe sepsis, they were randomized to receive within 2 hrs a loading dose of 6000 IU of AT III or placebo followed by a 96-hr continuous infusion of 250 IU/hr of AT III or placebo in addition to standard treatment. The treatment of patients with AT III did not influence lipoprotein parameters[5]. Heparin in anticoagulant doses, coumadin derivatives, nonsteroidal anti-inflammatory drugs in anti-inflammatory doses, and additional open label AT III concentrate were not permitted during the study. Otherwise, there were no further requirements, or restrictions, in intensive care medication or management. All patients received enteral nutrition, and none of the patients received parenteral

nutrition or lipid emulsions. Acute Physiology and Chronic Health Evaluation II (APACHE II) scores were calculated according to Knaus *et al*.[21] and multiple organ dysfunction syndrome scores according to Marshall *et al*.[22].

For healthy control subjects, blood was obtained with informed consent from healthy blood donors (n=18).

Blood Sampling and Analysis − Serial blood samples were drawn on day 0 (study entry) and on days 1, 2, 3, 7, 14, and 28 after study entry, by using an indwelling arterial catheter. Blood was collected in EDTA or heparin anticoagulated tubes or plain sterile glass tubes (Becton, Dickinson Vacutainer Systems, Meylan, Cedex-France) and immediately centrifuged twice at 3,200 rpm (1,000 x *g*) for 10 min at 4°C. Plasmad and sera were stored at –80°C until further analysis.
Cholesterol and triglyceride levels were determined using commercially available enzymatic kits (Roche Diagnostics Cholesterol Reagent and Roche Diagnostics Triglyceride Reagent, respectively; Roche Diagnostics, The Netherlands). LDL and HDL separation was performed using density gradient ultracentrifugation exactly as described previously[5]. The LDL and HDL fractions were pooled according to their density and the cholesterol content was determined. NonHDL-cholesterol was calculated by extracting the HDL-cholesterol levels from the total cholesterol levels.

Plasma apoAI and apoB levels were determined by turbidometric analysis or Cobas Mirea (ABX, Montpellier, France). Plasma apoCI and apoCIII concentrations were determined using sandwich ELISAs specific for human apoCI[23] and apoCIII[24] as described previously. For determination of the distribution of apoCI and apoCIII over lipoproteins after fast performance liquid chromatography (FPLC), plasma obtained at day 3 from survivors and non-survivors (n=3 per group) were injected onto a Superose 6 column (Äkta System; Amersham Pharmacia Biotech, Piscataway, NJ), and eluted with PBS, 1 mM EDTA, pH 7.4. Collected fractions were assayed for apoCI and apoCIII as described above.

Statistical Analysis − The data were analyzed using nonparametric Mann-Whitney *U* tests (SPSS version 11.0; SPSS, Chicago, IL). $P<0.05$ was regarded as significant. Results are expressed as means ± SEM.

Results

Patient Characteristics − The study included 17 consecutive patients (9 males and 8 females) admitted to the ICU who fulfilled the criteria of severe sepsis. The demographic and clinical characteristics are presented in Table 1.

Table 1. Demographic and clinical characteristics of 17 patients with severe sepsis.

Patient (No.)	Sex	Age (years)	APACHE II (Score)	MODS (Score)	Diagnosis	Microorganism	Renal Replacement Therapy	Mechanical Ventilation	Survival
1	Male	92	27	8	CAP	*Streptococcus pneumoniae*	No	Yes	No
2	Female	68	24	9	CAP	*Legionella pneumophila*	No	Yes	No
3	Female	71	31	5	CAP	*Streptococcus pneumoniae*	No	Yes	Yes
4	Male	63	18	6	VAP	*Escherichia coli*	No	Yes	Yes
5	Male	68	34	11	CAP	*Streptococcus pneumoniae*	Yes	Yes	No
6	Male	84	23	3	Fourniers gangrene	Culture negative	No	Yes	Yes
7	Male	48	29	9	HAP	*Staphyloccus aureus*	No	Yes	No
8	Female	35	40	13	ARDS (drowning)	Polymicrobial	No	Yes	Yes
9	Female	51	30	8	HAP	*Pseudomonas aeruginosa*	No	Yes	Yes
10	Male	67	22	6	HAP	*Enterobacter cloacae*	No	Yes	Yes
11	Male	67	22	4	CAP	*Moraxella catarrhalis*	No	Yes	No
12	Female	78	17	5	Peritonitis	*Escherichia coli*	No	Yes	Yes
13	Male	76	33	11	UTI	*Proteus mirabilis*	Yes	Yes	No
14	Female	64	41	10	Meningitis	Culture negative	Yes	Yes	No
15	Female	65	22	3	CAP	*Escherichia coli*	No	No	Yes
16	Male	49	12	3	CRI	*Pseudomonas aeruginosa*	No	Yes	Yes
17	Female	68	32	8	CAP	*Streptococcus pneumoniae*	No	Yes	No
Mean		65.5	26.9	7.2					

CAP, community-acquired pneumonia; VAP, ventilator-associated pneumonia; HAP, hospital-acquired pneumonia; ARDS, acute respiratory distress syndrome; UTI, urinary tract infection; CRI, catheter-related infection.

The patients were all severely ill with a mean Acute Physiology and Chronic Health Evaluation II (APACHE II) score of 26.9 ± 1.9 and a mean multiple organ dysfunction syndrome (MODS) score of 7.2 ± 0.8. All patients except one received mechanical ventilation, and three of the 17 patients received renal replacement therapy. Eight patients (47%) died within 30 days after entering the study and were classified as “non-survivors”. The other 9 patients (53%) were defined as “survivors”. Compared to the survivors, the non-survivors were more severely ill with higher MODS scores (8.8 ± 0.8 vs. 5.8 ± 1.1; P<0.05) and a tendency towards higher APACHE II scores (30.3 ± 2.2 vs. 23.9 ± 2.8; P=0.067). No statistical difference was found between the age of survivors and of non-survivors (62.6 ± 5.1 vs. 68.9 ± 4.3 years, respectively).

Plasma ApoCI Levels − At the entry of the study, plasma apoCI levels were 3-fold lower in septic patients (2.0 ± 0.5 mg/dL; n=17) as compared to healthy volunteers (6.0 ± 0.6 mg/dL; n=18; P<0.00001) (Fig. 1A). ApoCI levels further declined to a nadir of 1.7 ± 0.3 mg/dL after 2 days, upon which a gradual increase in apoCI was observed reaching control levels after 28 days (5.8 ± 1.1 mg/dL). When non-survivors were compared to survivors, apoCI levels tended to be lower in non-survivors in the first few days of the study (Fig. 1B). Interestingly, plasma apoCI levels remained low in non-survivors, whereas apoCI levels recovered in survivors to normal levels within 4 weeks, reaching statistical difference between survivors and non-survivors on day 7 and 14.

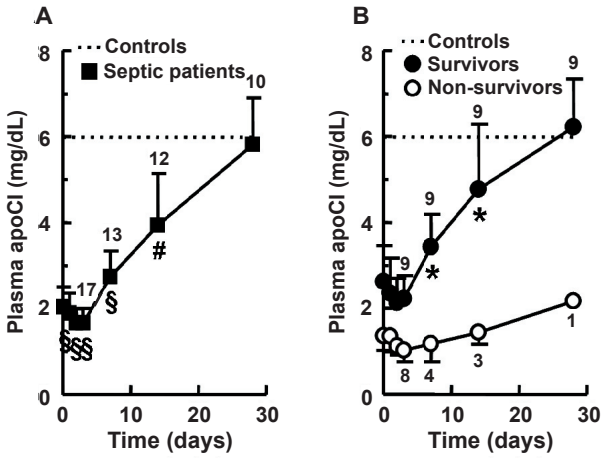

Figure 1. High plasma apoCI is associated with increased survival of patients with severe sepsis. ApoCI levels were determined in plasma of 17 patients with severe sepsis (squares) during 28 days after diagnosis (A). Patients are divided in 30-day-survivors (closed circles; n=9) and non-survivors (open circles; n=8) (B). Dotted lines indicate apoCI levels in healthy subjects (n=18). Inserted numbers represent patient numbers at the individual time points. Values indicate means ± SEM. Statistical differences were assessed between septic patients and healthy subjects ($^{\#}P$<0.01, $^{\S}P$<0.0001; (A)), and between survivors and non-survivors ($^{*}P$<0.05; (B)).

Plasma Lipid Levels − Such a difference between survivors and non-survivors could not be detected for total cholesterol (Fig. 2A). Likewise, no differences with respect to LDL-cholesterol (Fig. 2B) and HDL-cholesterol (Fig. 2C) were found between survivors and non-survivors. Triglycerides tended to be increased in non-survivors (Fig. 2D), albeit that statistical significance was not reached.

Adjusted Apolipoprotein Levels − Next, we examined the lipoprotein distribution of apoCI at day 3 in survivors and non-survivors of severe sepsis. The majority of plasma apoCI in survivors could be found in the HDL-fraction (approx. 80%), and only a minor part in the VLDL and LDL fractions (Fig. 3). Interestingly, the lipoprotein distribution of apoCI in the non-survivors differed from the survivors

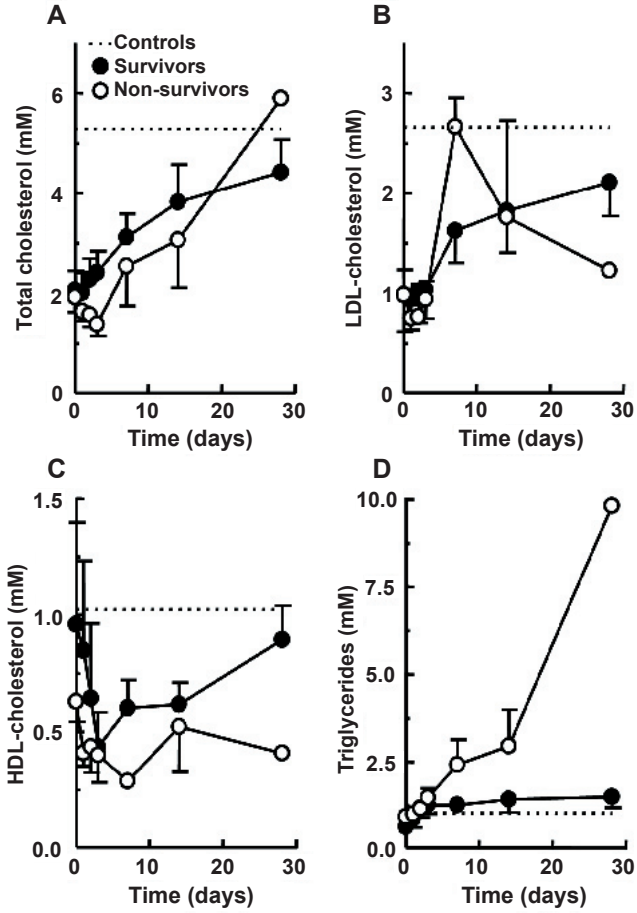

Figure 2. Plasma lipids do not correlate with survival in patients with severe sepsis. Blood was collected from critically ill 30-day-survivors (closed circles; n=9) and non-survivors (open circles; n=8). The plasma concentrations of total cholesterol (A), HDL-cholesterol (B), LDL-cholesterol (C), and triglycerides (D) were determined. Dotted lines indicate levels in healthy subjects (n=18). Values indicate means ± SEM.

in that the relative VLDL-associated apoCI fraction increased 7.8-fold (61.9 ± 5.6 vs. 8.0 ± 7.0% in survivors; *P*<0.05), whereas the HDL-associated apoCI was significantly decreased by a factor of 2.8 (30.2 ± 5.5 vs. 83.4 ± 12.8% in survivors; *P*<0.05). The apoCI content found in the LDL fraction was similar in survivors and non-survivors (8.7 ± 5.9 and 8.3 ± 2.9%, respectively). Since apoCI is present on all lipoproteins, we adjusted the plasma apoCI levels for common lipoprotein core lipid levels (*i.e.* triglycerides and cholesterol). As shown in Fig. 4, the predictive value of apoCI in determining the outcome of severe sepsis persisted after adjustment. In fact, the difference between survivors and non-survivors was even enlarged, indicating that the changes in apoCI levels are not simply due to alterations in lipoprotein levels.

To examine whether this effect is specific for apoCI and cannot be found for other plasma apolipoproteins, we also studied plasma levels of the main protein constituent of HDL (*i.e.* apoAI), VLDL and LDL (*i.e.* apoB), and a structurally closely related apolipoprotein (*i.e.* apoCIII) between survivors and non-survivors. The plasma levels of apoAI and apoB were adjusted for HDL-cholesterol and LDL-cholesterol, respectively. As shown in Figs. 5A and 5B no differences were found between survivors and non-survivors. Since apoCIII showed a similar lipoprotein distribution pattern as apoCI, with a distribution over HDL (83.9 ±

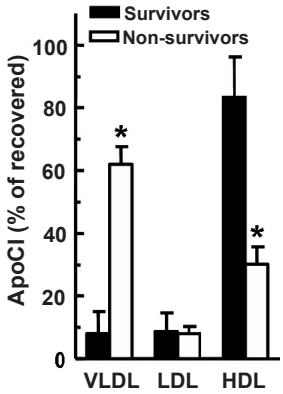

Figure 3. The lipoprotein distribution of apoCI differs between survivors and non-survivors of severe sepsis. On day 3, the plasma lipoproteins of survivors (black bar; n=3) and non-survivors (white bar; n=3) were separated with fast performance liquid chromatography and apoCI was determined in the different lipoprotein fractions. Values indicate mean percentages of total recovered apoCI ± SEM. **P*<0.05, significant difference between survivors and non-survivors.

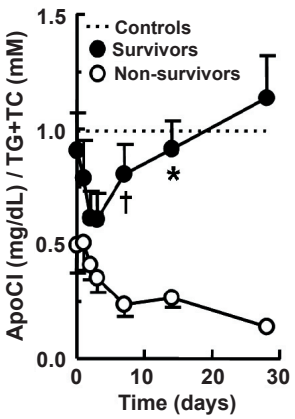

Figure 4. Plasma apoCI is associated with increased survival of patients with severe sepsis independent of lipoprotein core lipids. The plasma concentration of apoCI in critically ill survivors (closed circles; n=9) and non-survivors (open circles; n=8) was adjusted for triglycerides (TG) and total cholesterol (TC). Dotted lines indicate levels in healthy subjects (n=18). Values indicate means ± SEM. **P*<0.05, †*P*<0.01, significant difference between survivors and non-survivors.

8.7 and 48.5 ± 25.8%), LDL (11.7 ± 8.2 and 15.3 ± 7.8%), and VLDL (4.4 ± 0.5 and 36.2 ± 18.3%) in survivors and non-survivors, respectively, plasma apoCIII levels were adjusted for lipoprotein core lipid levels (triglycerides and cholesterol) similarly as apoCI, but again no differences were found between survivors and non-survivors (Fig. 5C). These results indicate that apoCI, and not apoAI, apoB and apoCIII, is a major determinant for survival in patients with severe sepsis.

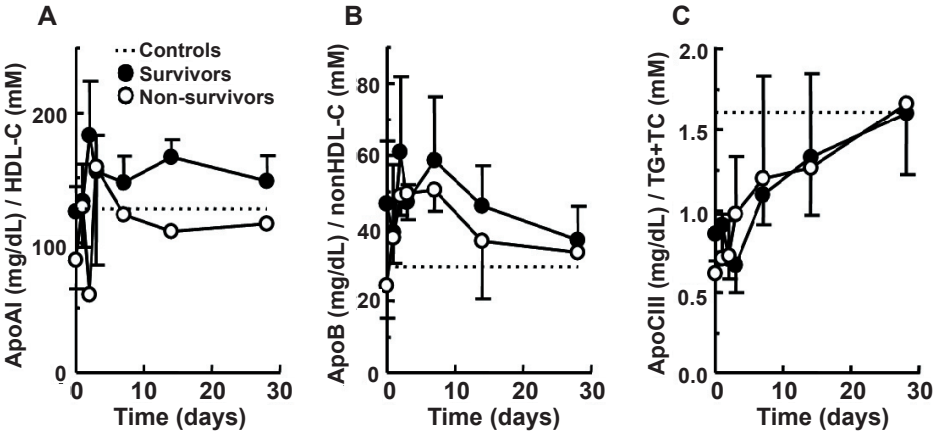

Figure 5. Plasma apoAI, apoB, and apoCIII do not correlate with survival in patients with severe sepsis. The plasma concentration of apoAI (A), apoB (B), and apoCIII (C), adjusted for their lipoprotein lipids, were determined in critically ill survivors (closed circles; n=9) and non-survivors (open circles; n=8). Dotted lines indicate levels in healthy subjects (n=18). Values indicate means ± SEM. HDL-C, HDL-cholesterol; nonHDL-C, nonHDL-cholesterol; TG, triglycerides; TC, total cholesterol.

Discussion

This is the first study to examine plasma apoCI levels in patients with severe sepsis and to determine its prognostic value in the survival outcome. We found largely reduced plasma apoCI levels in patients with severe sepsis upon entry in the study. In 30-day-survivors, apoCI levels recovered within 4 weeks to levels observed in healthy subjects, whereas in non-survivors the apoCI levels remained low. Since this marked difference between survivors and non-survivors could not be found for plasma lipid levels and other apolipoproteins (*i.e.* apoAI, apoB, and apoCIII), we conclude that high plasma apoCI levels are a predictor of survival in patients with severe sepsis.

The decreased plasma apoCI levels as found in the present study are in accordance with findings of Barlage *et al.*[3], who showed that apoCI was virtually absent from HDL during sepsis, suggestive of a concomitant decrease in plasma levels of apoCI. Furthermore, our findings indicate that apoCI may act as a negative acute phase protein during bacterial sepsis.

There are three physiologically relevant processes which would benefit from the observed decrease in apoCI levels. First, the decrease in apoCI may be an attempt of the host to protect itself against an overwhelming cytokine response. We recently found that apoCI induces an increased proinflammatory response against lipopolysaccharide (LPS), the main toxic constituent of Gram-negative bacteria, and against invading bacteria[19]. In the early phase of bacterial infection, an increased proinflammatory response is crucial to combat the infection[25]. Therefore, high apoCI levels will be beneficial in surmounting the first phase of bacterial sepsis, but an increased proinflammatory response may be detrimental once the infection has progressed into sepsis. The host may thus attempt to lower the overwhelming cytokine response by decreasing apoCI levels.

Second, decreasing apoCI levels may help to improve the liberation of free fatty acids (FFA) from circulating triglycerides as an energy source for peripheral cells during sepsis. During sepsis reduced levels of lipoprotein lipase (the rate limiting enzyme of plasma triglyceride lipolysis) have been observed[11,26-28], suggestive for decreased triglyceride lipolysis. However, it has been shown that during sepsis FFA generation and oxidation from VLDL-derived triglycerides in peripheral tissues is actually increased, and there is evidence that FFA are the preferred energy source during sepsis[29-32]. Since we and others have shown that apoCI is a potent inhibitor of FFA generation by blocking triglyceride lipolysis by lipoprotein lipase[23,33] and hepatic lipase[34,35], the reduced apoCI levels during sepsis may help to maintain efficient triglyceride lipolysis, despite lower lipoprotein lipase levels, and subsequently increased FFA availability.

Third, decreasing apoCI levels may help to improve the uptake of lipoproteins by peripheral tissues to satisfy an increased demand for phospholipids, suggested by Barlage *et al.*[3]. These phospholipids may be used for the regeneration of damaged cellular membranes of epithelial and endothelial cells. Work from several groups, including ours, have shown that apoCI is a well known inhibitor of lipoprotein particle uptake by the very-low-density lipoprotein (VLDL) receptor[36], the LDL receptor[37], and the LDL receptor related protein (LRP)[36,38,39]. The concomitantly increased processing of the lipoprotein particles by lipases as described above will further add to an increased uptake of the subsequently generated lipoprotein remnants. Since phospholipids are a major constituent of lipoproteins, decreased apoCI levels may result in increased lipoprotein particle uptake with a concomitantly increased phospholipid uptake.

The reduced apoCI levels during sepsis may thus be beneficial for several physiologic processes. The question emerges with respect to the molecular mechanism responsible for this reduction. A plausible mechanism may be related to serum amyloid A (SAA), an acute phase protein, which is highly upregulated

during sepsis, and is able to displace apolipoproteins from HDL particles[40-42]. ApoCI, which primarily resides on HDL in healthy subjects[17,18,43], may be displaced by SAA, hereby forcing apoCI to reside on VLDL particles. Since the plasma turnover of VLDL-associated apoCI is much faster than for HDL-associated apoCI[44], the increased presence of HDL-derived apoCI on VLDL particles will lead to increased apoCI catabolism, and may consequently result in decreased plasma apoCI levels.

Evidently, further studies are required to elucidate both the physiological relevance and the mechanism(s) responsible for these decreased plasma apoCI levels. In addition, it would be interesting to study during which phase of infection/sepsis the decrease in apoCI levels is initiated, since septic patients have already decreased levels when they enter the hospital. Does it occur directly in the first phase of infection, or does it occur when the primary attack towards the bacteria was insufficient and the infection progresses into sepsis?

Interestingly, plasma apoCI levels were lower in non-survivors as compared to survivors of severe sepsis, and adjustment of the plasma apoCI levels for lipoprotein lipids even enlarged this difference. Such an effect could not be found for other lipid parameters (*i.e.* total cholesterol, LDL-cholesterol, HDL-cholesterol, and triglycerides) and apolipoproteins (*i.e.* apoAI, apoB, and apoCIII). Only a few earlier studies have correlated the lipoprotein and/or apolipoprotein plasma levels with the outcome of severe sepsis[4,45,46]. Although in all of these studies LDL-cholesterol and HDL-cholesterol were lower in septic patients as compared to the controls, the general conclusion was that both LDL-cholesterol and HDL-cholesterol could not predict the outcome of severe sepsis, findings that we confirmed in our study. To our knowledge there is only one study that did show small differences between survivors and non-survivors. Chien *et al*.[47] recently observed that non-survivors had slightly decreased HDL-cholesterol and a concomitant decrease in apoAI levels as compared to survivors. However, they still concluded that serum levels of HDL-cholesterol were a poor prognostic factor for the outcome of severe sepsis. In addition, similarly to our study, they did not find any differences in plasma LDL-cholesterol and apoB levels between survivors and non-survivors. Although future studies in larger populations will have to confirm our current findings, so far apoCI appears to be the only lipoprotein parameter that predicts survival from severe sepsis.

In conclusion, plasma apoCI levels are markedly decreased in patients with severe sepsis upon hospitalization. The mechanism behind these decreased apoCI levels remains unsolved as yet, but may well be an attempt of the host to protect itself against an overwhelming apoCI-induced cytokine response as related to the recently identified proinflammatory actions of apoCI during

bacterial invasion. In non-survivors of severe sepsis plasma apoCI levels were lower upon hospitalization as compared to survivors and remained low, whereas in survivors apoCI levels recovered to normal levels within 4 weeks. Differences in lipoprotein levels could not account for this observation, since adjustment of apoCI levels for neutral lipoprotein lipids levels even enlarged the difference between survivors and non-survivors. Therefore, a high plasma apoCI level is a major prognostic factor for survival in patients with severe sepsis.

Acknowledgements

This study was supported in part by the Netherlands Organization for Scientific Research (NWO RIDE 014-90-001 to LMH and NWO VIDI 917-36-351 to PCNR), by the Netherlands Heart Foundation (NHS 2005B226 to PCNR), and by the Leiden University Medical Center (Gisela Thier Fellowship to PCNR). We thank Elly C.M. de Wit from TNO-Quality of Life for excellent technical assistance.

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