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Dysfunctional High-Density Lipoproteins in Children with Chronic Kidney Disease

Ryohei Kaseda[1], **Kathy Jabs**[1], **Tracy E. Hunley**[1], **Deborah Jones**[1], **Aihua Bian**[2], **Ryan M. Allen**[3], **Kasey C. Vickers**[3], **Patricia G. Yancey**[3], **MacRae F. Linton**[3,4], **Sergio Fazio**[3,5,#], and **Valentina Kon**[1,*]

[1]Department of Pediatrics, Vanderbilt University Medical Center, Nashville, Tennessee

[2]Department of Statistics, Vanderbilt University Medical Center, Nashville, Tennessee

[3]Department of Medicine, Vanderbilt University Medical Center, Nashville, Tennessee

[4]Department of Pharmacology, Vanderbilt University Medical Center, Nashville, Tennessee

[5]Department of Pathology, Vanderbilt University Medical Center, Nashville, Tennessee

Abstract

Objectives—Our aim was to determine if chronic kidney disease (CKD) occurring in childhood impairs the normally vasoprotective functions of high-density lipoproteins (HDL).

Materials and Methods—HDL were isolated from children with end-stage renal disease on dialysis (ESRD), children with moderate CKD and controls with normal kidney function. Macrophage response to HDL was studied as expression of inflammatory markers (MCP-1, TNF-α, IL-1β) and chemotaxis. Human umbilical vein endothelial cells were used for expression of adhesion molecules (ICAM-1, VCAM-1, E-selectin) and adhesion. Cellular proliferation, apoptosis, and necrosis of endothelial cells was measured by MTS/PMS reagent-based assay, flow cytometry, and ELISA. Cholesterol efflux was assessed by gas chromatographic measurements of cholesterol in macrophages exposed to HDL.

Results—Compared with $HDL^{Control}$, HDL^{CKD} and HDL^{ESRD} heightened the cytokine response and disrupted macrophage chemotaxis. $HDL^{Control}$ reduced endothelial expression of ICAM-1, VCAM-1, E-selectin, whereas HDL^{CKD} and HDL^{ESRD} were less effective and showed reduced capacity to protect endothelial cells against monocyte adhesion. Compared with a dramatically enhanced endothelial proliferation following injurious stimulus by $HDL^{Control}$, neither HDL^{CKD} nor HDL^{ESRD} caused proliferative effects. HDL of all three groups were equally protective against

*Correspondence: Valentina Kon, Department of Pediatrics, Vanderbilt University Medical Center, 1161 21st Avenue South, C-4204 Medical Center North, Nashville, TN 37232-2584. Phone: 615-322-7416; Fax: 615-322-7929; valentina.kon@vanderbilt.edu.
#Dr. Fazio's current address is: Center for Preventive Cardiology at The Knight Cardiovascular Institute of Oregon Health and Science University, 3881 SW Sam Jackson Park Rd, Portland, OR 97239

apoptosis assessed by flow cytometry and cleaved caspase-3 activity. Compared to $HDL^{Control}$, HDL^{CKD} and HDL^{ESRD} trended toward reduced capacity as cholesterol acceptors.

Conclusion—CKD in children impairs HDL function. Even in the absence of long-standing and concomitant risk factors, CKD alters specific HDL functions linked to control of inflammation and endothelial responses.

Keywords

HDL; CKD; child; macrophage; endothelial cell

1. INTRODUCTION

Chronic kidney disease (CKD) causes vascular dysfunction and increases risk of cardiovascular disease (CVD).[1–6] Typically, individuals with CKD present with CVD risk factors and comorbidities (age, obesity, smoking, diabetes, and hypertension) which by themselves disrupt vascular integrity, including endothelial function, and cause CVD. The detrimental impact of CKD prevails among all ages, however, it is most pronounced in the younger age groups, [3,7,8] and thus the direct effects of CKD on CVD are best studied in pediatric populations. While in the general pediatric population CVD mortality is extremely low, more than 25% of deaths in children with CKD are due to CVD.[9] Moreover, children with advanced CKD requiring dialysis who survive into adulthood live 40–50 years less than age-matched population.[9] Although these observations underscore the direct impact of CKD on development of vascular complications, the pathogenesis remains unclear.

HDL have been shown to have a variety of beneficial vasoprotective actions.[10–13] In addition to reverse cholesterol transport whereby HDL transfers cholesterol from the periphery to the liver for excretion, HDL has been shown to reduce inflammatory processes, limit oxidative stress, and inhibit blood clotting mechanisms.[10,1214] We and others have shown that adults with end stage renal disease (ESRD) requiring hemodialysis (HD) have a profoundly impaired HDL, which is less able to accept cholesterol from lipid-loaded macrophages and to control inflammation than HDL from normal controls.[15–22] Predictably, the individuals in our study and adult subjects reported by others had high rates of CVD co-morbidities, including diabetes (40–50%); underlying CVD (30–60%); hypertension (>90%), and smoking (25%). [15–17,19] In the current study, we sought to evaluate the direct effects of CKD per se on HDL functionality. To this end we examined whether moderate or end-stage CKD in children affects HDL functions relevant to vasculopathic processes.

2. MATERIALS AND METHODS

2.1 Participants and HDL isolation

The study was approved by the institutional review board of Vanderbilt University Medical Center, and written informed consent was obtained from all participants and their parents. Forty-six children, 1–18 years of age, with chronic kidney disease (CKD stage 3–4), end-stage kidney disease requiring dialysis (CKD stage 5), or normal kidney function were recruited. The underlying causes of CKD included urological disorders and dysplasia (n=15), chronic glomerulosclerosis (n=12), hemolytic uremic syndrome (n=2), unknown

(n=2). None of the children were receiving lipid lowering therapy and none had diabetes mellitus. Controls were recruited from children who had negative work-up for stones or other kidney disease. Five subjects were excluded, two samples were improperly handled and one yielded insufficient amount of HDL, one patient was maintained on hemodialysis, and a control subject turned out to have Hashimoto's disease.

Blood samples were obtained by venipuncture collected into ethylenediaminetetraacetic acid-containing tubes, centrifuged at 1700 *g* for 15 min at 4°C. Aliquots were stored at −80°C for lipid/chemical determinations while the HDL fraction (d = 1.063 to 1.22 g/ml) was isolated from fresh plasma by density gradient ultracentrifugation (DGUC).[23] Because of their size and small volume, we were initially concerned that the DGUR method would yield insufficient HDL fraction and began the studies using samples (three from each group) obtained by precipitating the apoB fraction with polyethylene glycol (PEG) solution as described.[24] HDL isolation method did not influence results of migration and cytokine expression studies and results were combined. All other assays used HDL prepared by DGUC.

Plasma total cholesterol, triglycerides and HDL were measured enzymatically (Cliniqa, CA). High-sensitivity C-reactive protein (hsCRP) was measured by high sensitivity immunoturbidimetric assay (Roche Modular System, Indianapolis, IN).[25] CKD stage was determined by calculating the estimated glomerular filtration rate (eGFR) by bedside Schwartz formula.[26]

2.2 Macrophage inflammatory cytokine response and chemotaxis assay

HDL modulation of inflammatory effect was measured using the established cytokine response in LPS-activated macrophages.[19] Briefly, THP-1 cells (American Type Culture Collection, Manassas, VA) were plated and differentiated by RPMI 1670 containing 10% fetal bovine serum and 50 ng/ml phorbol 12-myristate 13-acetate. THP-1 macrophages were exposed to HDL (50 μg/ml) and LPS (50 ng/ml) for 4 h. Total RNA was extracted from cells with Trizol reagent (Life Technologies, Carlsbad, CA). Quantification of human interleukin (IL)-1β, monocyte chemoattractant protein-1 (MCP-1), tumor necrosis factor-α (TNF-α), and endogenous control human Euk 18S rRNA levels was performed by real-time reverse transcriptase polymerase chain reaction (PCR) using CFX96 Real-Time System (BIO-RAD, Hercules, CA). Probes for IL-1β (Hs99999029_m1), MCP-1 (Hs00234140_m1), TNF-α (Hs99999043_m1), and 18S rRNA were obtained from Applied Biosystems (Foster City, CA).

Macrophage migration studies were performed in a microchemotaxis chamber as described.[19] THP-1 cells (5×10^5 /well) were exposed to HDL (50 μg/ml) for 1 h and then added to the upper compartment. MCP-1 0.1 μg/ml (Peprotech, Rocky Hill, NJ) was added to the lower compartment. Filters were fixed in methanol and stained with 1% crystal violet. Duplicate wells were used for each experimental condition, and > 5 fields (×40) were counted for each well in a blinded fashion.

2.3 Macrophage cholesterol efflux to HDL

THP-1 cells were plated and differentiated into macrophages then enriched with acetylated-low density lipoprotein (LDL) (100 μg/ml, Intracel, Frederick, MA) and exposed to HDL (50 μg/ml) and LPS (50 ng/ml) for 24 h.[19] Cholesterol content was measured by the most demanding and rigorous gas chromatography method [23,27] and cholesterol efflux determined as the percent at baseline versus after incubation with HDL.[28] Cell protein content was measured by bicinchoninic acid assay.

2.4 Endothelial cell adhesion assays

Assessment of endothelial cell adhesion molecules was performed in HUVEC (American Type Culture Collection, Manassas, VA) grown on 0.1% gelatin coated plates and exposed to HDL (50 μg/ml) and LPS (1μg/ml) for 5 h.[29] Quantification of VCAM-1, ICAM-1, E-selectin and endogenous control human GAPDH rRNA levels was performed by real-time PCR. Probes for ICAM-1(forward: 5'-CCACAGTCACCTATGGCAAC, Reverse: 5'-AGTGTCTCCTGGCTCTGGTT), VCAM-1(forward: 5'-GCTTCAGGAGCTGAATACCC, Reverse: 5'-AAGGATCACGACCATCTTCC), E selectin (forward: 5'-TGAACCCAACAATAGGCAAA, Reverse: 5'-CCTCTCATCATTCCACATGC) GAPDH (forward:5'-GAAGGTGAAGGTCGG AGTC Reverse:5'-GAAGATGGTGATGGGATTTC) were obtained from Integrated DNA technologies (Coralville, IA).

Monocyte adhesion to endothelial cells was studied using HUVEC grown on coated plates.[30] THP-1 cells were labeled with fluorescent dye calcein acetoxymethyl ester (4 μg/ml)(Life Technologies, Carlsbad, CA). HUVEC were exposed to HDL (50–300μg/ml) and TNF-α (1 ng/ml x 5h) (Peprotech, Rocky Hill, NJ) then calcein-labeled THP-1 cells. Fluorescence was measured at 494/520 nm (Abs/Em) by SpectraMax M5 Multi-Mode Microplate Readers (Molecular Devices, Sunnyvale, CA). Each experimental condition was done in triplicate and adhesion compared to the value obtained with TNFα only. In complementary microscopic studies, HUVEC grown on 0.1% gelatin coated glass culture slides (BD biosciences, San Jose, CA) were exposed to the fluorescence labeled THP-1 cells then fixed with 4% paraformaldehyde in phosphate buffered saline (Boston BioProducts Inc., Ashland, MA), mounted with VECTASHIELD Hard Set Mounting Medium with DAPI (Vector Laboratories Inc., Burlingame CA).

2.5 Endothelial cell proliferation, apoptosis, and necrosis assays

HUVECs were exposed to 50μg/ml of HDL and 10 ng/ml of TNF-α x 48 h. Cellular proliferation was measured with an MTS/PMS reagent based kit (Promega, Madison, WI). The quantity of formazan product was measured by 490nm absorbance using CellTiter 96 Aqueous One Solution Cell Proliferation Assay following the manufacturer's instructions.[31]

Apoptotic cells were determined by staining with the Annexin V and 7-AAD using FITC Annexin V Apoptosis Detection Kit with 7-AAD (BioLegend San Diego, CA). HUVEC were exposed TNF-α (10ng/ml) and HDL (50 μg/ml) x 16 h. Cells were resuspended in Annexin binding buffer at 1×10^6 cells/ml and incubated with Annexin V and 7-AAD for 15

min at room temperature in the dark. The cells were analyzed using a LSR II flow cytometer (BD Bioscience, San Jose, CA).[32]

Caspase 3 (Cleaved-Asp-175) was measured by ELISA (Assay Biotech, Sunnyvale, CA). HUVEC were exposed to HDL 50–300 μg/ml and 10 ng/ml of TNF-α x 48 h. After removing the supernatant, cells were fixed with 4% formaldehyde for 20 min at room temperature and incubated with quenching buffer for 20 min and blocking buffer for 1 h. Primary antibodies (Anti caspase 3 (Cleaved-Asp-175) rabbit polyclonal antibody) were added for overnight at 4C and secondary antibodies (HRP conjugated anti rabbit IgG antibody) were added for 1.5 h at room temperature. Cells were incubated with substrate for 30min before adding stop solution. Absorbance was measured at 450nm and results adjusted by cell numbers by staining with crystal violet measuring absorbance at 595 nm.

2.6 Statistics

Results are expressed as a mean of triplicate assays in endothelial cell adhesion, efflux and apoptosis assays, and duplicate assays in mRNA of inflammation cytokine response and adhesion molecules. Descriptive statistics are presented as frequencies and percentages for categorical variables and mean ± SD or median (interquartile range [IQR]) according to the distribution of the continuous variables. Demographic and clinical factors were compared between HDLs among control, patients with CKD, ESRD using Kruskal-Wallis test and unpaired t-test, as appropriate. Statistical analyses were performed using R version 2.10.0. A 2-sided significance level of 5% was required for consideration as statistically significant.

3. RESULTS

3.1 Characteristics of study participants

Demographic and clinical characteristics of study participants are summarized in Table 1. The groups were comparable in terms of age, gender and race. Because the study included children over a range of ages, the body mass index (BMI), systolic and diastolic blood pressure measurements are expressed as percentile normalized by age and height. There were no differences in BMI. The systolic and diastolic blood pressure values in the CKD and ESRD groups were not different than controls. Many children in CKD (9/16) and ESRD (5/15) groups were taking antihypertensive medications, including angiotensin converting enzyme inhibitor (ACEI) and/or an angiotensin receptor blocker (ARB) (16/31) or calcium channel blockers (1/31). Compared with controls, plasma levels of total cholesterol, LDL cholesterol and triglycerides were higher in CKD and ESRD, whereas HDL levels were not different among the groups. High sensitivity CRP was higher in CKD and ESRD subjects than controls.[33] Compositional analysis of HDL revealed that compared to children with normal kidney function, HDL of children with ESRD-PD have reduced phospholipid content (23%) and increased triglycerides (136%). Total cholesterol, free cholesterol, and cholesteryl ester levels were similar amongst ESRD-PD, CKD and Control groups (Table 1).

3.2 HDL effects on macrophage inflammatory and chemotactic responses

HDL from children with CKD and ESRD elicited an inflammatory cytokine response from THP-1 macrophages (Figure 1). Expression of MCP-1 in response to HDL^{CKD} was

increased 2-fold compared to $HDL^{Control}$. Similarly, HDL^{ESRD} caused >80% greater cytokine response than elicited by $HDL^{Control}$. Both HDL^{CKD} and HDL^{ESRD} enhanced cellular expression of TNF-α, 90% and 64% greater than $HDL^{Control}$, respectively, and IL-1β, 80% and 61% greater than $HDL^{Control}$, respectively. In addition, HDL^{CKD} and HDL^{ESRD} were found to have impaired anti-chemotactic functions. Whereas $HDL^{Control}$ dramatically inhibited MCP-1-induced THP-1 migration, neither HDL^{CKD} nor HDL^{ESRD} had anti-chemotactic effects (Figure 2). Indeed, the migratory response of HDL^{CKD} and HDL^{ESRD} was indistinguishable from chemotactic response elicited by MCP-1 alone.

3.3 HDL effect to vascular endothelial cells

A key vasoprotective feature of HDL is support of endothelial function.[10,14] To determine whether HDL from children with CKD and ESRD affect endothelial adhesiveness, we tested HDL's ability to suppress adhesion molecule expression, and found that compared to $HDL^{Control}$, both HDL^{CKD} and HDL^{ESRD} had blunted ability to suppress endothelial activation (Figure 3). $HDL^{Control}$ was found to dramatically reduced endothelial gene expression of ICAM-1, VCAM, and E-selectin. By contrast, HDL^{CKD} and HDL^{ESRD} resulted in significantly less suppression of ICAM-1 expression. VCAM-1 and E-selectin were similarly affected by HDL of all groups. These expression studies were complemented by functional experiments. $HDL^{Control}$ caused a 10.4% reduction in monocyte adhesion to endothelial cells compared to only 1.9 and 1.3% reduction following exposure to HDL^{CKD} or HDL^{ESRD}, respectively (Figure 4A). The extent of this impairment is visually illustrated in Figure 4B. Calcein-labeled THP-1 cells (green) were found to have greater adhesion to HUVECs (blue-DAPI) exposed to HDL^{CKD} or HDL^{ESRD} than cells exposed to $HDL^{Control}$.

We next examined the impact of CKD on vasoprotective properties of HDL that involve endothelial cell proliferation and death. Due to limited sample volumes, HDL samples were pooled in each group using the same quantity of HDL total protein concentration. $HDL^{Control}$ more than doubled HUVECs proliferation capacity following TNF-α activation (Figure 5). By contrast, neither HDL^{CKD} nor HDL^{ESRD} affected on cellular proliferation. Although HDL had a dramatic effect on endothelial cell proliferation, there was little difference in cell death, as HDL from all three groups repressed apoptosis. We also found little difference among $HDL^{Control}$, HDL^{CKD}, and HDL^{ESRD} on cell survival and necrosis over a range of HDL concentrations (50–300μg/ml) (Figure 6). Complementing the findings are our observations of cleaved caspase 3 activity in HDL-exposed HUVECs. Thus, similar to our flow cytometry results, HDL from the three groups reduced apoptosis, but there was no difference among $HDL^{Control}$, HDL^{CKD} and HDL^{ESRD} on cleaved caspase 3 activity (medium 26.3±1.2, 29.7±1.5, 29.6±1.2, 28.7±0.7, respectively, P=0.111). The lack of effect was observed over a range of HDL concentrations (50–300μg/ml).

3.4 HDL effects on cholesterol acceptor function

We and others have reported that the cholesterol acceptor function of HDL from adult ESRD patients on hemodialysis is impaired.[10,14,15,19] We therefore also analyzed the cholesterol efflux capacity of HDL in children with CKD. Both HDL^{CKD} and HDL^{ESRD} showed a non-significant trend toward reduced capacity to accept cholesterol from lipid-laden macrophages (Figure 7).

4. DISCUSSION

Our results suggest that children with moderate CKD and ESRD requiring dialysis have dysfunctional HDL and the dysfunction is not dependent on the level of plasma HDL cholesterol. Importantly, HDL dysfunction occurs in the absence of long-standing comorbidities and risk factors that plague adult CKD patients. Compared with $HDL^{Control}$, HDL^{CKD} and HDL^{ESRD} potentiate the macrophage inflammatory response and impair protective functions responsible for endothelial cell integrity, including cellular adhesion and proliferation. Notably, not all HDL functions are equally disrupted, as there were no significant differences among the HDL's on cholesterol efflux capacity or endothelial cell survival. The study suggests that CKD alone is sufficient to cause HDL dysfunction and that even moderate loss of renal function causes specific changes in HDL function that may be linked to early CVD in this population.

CKD is characterized by complex vasculopathy that includes endothelial dysfunction linked to inflammation.[33–36] HDL have important vasoprotective properties by providing anti-inflammatory actions and protecting the vascular endothelium.[10–14,37] The current study demonstrates that children with CKD and ESRD have dysfunctional HDL. Our data show that HDL^{CKD} and HDL^{ESRD} increase macrophage expression of IL-1β, TNF-α and MCP-1. Moreover, HDL^{CKD} and HDL^{ESRD} were not found to reduce MCP-1-induced chemotaxis, an effect clearly apparent with $HDL^{Control}$. These results significantly add to previous reports in adults with chronic disorders such as diabetes, coronary artery disease, metabolic syndrome, rheumatoid arthritis, showing that HDL loses its anti-inflammatory capacity.[38–40] Adults with CKD also have HDL with pro-inflammatory effects which in turn correlate with poor outcome.[38] Importantly, however, adults with CKD have a host of other potential confounders of HDL activity. Thus, more than half of the CKD subjects have diabetes mellitus, underlying cardiac disease, obesity, long-standing hypertension.[15–19] The current results are the first to describe enhanced macrophage inflammatory cytokine response to HDL from children with CKD, in the absence of concomitant conditions and risk factors. The results also reveal that this impairment occurs before progression to kidney failure since even children with stage 3–4 CKD have HDL that amplified macrophage cytokine response and chemotaxis to a degree indistinguishable from HDL^{ESRD}.

CKD-associated vasculopathy is critically dependent on endothelial cell function.[4,36,41,42] Our study suggests that the effect of CKD on HDL contributes to endothelial dysfunction, as both HDL^{CKD} and HDL^{ESRD} were ineffective in suppressing endothelial cells expression of adhesion molecules, specifically, endothelial ICAM-1. These results complement previous observations that vascular expression and plasma levels of ICAM-1 predict CVD events among ESRD patients.[43,44] Our expression studies are supported by functional results showing HDL^{CKD} and HDL^{ESRD} have significantly reduced ability to prevent monocyte adhesion to endothelial cells.

Our results reveal a more pronounced inhibitory effect of HDL of children with CKD on cellular proliferation than on protection against cell death. Compared with $HDL^{Control}$, HDL^{CKD} and HDL^{ESRD} were significantly less effective in restoring endothelial cell proliferation following TNF-α stimulus. The results are in line with previous observations

that uremic serum impairs endothelial cell proliferation.[45,46] Our novel results suggest that the endothelial dysfunction commonly seen across the spectrum of CKD may be influenced by dysfunctional HDL. Conversely, HDL from all three groups provided similar degree of protection against apoptosis and necrosis of endothelial cells. The divergent effects of HDL^{CKD} and HDL^{ESRD} on cellular proliferation versus death echo observations that uremic serum regulates endothelial cell proliferation, but has little impact on endothelial apoptosis.[46] The variable effect of HDL on proliferation versus survival has been documented in different cells, including, hematopoietic, adipocytes, cardiomyocytes, and cancer cells.[47,48] Taken together, these results suggest that HDL of CKD patients retain its ability to protect against apoptosis and necrosis; however, HDL-directed proliferation of endothelial cells in response to injurious stimuli occurring in CKD (toxic metabolites, oxidant stress, and inflammation) is markedly compromised.

Although we and others have reported that HDL from adults with ESRD on dialysis have impaired cholesterol efflux capacity,[15,19] we now report that HDL from children with CKD and ESRD showed only a non-significant trend for reduced efflux. It is possible that CKD may preferentially disrupt cholesterol efflux to phospholipid-poor apolipoprotein A-I. However, in our system of cholesterol-loaded macrophages, cholesterol is mobilized through scavenger receptor class-BI (SR-BI), ATP-binding cassette transporter G1 (ABCG1), and in particular ATP-binding cassette transporter A1 (ABCA1).[49–51] Further, density-gradient ultracentrifugation-isolated HDL fractions have been reported to stimulate ABCA1 cholesterol efflux due to the presence of small phospholipid-poor/apoA-1 particles (pre-β-1 HDL) and the ability of apoA-I to dissociate from mature HDL.[52–55] Using these HDL fractions, we did not observe a difference in efflux with HDL from control versus CKD or ESRD-PD subjects suggesting that ABCA1-mediated efflux is not affected. It is possible that other lipid-poor apoproteins/particles (d >1.22g/ml) could be impaired in their ability to stimulate ABCA1 efflux. However, using serum fractions depleted of apoB lipoproteins by PEG precipitation, i.e. whereby all of the HDL acceptors for ABCA1 would be present, revealed that, similar to the gradient ultracentrifugation-isolated HDL fractions, there were no difference in cholesterol efflux using the serum fractions. Interestingly, while the composition of HDL from ESRD-PD group were directionally similar to those reported in adult ESRD hemodialysis patients (i.e. reduction in in phospholipids and increase in triglycerides) they were not statistically significant and suggest that the more profound alterations in composition of HDL particles are linked to impaired cholesterol acceptor capacity. Indeed, in adults, changes in the phospholipid and triglyceride content of HDL particles were shown to correlate with impairment in cholesterol efflux.[15] It is therefore possible that alterations in HDL composition observed in children in the current study are insufficiently disturbed to impair efflux capacity. It is also possible that duration and the associated conditions that characterize adult CKD provoke the disruption in HDL efflux capacity. Furthermore, the children in our study were on peritoneal dialysis while adult patients in the published reports were on hemodialysis. Whether the mode of dialysis affects cholesterol acceptor capacity remains to be determined and requires further investigation. Recently, Khera *et al.* reported that cholesterol efflux capacity was inversely associated with CVD[56]; however, it is possible that reduced efflux is a consequence and not a predictor of future development in atherosclerosis. The median age of our population was about 9 years

of age, which may not be sufficient duration to establish atherosclerotic vasculopathy linked to impairment in cholesterol efflux. In this regard, Li *et al*. have reported that increased, rather than decreased, cholesterol efflux in adults with CKD is associated with risk of myocardial infarction, stroke, and death, while confirming the inverse association between cholesterol efflux and prevalent coronary artery disease.[57]

In summary, even in the absence of long-standing CKD and concomitant risk factors, CKD in childhood causes specific HDL abnormalities linked to maintenance of normal vascular function, including inflammatory responses in macrophages and production of adhesion molecules by endothelial cells.

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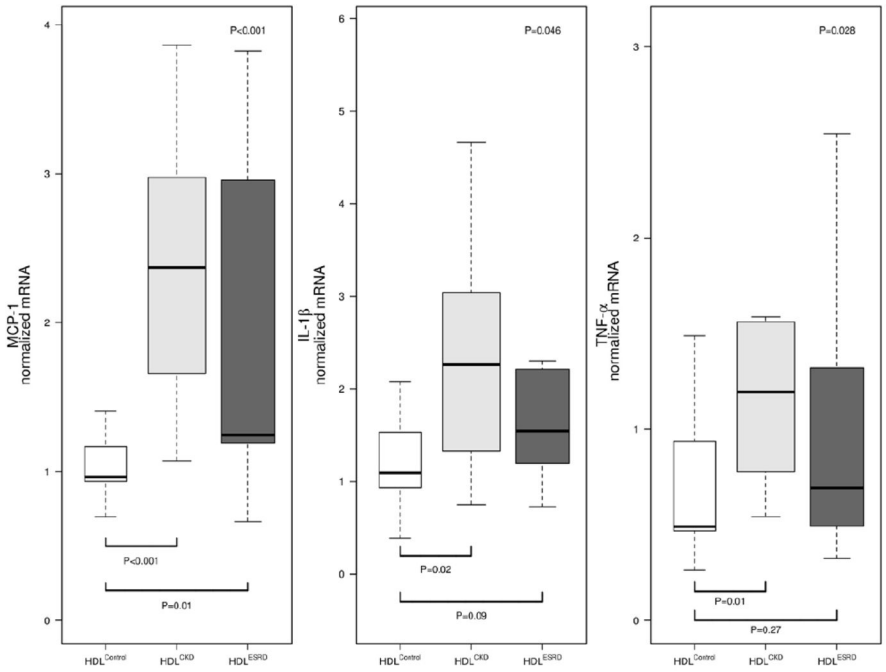

Figure 1.

Cytokine response of THP-1 macrophages to $HDL^{Control}$, HDL^{CKD}, and HDL^{ESRD} relative to cells exposed to LPS alone. mRNA expression for monocyte chemoattractant protein (MCP)-1, interleukin-1β (IL-1β), and tumor necrosis factor (TNF)-α measured by real-time PCR. N=9, 16, 14 for $HDL^{Control}$, HDL^{CKD} and HDL^{ESRD}, respectively. Comparison of HDL effects among control, CKD, ESRD subjects used Kruskal-Wallis test (p value shown in upper right corner of each panel) and unpaired t-test (shown as bars between individual groups).

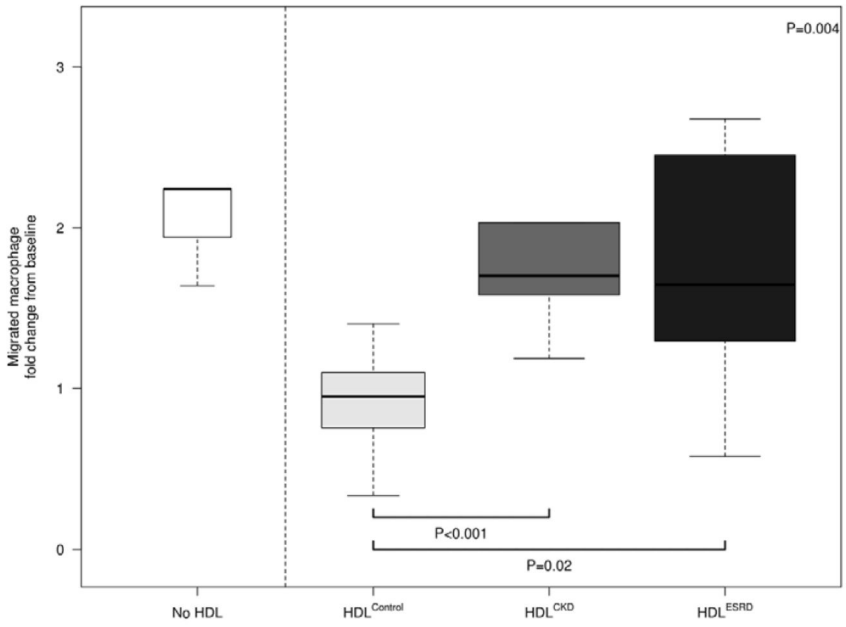

Figure 2.

Chemotactic effects of $HDL^{Control}$, HDL^{CKD} and HDL^{ESRD}. THP-1 cells in the upper compartment of a microchemotaxis chamber are separated by a filter from the lower compartment containing MCP-1. N=7, 13, 13 for $HDL^{Control}$, HDL^{CKD} and HDL^{ESRD}, respectively. Bars show median and interquartile range values. Comparison of HDL effects among control, CKD, ESRD subjects used Kruskal-Wallis test (p value shown in upper right corner of each panel) and unpaired t-test (shown as bars between individual groups).

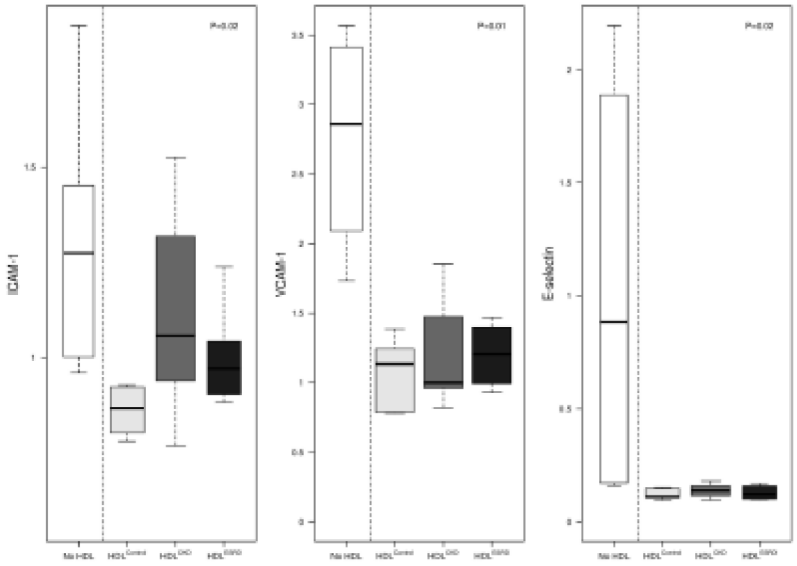

Figure 3.

Adhesion molecule response of endothelial cells to $HDL^{Control}$, HDL^{CKD}, and HDL^{ESRD}. mRNA expression for adhesion molecule (ICAM)-1; vascular cell adhesion molecule (VCAM)-1; and E-selectin measured by real-time PCR. N=6, 8, 6 for $HDL^{Control}$, HDL^{CKD} and HDL^{ESRD}, respectively.

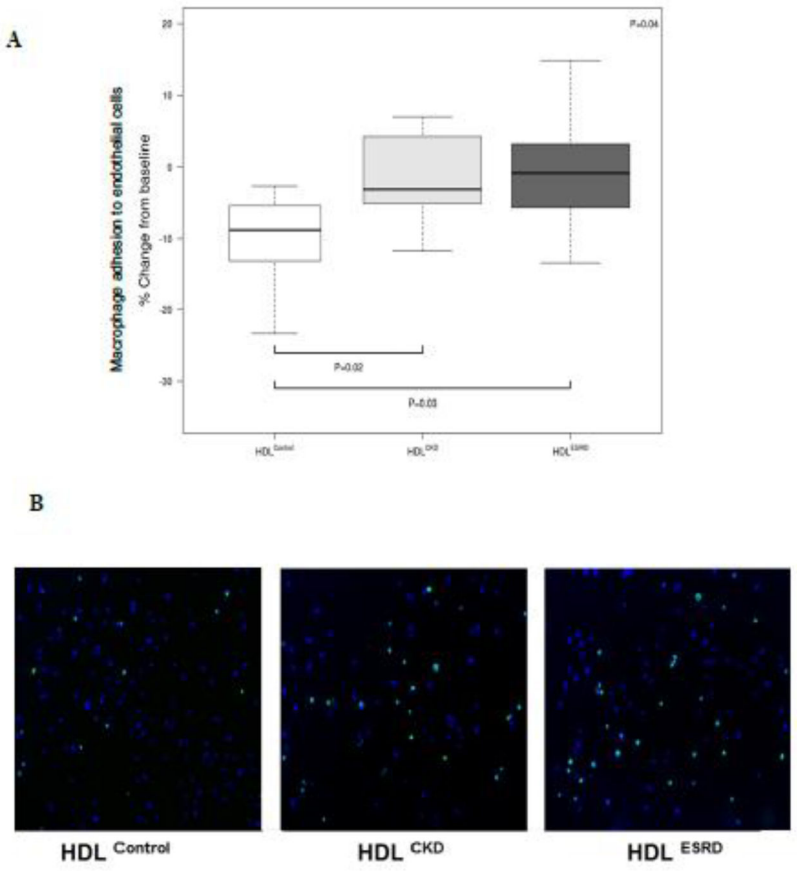

Figure 4.

Quantitated adhesion of THP-1 cells and HUVECs. (A) Cellular fluorescence was measured by SpectraMax M5 Multi-Mode Microplate Reader with each experimental condition done in triplicate and adhesion compared to the value obtained with TNF-α only. N=7, 13, 13 for $HDL^{Control}$, HDL^{CKD} and HDL^{ESRD}, respectively. Bars show median and interquartile range values. Comparison of HDL effects among control, CKD, ESRD subjects used Kruskal-Wallis test (p value shown in upper right corner of each panel) and unpaired t-test (shown as bars between individual groups). (B) Microscopic appearance of adhesion of calcein-labeled THP-1 to TNF-α-exposed HUVEC (Green: THP-1; Blue: DAPI).

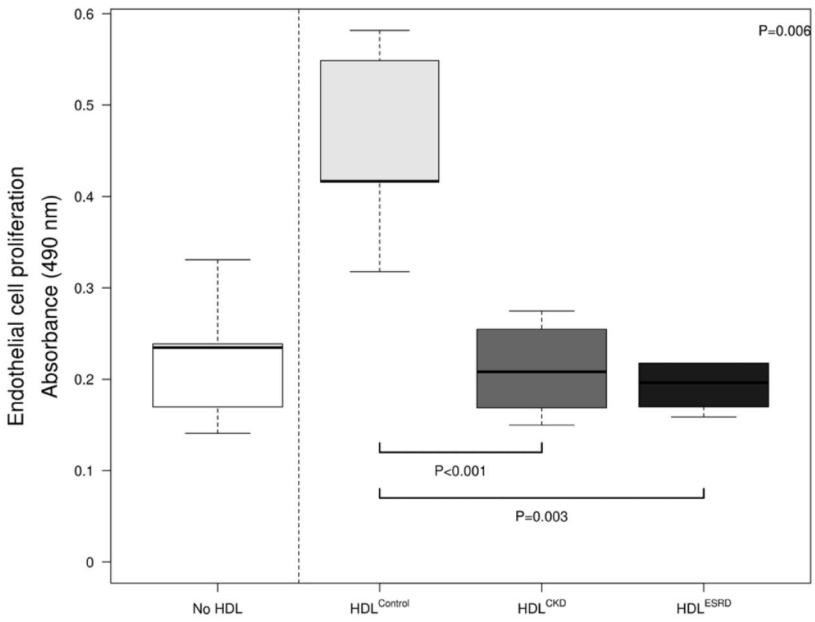

Figure 5.

Endothelial cell proliferation showing impaired proliferation effects of HDL^{CKD} and HDL^{ESRD}. Due to limited sample volumes, HDL samples were pooled in each group using the same quantity of HDL protein concentration; each experiment was done in triplicate and repeated 2–4 times. Bars show median and interquartile range values. Comparison of HDL effects among control, CKD, ESRD subjects used Kruskal-Wallis test (p value shown in upper right corner) and unpaired t-test (shown as bars between individual groups).

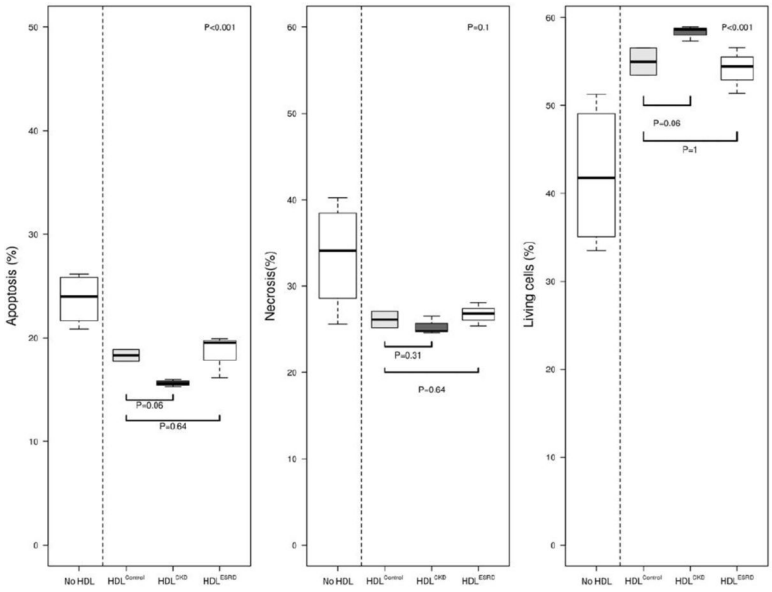

Figure 6.

HDL effects on endothelial cells survival, apoptosis, and necrosis was measured by flow cytometry using FITC Annexin V Apoptosis Detection Kit with 7-AAD showing similar effects among $HDL^{Control}$, HDL^{CKD}, and HDL^{ESRD}. HDL samples pooled in each group using the same quantity of HDL protein concentration and each experiment was done in triplicate and repeated 2–4 times. Bars show median and interquartile range values. Comparison of HDL effects among control, CKD, ESRD subjects used Kruskal-Wallis test (p value shown in upper right corner of each panel) and unpaired t-test (shown as bars between individual groups).

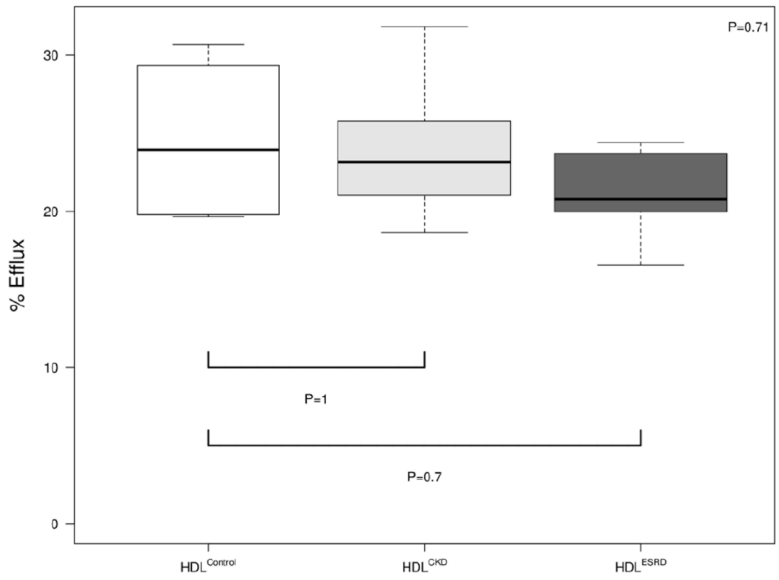

Figure 7.

Cholesterol acceptor capacity of $HDL^{Control}$, HDL^{CKD} and HDL^{ESRD} measured by gas chromatography. Cholesterol efflux was determined as the percent at baseline versus after incubation with HDL. N=4, 6, 6 for $HDL^{Control}$, HDL^{CKD} and HDL^{ESRD}, respectively. Bars show median and interquartile range values. Comparison of HDL effects among control, CKD, ESRD subjects used Kruskal-Wallis test.

Table 1

Characteristics of Study Subjects

	Control (N=10)	CKD (N=16)	ESRD-PD N=15	P Value
Age, years	9.3±2.0	9.8±5.5	9.6±5.0	P=0.72
Body mass index (percentile)	59±23	54±27	54±27	P=0.93
Systolic blood pressure (percentile)	70±24	72±20	76±22	P=0.64
Diastolic blood pressure (percentile)	62±17	59±27	72±28	P=0.26
Total cholesterol (mg/dl)	123±16	149±28	171±46	P=0.037
TG (mg/dl)	82±22	143±89	269±161	P=0.004
HDL, mg/dl	41.2±12.3	39.8±7.2	33.8±9.3	P=0.14
LDL, mg/dl	65±11	83±25	91±33	P=0.096
hsCRP (mg/dl)	0.40±0.20	0.76±0.76	1.65±1.40	P=0.002
HDL composition (μg/mg protein)				
Total cholesterol	0.291±0.071	0.264±0.052	0.293±0.098	P=0.87
Cholesteryl ester	0.225±0.051	0.204±0.041	0.228±0.091	P=0.85
Free cholesterol	0.065±0.027	0.060±0.014	0.065±0.007	P=0.36
Phospholipids	0.549±0.129	0.424±0.045	0.493±0.068	P=0.17
Triglycerides	0.065±0.023	0.087±0.036	0.154±0.108	P=0.06

Continuous variables are presented as mean±SD. T test used: Kruskal-Wallis. TG=triglyceride; HDL=high density lipoprotein; LDL=low density lipoprotein; hsCRP=high - sensitivity C reactive protein.