INCREASED CARBONYLATION OF HDL IN RABBITS WITH CKD: CARBONYLATED-HDL EXHIBIT BLUNTED ANTI-AGGREGATIVE ACTIVITY ON HUMAN PLATELETS THROUGH CD36 PATHWAY.

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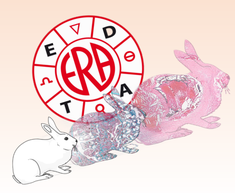

INTRODUCTION AND AIMS: Recent studies have shown altered biological properties of HDL in chronic kidney disease (CKD). As cardiovascular mortality remains the major cause of death in CKD and as oxidative stress is raised in CKD, we aimed to explore the specific role of oxidative modifications of HDL in CKD and their impact on anti-aggregant phenotype of HDL.

Biometry	Control (n=9)			CKD (n=8)			P-value
Body wt, kg	3.54	±	0.15	3.11	±	0.09	0.028
Kidney wt, g	19.8	±	2.1	14.2	±	0.9	0.017
Plasma analysis							
Urea, mM	7.00	±	0.51	17.58	±	3.64	0.021
Creatinine, μM	62.89	±	4.75	214.3	±	25.23	< 0.0001
Glucose, mM	5.78	±	0.55	7.54	±	0.20	0.006
Total cholesterol(TC), mM	1.40	±	0.19	3.48	±	0.59	0.006
HDL-C/TC ratio, mM	0.62	±	0.09	0.43	±	0.06	0.104
Triacylglycerols, mM	5.74	±	1.70	6.20	±	2.18	0.869
MDA, µM	0.14	±	0.07	1.45	±	0.10	< 0.001
AOPP, nmol/mg	1.00	±	0.19	0.72	±	0.10	0.292
AOA, mM	0.98	±	0.06	0.83	±	0.04	< 0.05

Table 1 — Main characteristics and biomarkers of oxidative stress of sham-operated and 5/6 nephrectomized rabbits
Differences were considered significant at the P<0.05 level, Student t tests
Data are expressed as means ± SEM, Student t tests. MDA: malondialdehyde; AOPP:advanced-oxidized protein products; AOA: anti-oxidant activity of the plasma. Differences were considered significant at the P<0.05 level

RESULTS: 8 CKD were compared with 9 Sham operated rabbits. Creatinine levels were significantly higher in CKD group (2.3-folds, p<0.001, **Table 1**) as well as plasma level of MDA (0,14 ± 0.07 vs 1.45 ± 0.10 μM, p<0.001, **Table 1**). MDA contents were significantly higher in the HDL from CKD group (0.89 ± 0.18 vs 2.64 ± 0.67 nmol/mg of proteins, p<0.05) while 8-isoprostane levels were not different (**Figure 1**). HNE-adducts were also increased in HDL (**Figure 2**) from CKD animals while HHE-adducts and 8-isoprostane levels were not different. Percentage of platelet aggregation compared to collagen alone was 65% when were incubated with HDL from CKD group while it was 30% with HDL from the control group (p<0.05)(**Figure 3**) evidencing a blunted anti-aggregative effect. Platelet aggregation in presence of HNE-modified HDL was 85% (p<0.05 compared to Control group). Incubation with Ab-CD36 induced a significant decrease in aggregation with CKD and HNE-modified HDL to 26 and 22%, respectively (p<0.05) (**Figure 3**).

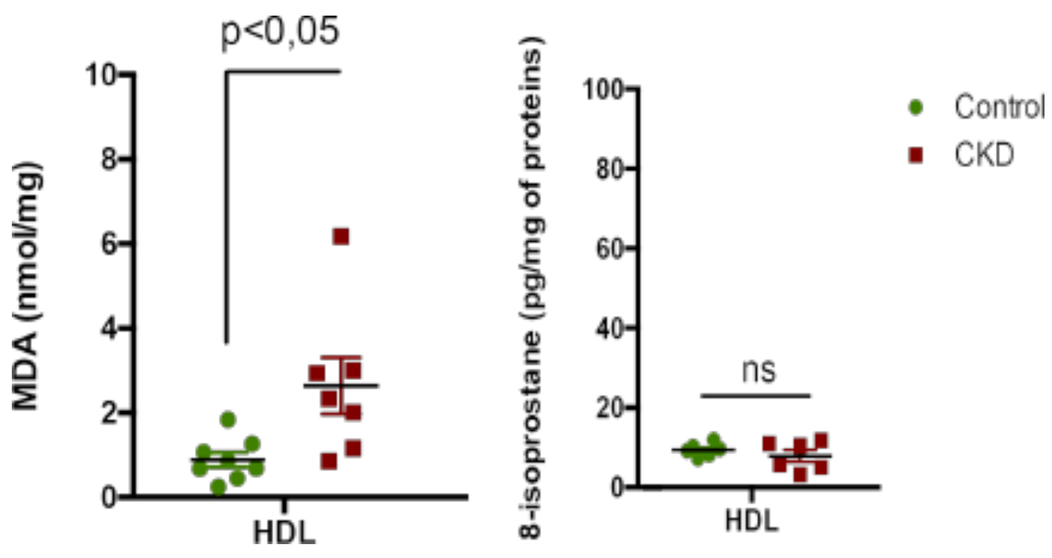

Figure 1 — Malondialdehyde (MDA) and 8-isoprostane levels in HDL from control and CKD rabbits
MDA levels in HDL. Amount is expressed as nmol/mg of proteins.
8-isoprostane level in HDL. Amount is expressed as pmol/mg of proteins.
Data are represented as mean ± SEM. Fischer exact t test, p< 0,05 was considered significant.

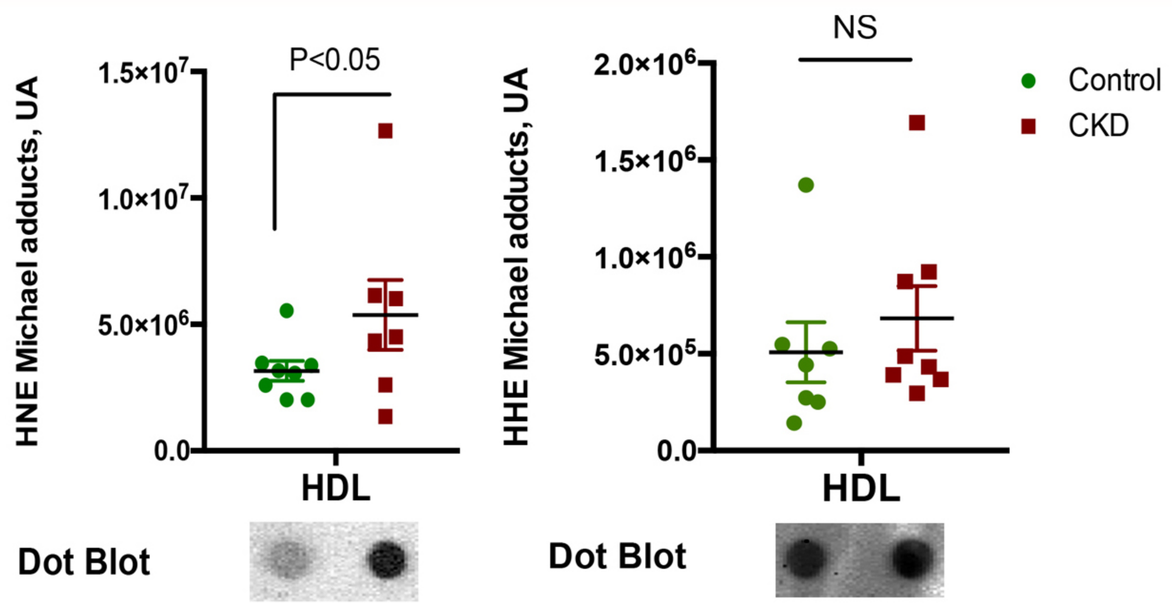

Figure 2 — Immunoblots (dot blots) of HNE and HHE-adducts in HDL from control and CKD rabbits
A: 4-hydroxy-nonenal (4-HNE) Michael adducts on HDL and LDL.
B: 4-hydroxy-2-hexenal (4-HHE) Michael adducts on HDL and LDL.
Mann-Whitney test, p< 0,05 was considered as significant.

METHODS: Rabbits were nephrectomized with a surgical 5/6 reduction technique. HDL were separated from plasma by potassium bromide stepwise density gradient ultracentrifugation. In rabbits, Malondialdehyde (MDA), 4-hydroxy-nonenal (HNE), 4-hydroxy-2-hexenal (HHE) protein adducts and 8-isoprostane levels were assayed. Platelet aggregation was measured after 5 min of incubation with HDL from each group in an aggregometer with or without anti-CD36 antibody (Ab-CD36). HDL from control were modified by an incubation overnight at 37°C with 100mM of HNE and platelet aggregation was assayed with or without Ab-CD36.

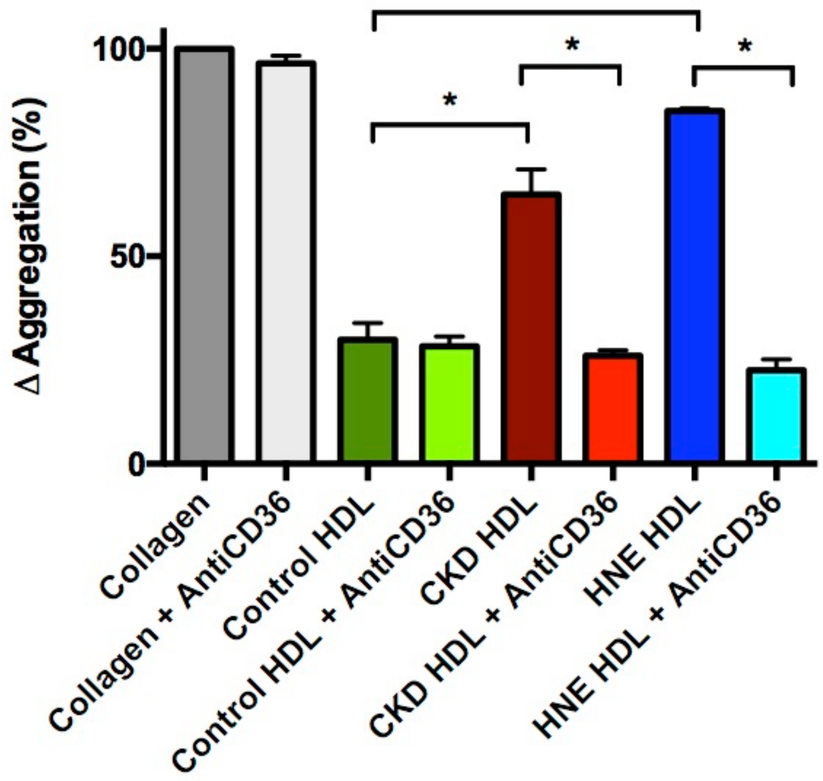

Figure 3 — Effects of HDL from Control and CKD rabbits on human platelet aggregation induced by collagen.
CKD HDL exhibited a blunted anti-aggregative phenotype compared to Control HDL. Control HDL modified by an incubation overnight with HNE solution (100mM) at 37°C (HNE HDL) showed a similar phenotype to CKD HDL. Pre-incubation with anti-CD36 antibody restored for CKD and HNE HDL an anti-aggregant phenotype similar to Control HDL (CKD HDL + Anti CD36 and HNE HDL + Anti CD36). Pre-incubation with anti-CD36 did not significantly modify the percentage of aggregation induced by the collagen. * p<0.05
Mann-Whitney test, p< 0,05 was considered as significant. Data are expressed as median and interquartile range for n=8-9.

CONCLUSIONS: CKD is associated with oxidative modifications of HDL among which carbonylation such as formation of HNE adducts. HDL from CKD rabbits exhibited an impaired ability to prevent platelet aggregation suggesting that altered HDL properties could contribute to the increased cardiovascular risk in this population.

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