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Inflammation Modulates Human HDL Composition and Function *in vivo*

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Abstract

Objectives—Inflammation may directly impair HDL functions, in particular reverse cholesterol transport (RCT), but limited data support this concept in humans.

Methods and Results—We employed low-dose human endotoxemia to assess the effects of inflammation on HDL and RCT-related parameters *in vivo*. Endotoxemia induced remodelling of HDL with depletion of pre- β 1a HDL particles determined by 2-D gel electrophoresis ($-32.2 \pm 9.3\%$ at 24h, $p < 0.05$) as well as small ($-23.0 \pm 5.1\%$, $p < 0.01$, at 24h) and medium ($-57.6 \pm 8.0\%$ at 16h, $p < 0.001$) HDL estimated by nuclear magnetic resonance (NMR). This was associated with induction of class II secretory phospholipase A2 (~36 fold increase) and suppression of lecithin:cholesterol acyltransferase activity ($-20.8 \pm 3.4\%$ at 24h, $p < 0.01$) and cholesterol ester transfer protein mass ($-22.2 \pm 6.8\%$ at 24h, $p < 0.001$). The HDL fraction, isolated following endotoxemia, had reduced capacity to efflux cholesterol *in vitro* from SR-BI and ABCA1, but not ABCG1 transporter cell models.

Conclusions—These data support the concept that “atherogenic-HDL dysfunction” and impaired RCT occur in human inflammatory syndromes, largely independent of changes in plasma HDL-C and ApoA-I levels.

Keywords

inflammation; atherosclerosis; cholesterol; lipoproteins; macrophages

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Introduction

Reverse cholesterol transport (RCT) is considered a major anti-atherogenic function of high density lipoprotein (HDL)¹. Loss of HDL RCT functions may contribute to atherosclerosis in chronic inflammatory states including obesity and insulin resistance. In fact, inflammation may directly impair HDL function and RCT^{2, 3}, but limited data support this concept in humans^{4, 5}.

Experimental studies in rodents and primates suggest that acute inflammation alters HDL composition^{6, 7}, with increased catabolism of HDL particles⁸, enrichment of acute phase HDL with serum amyloid-A (SAA)⁹, and loss of apolipoprotein (apo) A-I². These changes combined with inflammatory down-regulation of the hepatic scavenger receptor BI (SR-BI)¹⁰, and reduced transport of cholesterol to bile and feces^{3, 11}, provide indirect support for an integrated effect *in vivo* of inflammation on RCT. A few human studies have characterized inflammatory changes in plasma lipoproteins^{4, 5, 12, 13}.

We published the first direct *in vivo* evidence in rodents that inflammation impairs multiple steps of the RCT pathway¹¹, which was recently validated by others^{14, 15}. We also provided preliminary proof of this concept in humans¹¹ and found that human endotoxemia induced a loss of HDL cholesterol efflux functions coincident with HDL depletion of phospholipid and enrichment with SAA¹¹.

In this paper, we extend our human endotoxemia studies¹¹ to examine (1) inflammatory change in HDL-particle size and distribution of ApoA-I-containing HDL particles at nuclear magnetic resonance (NMR) and 2D gel electrophoresis, (2) change in HDL modulating enzymes and proteins, and (3) relation of change in HDL parameters with loss of HDL cholesterol efflux functions *in vitro* in ABCA1, SR-BI and ABCG1 model systems. We define specific inflammatory changes in HDL particles as well as key proteins in HDL metabolism and RCT that may drive selective modulation of cholesterol efflux functions. These studies suggest potential for development of specific HDL functional parameters, distinct from HDL-C and ApoA-I, for prognostic and therapeutic applications.

METHODS

Descriptions of the human study protocol, laboratory methods and statistical analysis are provided in the online supplement. The University of Pennsylvania Institutional Review Board (IRB) approved the human study and written informed consent was obtained.

RESULTS

Baseline Characteristics and Inflammatory Responses

Participants' baseline characteristics have been published previously¹⁶. Briefly, subjects were young healthy adults (N=20, 50% male, 80% Caucasian, age 25.7±3.9) with normal blood pressure, plasma lipoproteins, and BMI (Supplement Table 1). As expected¹⁶ endotoxemia induced an acute febrile illness largely resolving in 8–12 hrs (Supplement Table 2).

Endotoxemia modulates HDL composition and size

Previously, we demonstrated that human endotoxemia reduced HDL phospholipids (~25%) while coincidentally increasing HDL-SAA (~80 fold) but with small impact on HDL cholesterol (+2.6%) and apoA-I (-11%)¹¹. Indeed, relative to apoA-I, there was a much greater loss in HDL phospholipids than change in cholesterol and apoA-II. Conversely, the

increase in HDL SAA was out of proportion to the reduction in apoA-I (Supplement Figure 1A-D).

Endotoxemia-induced change in mean HDL-particle size at NMR and in the distribution of apoA-I -containing HDL particles by 2D gel electrophoresis (Figure 1 and Supplement Table 3). Although the total HDL particle number at NMR did not change during endotoxemia, we observed significant decreases in the numbers of small and medium sized particles (Figure 1A). HDL separation by 2D gel electrophoresis also showed significant reductions in the small pre- β 1a and α 3 as well as in the medium-sized α 2 HDL subpopulations but no change in the large α 1 particles (Figure 1B).

Potential mechanisms of HDL particle remodeling by inflammation

We examined HDL-related lipases, transfer proteins and regulatory enzymes that may contribute to HDL remodeling^{3, 4, 17, 18}. Previously, our group has shown increased plasma endothelial lipase (EL) levels during endotoxemia¹⁷ and now we also demonstrate a marked increase in circulating class II secretory phospholipase A2 (sPLA2-IIA) (Figure 2A), lipases known to selectively modulate HDL phospholipids. Indeed, increases in both plasma EL (Spearman rho -0.50, p=0.03) and sPLA2-IIA (Spearman rho -0.43, p=0.2) tended to correlate with reductions in HDL phospholipids. In addition, modulation of the cholesterol content of HDL particles during endotoxemia is suggested by a reduction in plasma CETP levels (Figure 2B) and plasma LCAT (Figure 2C).

Endotoxemia impairs the capacity of HDL to efflux cellular cholesterol

Inflammatory remodeling of human HDL may impair its anti-atherogenic functions, in particular the capacity to remove cholesterol from peripheral cells, a critical step in the RCT process. We reported previously that endotoxemia induced a progressive reduction in the capacity of mouse and human HDL to promote ³H-cholesterol efflux *ex vivo* from J774 macrophages, an ABCA1-dependent efflux model (~60% reduction in efflux at 24h, Figure 3A)¹¹. We now demonstrate a specific loss (~20% reduction in efflux at 12 hrs) in the capacity of inflammatory HDL to remove cholesterol in an SR-BI dependent model (Figure 4A) but no change in an ABCG1 over-expressing BHK-1 cell model (e.g., pre-LPS ABCG1 efflux of $2.60 \pm 0.25\%$ vs. 12h post-LPS efflux of $2.58 \pm 0.25\%$; mean $\pm$ SEM, n=6 sera).

We explored the relationship of HDL particles with efflux in ABCA1 and SR-BI model systems. Pre-endotoxemia ABCA1-mediated efflux correlated with HDL-C, phospholipids, apoA-I, small HDL and pre- β 1a and pre- β 1b particles but these correlations were markedly reduced 24h following LPS coincident with reduced efflux (Figure 3B). These data are consistent with a loss of HDL particles that interact with ABCA1. Pre-endotoxemia SR-BI-mediated efflux correlated with HDL-C, apoA-I, phospholipids and mostly medium sized particles; these correlations were also attenuated following LPS (Figure 4B) consistent with remodeling of particles that are known to interact with SR-BI.

Although post-endotoxemia human HDL was significantly enriched in SAA, we found that these HDL fractions had reduced cholesterol efflux capacity in both ABCA1 and SR-BI model systems. In fact, the SAA content of post-LPS inflammatory human HDL was neither correlated with ABCA1 nor SR-BI dependent efflux.

DISCUSSION

Although epidemiological studies suggest an inverse relation between circulating HDL-C and cardiovascular disease (CVD), whether low HDL-C is merely a bystander or is causative remains controversial. Unlike LDL-C, genetic factors that influence HDL-C levels are not associated with coronary artery disease (CAD) in genome wide association studies

(GWAS)¹⁹. Failure of the cholesterol ester transfer protein (CETP) inhibitor torcetrapib and more recently niacin to reduce CVD despite their HDL-C raising effects, has raised doubts about the therapeutic potential of raising HDL-C²⁰. However, HDL is the most complex lipoprotein family and its function and metabolism are not well understood as yet. Global measures such as HDL-C or apoA-I likely fail to capture functionality that may reside in HDL sub-fractions. We present novel data demonstrating that inflammation in humans modulates HDL composition and function in the absence of substantial change in HDL-C or apoA-I thereby reinforcing the concept that alternative measures of HDL function need to be applied in order to address rigorously whether HDL is causally involved in atherosclerosis.

Previously, we reported the first *in vivo* functional evidence that acute inflammation retards RCT in rodents and provided proof-of-principle for similar effect in humans¹¹. In the current paper we demonstrate that human endotoxemia (a) remodels HDL with a characteristic decrease in lipid-poor pre- β 1 and other small and medium sized HDL particles, (b) induces plasma sPLA₂-IIA and suppresses CETP mass and LCAT activity, biochemical consequences known to remodel HDL phospholipid and cholesterol, and (c) reduces HDL capacity to efflux cholesterol from SR-BI and ABCA1, but not ABCG1, model systems.

Low-dose experimental endotoxin, which transduces toll-like receptor 4 (TLR-4) signaling *in vivo*, may be an informative model of cardio-metabolic pathologies in humans^{4, 21}. Sepsis and chronic infections^{12, 22} in humans induce insulin resistance, glucose intolerance and lipoprotein changes similar to the metabolic profile in obesity, type-2 diabetes and CAD. We and others have shown that experimental endotoxemia induces insulin resistance^{16, 21}, adipose inflammation²³ and lipoprotein changes^{4, 6, 7, 11, 24} that resemble those in CVD risk states. A role for TLR4 is specifically suggested by work demonstrating reduced diet induced obesity²⁵ and atherosclerosis in TLR4 deficient mouse models. In fact, TLR4 may be activated by endogenous ligands including fatty-acids that are increased in diet induced obesity and insulin resistance²⁵. Finally several groups, including ours¹¹, have defined mechanisms of altered HDL function and impaired reverse cholesterol transport during inflammation in rodent models^{14, 15}.

Hudgins *et al.* were the first to examine diverse lipoprotein changes during placebo-controlled, experimental endotoxemia in humans⁴. In contrast to studies in rodents^{6, 26}, they found no change in HDL-C and apoA-I levels, but did observe increased SAA and loss of HDL phospholipids. The functional consequences of HDL remodelling were not directly addressed. In our published work, we have confirmed marked increase in HDL-SAA with reduction in HDL phospholipids and a relative preservation of HDL apoA-I. Here, we present novel data on the effects of innate inflammatory responses on the HDL subpopulation profiles determined by NMR and 2D gel analyses and how those changes influence HDL-mediated cellular cholesterol efflux.

We observed a marked increase of circulating sPLA₂-IIA during human endotoxemia. Our prior finding of increased plasma EL after LPS administration¹⁷ and others' work showing suppressed hepatic lipase (HL) during inflammation²⁷ support a role for these lipases in HDL phospholipid turnover in human inflammatory syndromes^{18, 28, 29}. While changes in HDL-C, apoA-I and apoA-II were minor, HDL phospholipids and small HDL particles decreased substantially suggesting impaired HDL maturation and increased catabolism of the small, less lipidated particles during acute inflammation.

Characterization of HDL particle size and the distribution apoA-I containing HDL particles during human inflammation may provide insight into specific changes in HDL that reduce HDL function and RCT leading to increased CVD risk. The majority of apoA-I in plasma has α mobility. These HDL particles have been classed as α_1 , α_2 , α_3 , and α_4 , with median

sizes of 11.0 , 9.2 , 8.05, and 7.43 nm, respectively³⁰. Smaller amounts of HDL particles contain apoA-I with pre- β mobility (pre- β_1 and pre- β_2). Four distinct particles have pre- α mobility (pre- α 1–4). Prior to LPS treatment, we have found only modest correlations of selective NMR HDL measures and 2D-gel parameters (e.g., medium HDL at NMR with α_2 particles on 2D gels, $r^2=0.35$, $p<0.01$) perhaps because these two methods provide distinct information regarding HDL particles. NMR estimates lipid content while non-denaturing 2D gel analyses measure apoA-I content. During endotoxemia, there was a substantial loss of small and medium sized HDL particles without significant reduction in levels of large HDL at NMR while 2D-gel electrophoresis suggested a reduction in smaller pre- β_1 and α_3 HDL while large α_1 HDL particle levels were not decreased. These selective changes are consistent with induction of inflammatory lipases coincident with the suppression of LCAT⁵, PLTP⁴ and CETP^{4, 5, 31} (and Figure 2).

We determined the functional impact of endotoxemia on human HDL by measuring the *ex vivo* capacity of the isolated HDL fraction to efflux cholesterol. We and others have reported an inflammation-induced loss of HDL's capacity to efflux cholesterol from an ABCA1 macrophage cell model^{11, 14}. This is consistent with our finding of reduced levels of pre- β_1 particles, major ABCA1 interacting HDL particles. We also report a significant loss of HDL cholesterol efflux from a SR-BI cell model. This loss is likely driven by reduced HDL phospholipids and an unfavorable remodeling of α_2 HDL particles which are preferential substrates of SR-BI³². Endotoxemia increased HDL-associated SAA by almost 100-fold. Our observation of reduced ABCA1 and SR-BI efflux despite *in vivo* increases in HDL SAA, coupled to our recent findings of reduced efflux and RCT during inflammation in rodents¹¹, suggest that inflammatory increases in HDL-SAA do not enhance HDL acceptor function *in vivo*³³. Notably, we did not observe any loss of HDL acceptor capacity in an ABCG1-model arguing against a non-specific effect on all HDL efflux pathways. The reduction in HDL efflux from ABCA1 and SR-BI systems was remarkable also because it was associated with only minimal change in HDL-C and apoA-I suggesting that these traditional HDL parameters may be poor markers of important HDL functions in humans.

Recently Khara et al., demonstrated that measurement of the capacity of plasma to induce cholesterol efflux (in an ABCA1 model) was a stronger and independent inverse predictor of CAD than HDL-C levels³⁴, supporting the argument that measures of HDL function may be more useful than HDL-C levels in CVD risk prediction. It remains to be established in prospective studies whether any measures of HDL function and genetic correlates of these measures predict incident CVD and if therapies that modulate these HDL functions reduce CVD. That none of the CAD genes discovered to date through GWAS relate to circulating HDL-C levels should not be surprising if HDL-C is truly a poor surrogate for HDL function in atherosclerosis. Notably, no GWAS studies have been published yet using measures of HDL function. Our experimental data reinforces the notion that alternative measures of HDL function need to be applied in order to address more rigorously whether HDL is causally involved in atherosclerosis.

This study has several limitations. It is correlative and does not permit causal inference. Although we demonstrate alterations in HDL composition and function during induced inflammation, we have not examined the effect of inflammation on HDL biogenesis *in vivo* nor on ABCA1, SR-BI, or ABCG1 expression and function within the liver, peripheral tissues or macrophages. Induction of acute inflammation, even low grade, may not reflect accurately the chronic inflammatory pathophysiology that is a feature of obesity, insulin resistance, and CAD. However, several lines of data suggest that low-dose experimental endotoxin may be an informative model of cardio-metabolic disease in humans^{4, 21}. A major additional advantage is that the model allows a direct assessment of the directional impact of induced inflammation on metabolic parameters including HDL composition and function.

This avoids confounding and reverse causation that are features of observational studies where inflammatory changes may result from risk factors and disease rather than be causal.

In summary, we present evidence that human acute inflammation can induce selective remodelling of HDL with induction of specific HDL lipases, suppression of CETP and LCAT activity, HDL enrichment with SAA, loss of specific small-medium sized HDL particles, and reduction in selective HDL cholesterol efflux functions. These data support the concept that atherogenic HDL dysfunction and impaired RCT occur in human inflammatory syndromes, independent of significant change in plasma HDL-C levels. Overall, our work suggests that experimental human inflammation induces HDL remodeling and loss of HDL atheroprotective functions in a model that is broadly relevant to diverse human inflammatory disorders.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Effects of endotoxin (3ng/kg, iv) on (A) HDL sub-populations by nuclear magnetic resonance spectroscopy (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ vs time 0, analyzed by repeated measures one-way ANOVA) and (B) selective ApoA-I containing subpopulations of HDL particles by 2D gel electrophoresis (** $p < 0.01$, *** $p < 0.001$ vs pre-LPS, analyzed by repeated measures one-way ANOVA). Data presented as mean $\pm$ SEM (n=20).

Figure 2.

Plasma levels of (A) secretory phospholipase A₂ and endothelial lipase (***) $p < 0.001$ vs. time 0, assessed by repeated measures one-way ANOVA), (B) plasma cholesterol esterification rate (nmols/h/ml plasma), an index of lecithin:cholesterol acyltransferase (LCAT) activity, and (C) cholesterol ester transport protein levels (** $p < 0.01$ vs pre-LPS; assessed by t-test). Data presented as mean $\pm$ SEM (n=20).

Figure 3.

(A) ABCA1-dependent cholesterol efflux (over 4h) to HDL fraction during endotoxemia (** $p < 0.01$ vs. pre-LPS efflux, $n = 12$, assessed by repeated measures one-way ANOVA). (B) ABCA-1 efflux correlations (r^2) with lipid parameters pre-LPS and 24h post LPS. Figure 3A data, published previously¹⁴, is presented for comparative interpretation with Figure 3B and Figure 4.

Figure 4.

(A) SR-BI-dependent cholesterol efflux (over 4h) to HDL fraction during endotoxemia (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ vs. pre-LPS, $n = 20$, assessed by repeated measures one-way ANOVA). (B) SR-BI efflux correlations (r^2) with lipid parameters pre-LPS and 24h post LPS.