Brief report

Impaired high-density lipoprotein function and endothelial barrier stability in severe anaphylaxis

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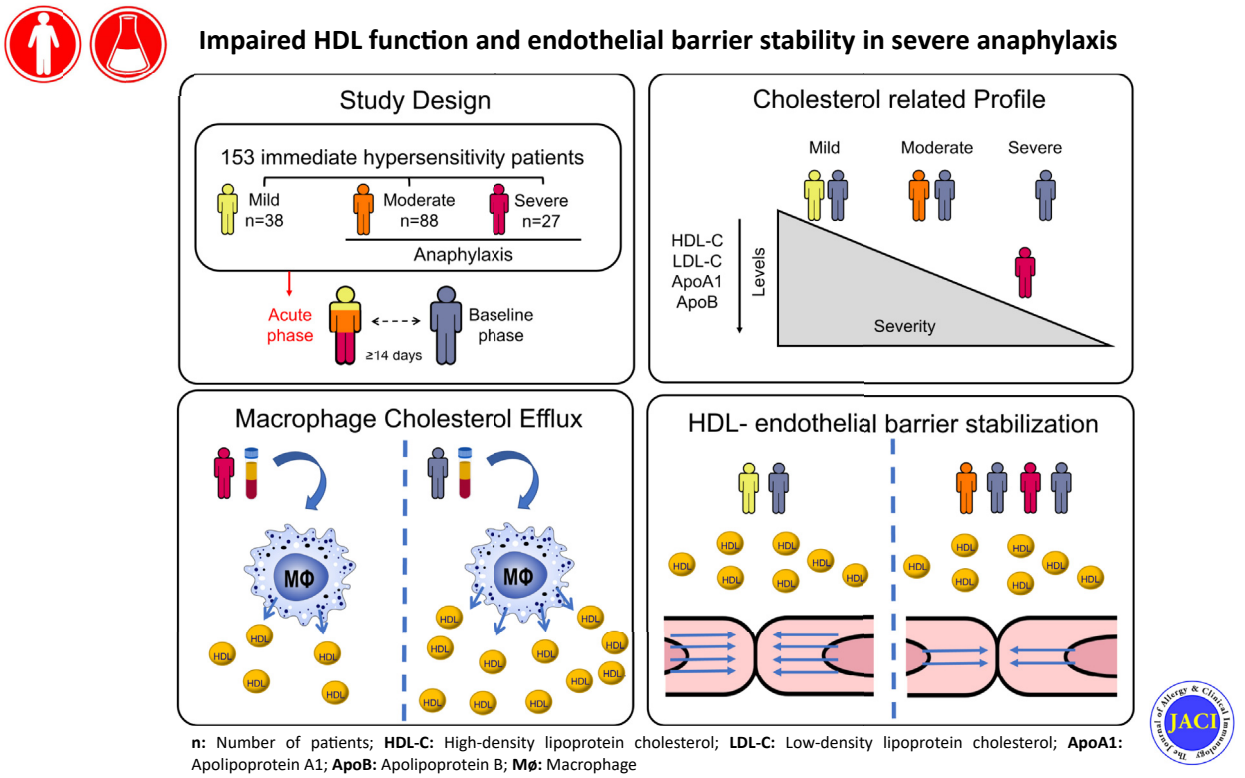
GRAPHICAL ABSTRACT



Capsule summary: Low serum levels of apolipoprotein and lipoprotein are observed in severe anaphylactic reactions. Specifically, the poor capacity of high-density lipoprotein to induce macrophage cholesterol efflux and endothelial barrier stability encourages further research, potentially leading to new diagnostic and therapeutic approaches.

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Impaired high-density lipoprotein function and endothelial barrier stability in severe anaphylaxis

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Background: Growing evidence demonstrates the importance of high- and low-density lipoprotein cholesterol in certain immune and allergy-mediated diseases.

Objective: This study aimed to evaluate levels of high- and low-density lipoprotein cholesterol and apolipoproteins A1 and B in sera from a cohort of patients presenting with hypersensitivity reactions. We further assessed the function of high-density lipoprotein particles as well as their involvement in the molecular mechanisms of anaphylaxis.

Methods: Lipid profile determination was performed in paired (acute and baseline) serum samples from 153 patients. Thirty-eight experienced a non-anaphylactic reaction and 115 had an anaphylactic reaction (88 moderate and 27 severe). Lecithin cholesterol acyl transferase activity was assessed in patient sera, and we also evaluated macrophage cholesterol efflux in response to the serum samples. Last, the effect of anaphylactic-derived high-density lipoprotein (HDL) particles on the endothelial barrier was studied. Detailed methods are provided in the Methods section in this article's Online Repository available at www.jacionline.org.

Abbreviations used
Apo: Apolipoprotein
HDL: High-density lipoprotein
HDL-C: HDL lipoprotein cholesterol
LCAT: Lecithin cholesterol acyl transferase
LDL: Low-density lipoprotein
LDL-C: LDL cholesterol
MCE: Macrophage cholesterol efflux

Results: Serum samples from severe anaphylactic reactions show statistically significant low levels of HDL cholesterol, low-density lipoprotein cholesterol, and apolipoproteins A1 and B, which points to their possible role as biomarkers. Specifically, HDL particles play a protective role in cardiovascular diseases. Using functional human serum cell assays, we observed impaired capacity of apolipoprotein B–depleted serum to induce macrophage cholesterol efflux in severe anaphylactic reactions. In addition, purified HDL particles from human anaphylactic sera failed to stabilize and maintain the endothelial barrier.

Conclusion: These results encourage further research on HDL functions in severe anaphylaxis, which may lead to new diagnostic and therapeutic strategies. (J Allergy Clin Immunol 2024;154:827-32.)

Key words: *Anaphylaxis, high-density lipoprotein cholesterol, macrophage cholesterol efflux, vascular permeability*

INTRODUCTION

Molecular and clinical findings reveal an association between allergic and cardiovascular diseases.[1] Traditionally, high- and low-density lipoprotein cholesterol (HDL-C and LDL-C, respectively) levels have been considered causal risk factors for cardiovascular diseases.[2] Recent evidence suggests that these lipoproteins and their major apolipoproteins might be associated with the development of immune-mediated diseases (rheumatoid arthritis, multiple sclerosis), inflammatory disorders (sepsis), and allergic diseases such as asthma or atopic dermatitis.[3-5] Specifically, a decrease in the levels of apolipoprotein (Apo) A1 and ApoB, the major protein moieties of HDL and LDL, respectively, has been observed in anaphylactic sera from allergic patients.[6,7] However, there is insufficient evidence supporting the molecular

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TABLE I. Characteristics of patients and their reactions

Characteristic	Patients			
	Total	Grade 1	Grade 2	Grade 3
No. of subjects	153	38	88	27
Sex*	62%	26%	52%	17%
Age (years) mean ± SD	39.05 ± 18.19	46.02 ± 18.77	35.77 ± 16.72	42.48 ± 19.49
Trigger (%)				
Food	29	0	39	37
Drug	66	100	53	59
Other	5	0	8	4
Symptom (%)				
Cutaneous	87	92	86	93
Mucosal	48	42	55	44
Gastrointestinal	37	0	48	56
Respiratory	63	0	85	78
Neurological	18	0	18	37
Cardiovascular	22	0	10	85
Treatment (%)†	61	24	73	78
Epinephrine	63	11	64	81
H1R antagonist	86	100	88	76
H2R antagonist	15	11	13	29
Corticosteroids	88	100	86	71
β_2-Adrenergic agonist	15	0	17	14

H1R, H_1 receptor; *H2R*, H_2 receptor.
*Percentage of female patients.
†Percentage of patients treated before acute-phase sample was collected; each column shows percentage of those patients treated with each specific drug.

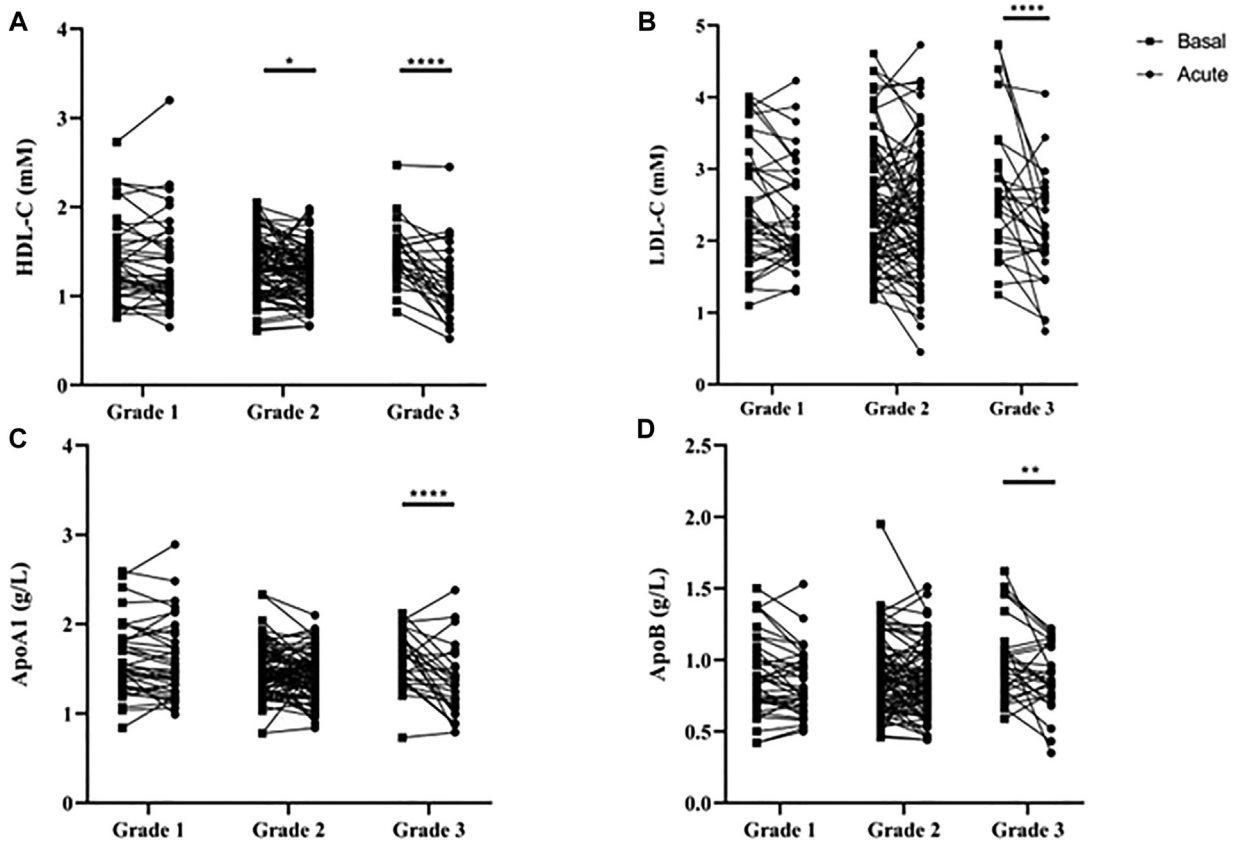

FIG 1. Serum from severe anaphylactic reactions exhibits low levels of HDL-C, LDL-C, ApoA1, and ApoB. Bulk values of **(A)** HDL-C (mM), **(B)** LDL-C (mM), **(C)** ApoA1 (g/L), and **(D)** ApoB (g/L) measured in paired serum samples of 38 non-anaphylactic reactions (grade 1) and 115 anaphylactic reactions. Of these reactions, 88 were moderate (grade 2) and 27 were severe (grade 3). *$P = .0187$, **$P = .0046$, ****$P < .0001$. Šídák multiple comparison test was used.

TABLE II. Effect of treatment and triggers on lipid profiles during acute reaction phase

Characteristic	Treatment		Trigger	
	Treated	Untreated	Food	Drug
HDL-C (mM)	1.185 (0.995-1.410)	1.230 (1.043-1.505)	1.200 (1.008-1.515)	1230 (1.010-1.460)
P value	.2382	.9317		
LDL-C (mM)	2.150 (1.830-2.728)	2.220 (1.780-2.940)	2.120 (1.583-2.673)	2.20 (1.840-2.840)
P value	.7387	.0844		
ApoA1 (g/L)	1.390 (1.188-1.590)	1.460 (1.300-1.620)	1.445 (1.288-1.628)	1.400 (1.210-1.590)
P value	.2651	.7150		
ApoB (g/L)	0.830 (0.720-0.967)	0.790 (0.640-1.020)	0.795 (0.6375-0.9325)	0.830 (0.720-1.030)
P value	.1956	.1523		
No. of measured samples	92	59	46	99

Values in parentheses are the 25 and 75% percentiles, respectively. Values not in parentheses are the median of the values of each group.

involvement of HDL in allergic hypersensitivity. Here, we aim to evaluate the levels of HDL-C, LDL-C, ApoA1, and ApoB in a heterogeneous cohort of patients who experienced mild, moderate, or severe immediate allergic hypersensitivity reactions induced by different triggers, mainly food or drugs. We further evaluate 2 major functions of HDL and their involvement in the molecular mechanisms of anaphylaxis.

RESULTS AND DISCUSSION

We studied 153 patients with immediate hypersensitivity reactions, 38 of whom experienced non-anaphylactic reactions (grade 1) and 115 of whom experienced anaphylactic reactions (88 moderate or grade 2, 27 severe or grade 3) according to the Brown classification system (Table I, and see the Methods section in this article's Online Repository available at www.jacionline.org). Grade 1 reactions only involved the integumentary system. Anaphylactic reactions (moderate or severe) with 2 or more systems affected were graded depending on the patient's specific symptoms. Paired serum samples collected from each patient during the acute reaction and at baseline (≥14 days) were analyzed. A significant reduction of HDL-C, LDL-C, ApoA1, and ApoB levels was observed in acute-phase samples from patients with severe anaphylactic reactions (grade 3) (Fig 1) compared to their baseline levels. These parameters were unable to distinguish between non-anaphylactic (grade 1) and anaphylactic reactions across all patients. Acute lipid profile was not influenced by either the trigger of the reaction or the treatment received (Table II).

An increase in extravasation is one of the likely reasons for the decrease in plasma levels observed in severe anaphylaxis.[8] In addition, the degradation of ApoA1 in the nascent HDL particles caused by the action of proteolytic mediators released by mast cells has been previously reported in experimental models.[9] Stimulation of cholesterol efflux from macrophages, regarded as the main atheroprotective function of HDL, initiates reverse cholesterol transport to the liver and intestine for excretion. The conversion of discoidal nascent HDL into spherical, mature HDL primarily occurs through the cholesterol esterification process facilitated by the enzyme lecithin cholesterol acyltransferase (LCAT). In our analysis, we assessed the capacity of anaphylactic ApoB-depleted sera, which contain nascent and mature HDL, as well as HDL regulatory proteins like LCAT, to stimulate macrophage cholesterol efflux (MCE). We also measured their LCAT activity. Interestingly, we observed no changes in LCAT activity (Fig 2, *A*). However, marked impairment was observed in the ability of ApoB-depleted serum to induce MCE in the severe reactions group (Fig 2, *B*). Moreover, no functional alterations were observed when we measured the capability of purified mature HDL to induce MCE (Fig 2, *C*). Consistent with these findings, there was a positive correlation between the decrease in serum levels of HDL-C and ApoA1 and the capacity of ApoB-depleted serum to induce MCE, thereby emphasizing that imbalanced circulating levels of HDL disrupt cholesterol metabolism in macrophages during anaphylaxis (Fig 2, *D*).

The quantity of HDL particles in blood appears to be overlooked in current research; instead, emphasis is placed on particle structure and composition. HDL particles contribute stability to the endothelium, which plays an essential role in anaphylaxis due to the loss of cell adherence caused mainly by the effect of vasoactive mediators, which in turn increase permeability.[8] Interestingly, we observed a loss of endothelial resistance in human dermal microvascular endothelial cells when exposed to purified HDL particles from the serum of moderate and severe anaphylaxis patients when compared to particles derived from non-anaphylactic reactions (Fig 3).

In summary, sera from patients presenting with severe anaphylactic reactions show lower levels of HDL and LDL particles and an impaired capacity for MCE. In addition, anaphylactic HDL particles are deficient in stabilizing and maintaining the endothelial barrier during anaphylaxis.

DISCLOSURE STATEMENT

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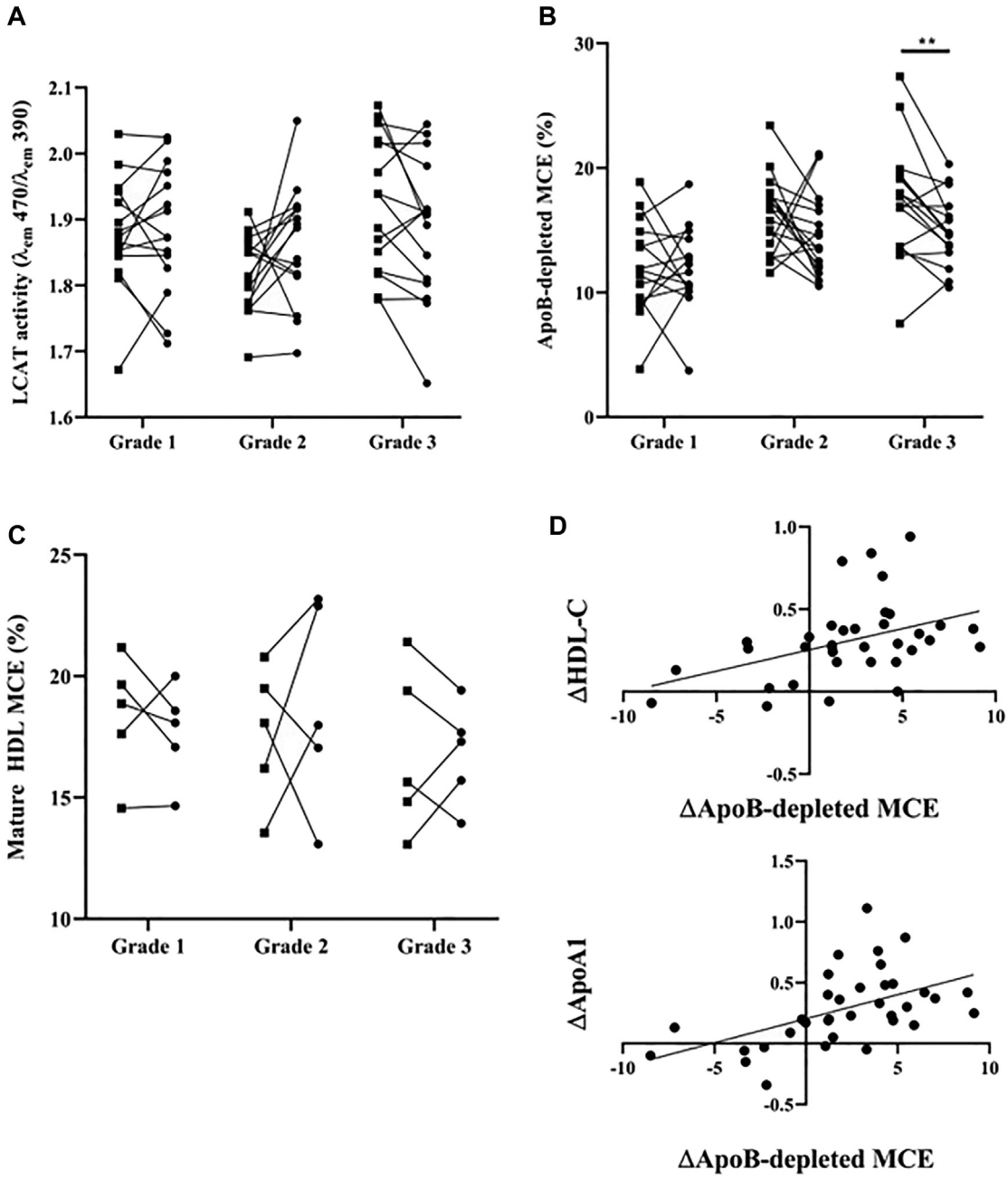

FIG 2. Serum from severe anaphylactic reactions displayed impaired capacity of HDL to induce MCE. **(A)** LCAT activity was measured in 15 paired serum samples of non-anaphylactic reactions (grade 1) and 30 anaphylactic reactions (15 grade 2 and 15 grade 3). Šídák multiple comparison test was used. **(B)** Evaluation in macrophages stimulated with 20 paired ApoB-depleted serum samples from non-anaphylactic reactions (grade 1) and 35 paired ApoB-depleted anaphylactic serum samples (18 grade 2 and 17 grade 3). $^{**}P = .01$. Student *t* test using paired samples was performed. **(C)** MCE was assessed in macrophages stimulated with purified mature HDL from paired serum samples of 5 non-anaphylactic reactions (grade 1) and 10 anaphylactic reactions (5 grade 2 and 5 grade 3). Šídák multiple comparison test was used. **(D)** Positive ΔHDL-C and ΔApoA1 correlation with ΔApoB-depleted MCE. ΔHDL-C–ΔApoB-depleted MCE ($R = 0.4565$, $^{**}P = .005839$) and ΔApoA1–ΔApoB-depleted MCE correlation ($R = 0.5114$, $^{**}P = .001691$) in paired serum samples of 20 non-anaphylactic reactions (grade 1) and 35 anaphylactic reactions (18 grade 2 and 17 grade 3). Spearman nonparametric (for ΔHDL-C with ΔApoB-depleted MCE) and Pearson parametric (for ΔApoA1 with ΔApoB-depleted MCE) tests were used.

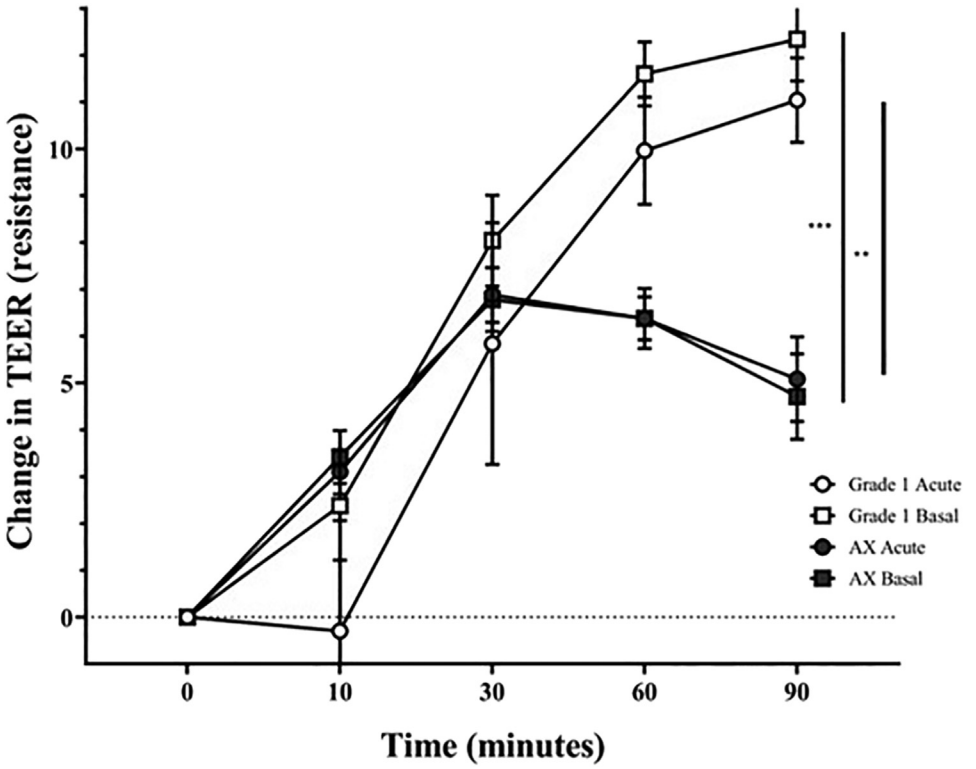

FIG 3. Functional impairment of anaphylactic HDL particles in endothelial barrier maintenance. TEER measurements in endothelial cells stimulated with purified HDL from paired serum samples of 5 non-anaphylactic reactions (grade 1) and 10 anaphylactic reactions (5 grade 2 and 5 grade 3). Grade 1 acute versus AX acute, $^{**}P = .0032$; grade 1 basal versus AX basal, $^{***}P = .0004$. Tukey multiple comparison test was used. *AX,* Anaphylaxis; *TEER,* transendothelial electrical resistance.

Disclosure of potential conflict of interest: The authors declare that they have no relevant conflicts of interest.

Key messages

- **Serum levels of HDL-C, LDL-C, ApoA1, and ApoB diminished in severe anaphylactic reactions.**
- **We found an impaired capacity of HDL to induce MCE and endothelial barrier stability during severe reactions.**

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