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Apolipoprotein A-V is not a major determinant of triglyceride levels during human sepsis☆,☆☆

Kanchana Ngaosuwan, MD, Natnicha Houngngam, MSc, Pichapa Limpisook, MD, Wanee Plengpanich, PhD, Weerapan Khovidhunkit, MD, PhD*

Hormonal and Metabolic Disorders Research Unit, Excellence Center for Diabetes, Hormone, and Metabolism, and Division of Endocrinology and Metabolism, Department of Medicine, Faculty of Medicine, Chulalongkorn University, and King Chulalongkorn Memorial Hospital, Thai Red Cross Society, Patumwan, Bangkok 10330, Thailand

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ABSTRACT

Purpose: During critical illnesses, alterations in lipid metabolism occur. We examined levels of apolipoprotein A-V, a novel regulator of triglyceride metabolism, during sepsis in humans.
Methods: Seventy-five cases of sepsis and 75 cases of acute illnesses not associated with infection were recruited. Lipids and apolipoprotein A-V levels were measured by enzymatic methods and enzyme-linked immunosorbent assay, respectively, within 24 hours of diagnosis. Fifty healthy controls were also enrolled.
Results: During sepsis and acute illnesses, serum total cholesterol and high-density lipoprotein cholesterol levels were significantly lower than those in controls. Serum triglyceride levels, however, were not significantly different. Similarly, serum apolipoprotein A-V levels during sepsis were not significantly different from those during acute illnesses and those in controls (expressed as median [interquartile range]: 149.6 [97.5-257.1] vs 157.9 [98.4-238.2] and 155.9 [91.5-253.8] ng/mL, respectively; $P = .98$); and they were not correlated with serum triglyceride levels. Low apolipoprotein A-V levels were associated with higher mortality, but the association became nonsignificant after adjusting for high-density lipoprotein cholesterol levels.
Conclusions: During sepsis or acute illnesses, serum apolipoprotein A-V levels were not significantly different from those in controls. Furthermore, apolipoprotein A-V levels were not linearly correlated with triglyceride levels, suggesting that it might not be a major determinant of triglyceride levels during sepsis.

1. Introduction

Sepsis is associated with multiple changes in lipid and lipoprotein metabolism [1,2], which typically encompass a rapid decrease in high-density lipoprotein (HDL) cholesterol levels and a delayed increase in triglyceride levels [3]. Alterations in several apolipoproteins also occur during sepsis, such as a decrease in apolipoprotein (apo) A-I and apo A-II and an increase in apo J. The underlying mechanisms and consequences of these changes are complex and not well understood. Nevertheless, changes in lipids and lipoprotein metabolism during sepsis are thought to be part of the acute-phase response; and lipoproteins have been shown to be one of the key players in innate immunity [1].

In patients with sepsis, there is a marked decrease in serum levels of HDL cholesterol and apo A-I; and several mechanisms have been proposed to contribute to these changes, including a decrease in ATP-binding cassette A1 [4], a key protein involved in cellular cholesterol efflux; a reduction in lecithin-cholesterol acyltransferase [5–7], a crucial enzyme in cholesterol esterification in lipoproteins; and an increase in secretory phospholipase A_2 [7], an enzyme that hydrolyzes phospholipids from HDL. Furthermore, circulating HDL during sepsis, also known as *acute-phase HDL*, exhibits different composition and functions from normal HDL. As a result, impairment in HDL-mediated cholesterol efflux and reverse cholesterol transport has been demonstrated during infection/inflammation [7–9]. These changes are thought to conserve cholesterol at peripheral sites, such as lymphocytes and macrophages, because extra cholesterol may be needed for lymphocyte activation and new membrane synthesis at the site of cellular injuries [10].

Triglyceride metabolism is closely linked to HDL metabolism as evidenced by an inverse correlation between triglyceride and HDL cholesterol levels in several conditions. During infection, circulating triglyceride levels are increased because of increased hepatic very low-density lipoprotein (VLDL) production and/or decreased triglyceride clearance

Abbreviations: apo, apolipoprotein; HDL, high-density lipoprotein; VLDL, very low-density lipoprotein.

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* Corresponding author. Hormonal and Metabolic Disorders Research Unit and Division of Endocrinology and Metabolism, Department of Medicine, Faculty of Medicine, Chulalongkorn University, Samakkee Payabarn Bldg, 2nd floor, King Chulalongkorn Memorial Hospital, Rama IV Rd, Patumwan, Bangkok 10330, Thailand. Tel.: +66 11 662 256 4101; fax: +66 11 662 652 5347.
E-mail address: wkhovid@gmail.com (W. Khovidhunkit).

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[11]. Both an increase in hepatic synthesis of triglyceride and a decrease in an activity of lipoprotein lipase, a pivotal enzyme in triglyceride hydrolysis, have been documented during infection [11–13]. An increase in triglyceride levels during sepsis might have beneficial effects on the host because triglyceride-rich lipoproteins, especially chylomicron and VLDL, have been shown to bind and neutralize the biological effects of endotoxin and protect animals against death from sepsis [14,15]. These data suggest that lipoproteins act as part of innate immunity providing immediate protection against infection. In addition to the lipid moieties of lipoproteins, apolipoproteins have also been shown to be responsible for the protective effects of lipoproteins [16–18].

Recently, apo A-V has been identified as a novel regulator of triglyceride metabolism. In the circulation, apo A-V is present on both triglyceride-rich lipoproteins and HDL particles. Deficiency of apo A-V, both in mice and in humans, leads to high triglyceride levels [19]. Apolipoprotein A-V may modulate triglyceride levels by inhibiting hepatic VLDL production and/or enhancing triglyceride clearance via lipoprotein lipase [20]. A previous study in rodents has shown that hepatic apo A-V mRNA expression was upregulated in response to endotoxin injection, and apo A-V protein was found to be increased in acute-phase HDL [21]. Investigations of how apo A-V is regulated during human sepsis may, therefore, provide mechanistic insights into the pathophysiology of triglyceride metabolism during critical illnesses. In our current study, we measured serum apo A-V levels in a large number of patients with confirmed sepsis and compared these with those in patients with acute illnesses not related to infection and in control subjects. On one hand, apo A-V levels might be increased during human sepsis as previously shown in mice. On the other hand, changes in apo A-V levels may be species specific; and a decrease in apo A-V levels might be observed, which could potentially explain an increase in triglyceride levels during human sepsis.

2. Materials and methods

2.1. Study subjects

We identified adult patients with sepsis at King Chulalongkorn Memorial Hospital using a list of potential subjects who had positive blood culture within 48 hours after incubation. *Sepsis* was defined as systemic inflammatory response syndrome in response to an infectious process according to the American College of Chest Physicians/Society of Critical Care Medicine Consensus Conference Committee [22]. We excluded subjects whose results of blood cultures were suspected to be contaminated. Written informed consent was obtained individually from the patient or their caregivers. The medical records were reviewed for clinical characteristics and the results of biochemical measurement. Blood samples that were collected at the same time of blood culture or within 24 hours after an initial collection and stored at 4°C at the main clinical pathology laboratory were retrieved. We chose a 24-hour time frame because our previous study in mice showed that apo A-V expression was rapidly increased at 8 hours of infectious stimuli and the circulating level of apo A-V was substantially increased at 16 hours [21]. Sera were prepared from the blood samples and stored at −20°C until assayed for lipids and apo A-V levels. A total of 75 subjects were enrolled.

To examine whether changes in apo A-V levels during sepsis was specifically due to infection or could occur in other critical illnesses as part of the acute-phase response, 75 age- and sex-matched subjects who were admitted for acute illnesses not related to infection were also recruited. All of these subjects had no clinical evidence of infection and did not receive any antibiotics within 48 hours after diagnosis. After an informed consent was obtained, blood samples collected within the first 24 hours of diagnosis were also retrieved from the main laboratory; and prepared sera were stored at −20°C until assayed. Conditions that might have affected apo A-V levels were used as exclusion criteria, such as history of severe hypertriglyceridemia (triglyceride levels >400 mg/dL), fibrate therapy within 2 weeks before enrollment, hypothyroidism, hyperthyroidism, L-thyroxine therapy, hepatitis (alanine aminotransferase and/or aspartate aminotransferase levels >200 IU/L), cirrhosis, and total bilirubin levels greater than 5 mg/dL. All subjects were followed until discharge from the hospital. Fifty healthy ambulatory subjects who were not receiving lipid-lowering therapy were recruited as controls. All subjects provided written inform consent, and the study protocol was approved by our Ethics Committee. The study was performed according to the Declaration of Helsinki for experiments involving humans.

2.2. Biochemical measurements

Lipid levels were measured using enzymatic methods in an automated system (Roche Diagnostics). Apolipoprotein A-V levels were measured using commercially available enzyme-linked immunosorbent assay (ELISA) (EMD Millipore, St Charles, MO, and Cusabio, China). We had also validated the ELISA by quantitative Western blotting using a monoclonal antibody against human apo A-V (sc-33081 from Santa Cruz Biotechnology, Dallas, TX). A significant positive correlation between apo A-V levels determined by ELISA and Western blotting was found ($r = 0.62$, $P = .03$).

2.3. Statistical analysis

SPSS (Chicago, IL) version 16 was used to perform statistical analyses. Data were presented as mean ± standard deviation for variables with normal distribution and median (interquartile range) for variables with a skewed distribution. Differences between 2 unrelated groups were compared using Student *t* test or Mann-Whitney test, where appropriate, for continuous variables and χ^2 test for categorical variables. Differences between 2 related groups were compared using Wilcoxon signed rank test. Comparisons among multiple groups were assessed using analysis of variance with the post hoc Bonferroni procedure for variables with normal distribution or Kruskal-Wallis test for variables with a skewed distribution. *P* values were 2-sided, and we used Sidak correction to adjust the *P* values for multiple testing involved. Correlations were investigated using Spearman correlation. Multivariate logistic regression analysis was performed to calculate the odds ratio of mortality of patients between different tertiles. Receiver operating characteristic curves were determined for serum apo A-V and other lipid levels.

3. Results

3.1. Clinical characteristics of the study subjects

The result from blood cultures showed that, among 75 patients with sepsis, 64 (85%) had gram-negative infection, whereas 8 (11%) had gram-positive infection and 3(4%) had polymicrobial infection. The most common organism identified was *Escherichia coli* (40%). Among 75 patients admitted for acute illnesses not related to infection, 30 (40%) had cardiovascular disease, 10 (13%) had congestive heart failure, 9 (12%) had acute hemorrhage, 5 (7%) had dysglycemic crisis, and 21 (29%) had other diagnoses.

Clinical characteristics of the study subjects are shown in Table 1. Patients with sepsis had significantly lower blood pressure and significantly higher body temperature, pulse rate, and respiratory rate compared to those with acute illnesses. In addition, patients with sepsis also had significantly lower hematocrit and platelet count but higher white blood cell count.

3.2. Lipids and apo A-V levels

Within 24 hours after diagnosis of sepsis or acute illnesses, blood samples were collected for measurement of lipid levels. We found that total cholesterol and HDL cholesterol levels were lowest in patients