see commentary on page 676

# Experimental coronary artery stenosis accelerates kidney damage in renovascular hypertensive swine

Dong Sun[1,2], Alfonso Eirin[1], Xiang-Yang Zhu[1], Xin Zhang[1], John A. Crane[1], John R. Woollard[1], Amir Lerman[3] and Lilach O. Lerman[1,3]

[1]Division of Nephrology and Hypertension, Mayo Clinic, Rochester, Minnesota, USA; [2]Department of Nephrology, The Affiliated Hospital of Xuzhou Medical College, Xuzhou, China and [3]Division Cardiovascular Disease, Mayo Clinic, Rochester, Minnesota, USA

**The impact of coronary artery stenosis (CAS) on renal injury is unknown. Here we tested whether the existence of CAS, regardless of concurrent atherosclerosis, would induce kidney injury and magnify its susceptibility to damage from coexisting hypertension (HT). Pigs (seven each) were assigned to sham, left-circumflex CAS, renovascular HT, and CAS plus HT groups. Cardiac and nonstenotic kidney functions, circulating and renal inflammatory and oxidative markers, and renal and microvascular remodeling were assessed 10 weeks later. Myocardial perfusion declined distal to CAS. Systemic levels of PGF2-α isoprostane, a marker of oxidative stress, increased in CAS and CAS plus HT, whereas single-kidney blood flow responses to acetylcholine were significantly blunted only in CAS plus HT compared with sham, HT, and CAS, indicating renovascular endothelial dysfunction. Tissue expression of inflammatory and oxidative markers were elevated in the CAS pig kidney, and further magnified in CAS plus HT, whereas angiogenic factor expression was decreased. Bendavia, a mitochondria-targeted peptide, decreased oxidative stress and improved renal function and structure in CAS. Furthermore, CAS and HT synergistically amplified glomerulosclerosis and renal fibrosis. Thus, mild myocardial ischemia, independent of systemic atherosclerosis, induced renal injury, possibly mediated by increased oxidative stress. Superimposed HT aggravates renal inflammation and endothelial dysfunction caused by CAS, and synergistically promotes kidney fibrosis, providing impetus to preserve cardiac integrity in order to protect the kidney.**

*Kidney International* (2015) **87,** 719–727; doi:10.1038/ki.2014.343; published online 22 October 2014

KEYWORDS: coronary artery stenosis; renovascular hypertension; renal fibrosis

**Correspondence:** *Lilach O. Lerman, Division of Nephrology and Hypertension, Mayo Clinic, 200 First Street SW, Rochester, Minnesota 55905, USA. E-mail: lerman.lilach@mayo.edu*

**Received 11 October 2013; revised 29 July 2014; accepted 14 August 2014; published online 22 October 2014**

Atherosclerotic coronary artery disease is the most common cause of death in the United States,[1] and it is often accompanied by diffuse vascular disease in other organs. An increase in systemic inflammation and oxidative stress may mediate some of the systemic manifestations of atherosclerosis,[2] yet myocardial ischemia may impose adverse effects on remote organs and vascular territories.[3] For example, myocardial infarction and stroke augment inflammation, and in turn atherosclerosis,[4] and they can trigger morphological and functional changes in the kidney.[5] However, the isolated effects on the kidney of myocardial ischemia dissociated from infarction and systemic atherosclerosis have been difficult to discern.

Patients with coronary artery disease often also have hypertension (HT), an important cause of chronic kidney disease.[6] Previous studies have shown increased prevalence of renovascular disease in patients with coronary artery disease,[7–9] in whom renal insufficiency is the main clinical predictor of renal artery stenosis. Renal artery stenosis is an important cause of secondary HT in the elderly population, which may ultimately lead to end-stage renal disease and represents a sizeable fraction of new patients entering dialysis. Importantly, hypertensive patients at risk for renovascular disease often present with severe coronary artery disease, and renal function correlates with its presence.[10] We have also recently shown that coexistence of coronary and renal artery diseases in human subjects magnifies renal injury.[11,12]

The preglomerular vasculature exposed to the HT develops progressive vascular pathology,[13] magnified by the loss of autoregulation with glomerular hypertrophy, hyperfiltration, and focal segmental glomerulosclerosis. Peritubular capillary loss and consequent hypoxia lead to tubular atrophy and interstitial fibrosis.[14] We have also shown that renovascular HT blunts antioxidant defense mechanisms in the nonstenotic kidney.[15] These functional and structural alterations may render kidneys exposed to HT susceptible to other comorbidities, and amplify renal damage.

However, whether nonatherosclerotic coronary artery stenosis (CAS) alone induces kidney injury or increases its vulnerability to adverse effects of coexisting HT remains unclear. The present study was therefore designed to test the hypothesis that isolated CAS elicits kidney inflammation and

oxidative stress, which are exacerbated by HT and impair renal function.

## RESULTS

Renal artery stenosis increased mean arterial pressure (MAP) in both HT and CAS + HT compared with sham (Table 1, $P = 0.03$ for both). Body weight and plasma renin activity were not significantly different among the groups, but serum creatinine was elevated in CAS, HT, and CAS + HT (Table 1, $P < 0.04$ each), affected by both CAS and HT. Urinary protein, affected by HT, increased significantly only in CAS + HT compared with sham and CAS (Table 1, $P = 0.006$ and $P = 0.045$, respectively). Circulating $PGF_2$-α isoprostane levels increased in CAS and CAS + HT compared with sham and HT (Figure 1a, $P < 0.05$ each), whereas systemic transforming growth factor (TGF)-β1 levels increased by HT in HT and CAS + HT (Figure 1b, $P = 0.02$ and $P = 0.03$ vs. sham, respectively). Norepinephrine levels were slightly reduced in CAS only compared with HT. Systemic levels of granulocyte–macrophage colony-stimulating factor, interleukin (IL)-1α, IL-1β, IL-1ra, IL-2, IL-4, IL-6, IL-8, IL10, IL12, IL18, and tumor necrosis factor (TNF)-α were not significantly different among the four groups (Supplementary Table S1 online, $P > 0.05$ for all).

### Cardiac function

Ten weeks after induction, the pigs developed significant and similar degree of CAS (Table 1, Figure 1d), but stroke volume and cardiac output decreased owing to CAS only in CAS + HT compared with sham and HT (Table 1, Figure 1e, $P \leqslant 0.03$ for each). The ejection fraction (albeit slightly affected by CAS) and early and late left ventricular (LV) filling velocities (*E/A*) were not significantly different among the four groups (Table 1). Myocardial perfusion distal to CAS and CAS + HT was lower than in sham (Figure 1f, $P = 0.02$ and $P = 0.01$, respectively), whereas LV muscle mass (LVMM) was increased in HT and CAS + HT (Figure 1c and g, $P \leqslant 0.03$ for both).

### Renal hemodynamics and function

The degree of renal artery stenosis was not different between HT and CAS + HT, but renal blood flow (RBF) and glomerular filtration rate (GFR) of the nonstenotic kidney were elevated only in HT; RBF was increased by HT, suppressed by CAS, and showed a significant interaction between the two (Table 2). GFR also tended to be blunted by the interaction HT×CAS (Figure 1h). RBF response to Ach was attenuated by CAS only in CAS + HT ($P = 0.08$ vs. baseline, Figure 1i), and became lower than sham ($P = 0.004$), by a significant interaction CAS×HT (Table 2).

Basal cortical perfusion in CAS and CAS + HT was lower than in HT, and after Ach infusion it was also lower than in sham (Table 2, $P < 0.05$ for each). Basal medullary perfusion was not significantly different among the four groups, but its response to Ach was blunted only in CAS + HT. Renal vascular resistance was elevated in CAS and CAS + HT compared with sham and HT (Table 2) owing to CAS. CAS also decreased proximal and distal tubular intratubular concentration in CAS + HT (Figure 1j), suggesting impaired tubular fluid reabsorption.

### Renal histology

Trichrome staining showed increased renal fibrosis in HT and CAS compared with sham pigs ($P = 0.003$ and $P = 0.001$, respectively). Compared with other groups, renal fibrosis and glomerular score in CAS + HT increased significantly (Figures 2a–c, $P < 0.01$ each), showing interactions between CAS and HT in both fibrosis (Figure 2b, $P = 0.0008$) and glomerulosclerosis (Figure 2c, $P = 0.04$). Dihydroethidium (DHE) staining was elevated in CAS ($P = 0.03$ vs. sham) and further exacerbated in CAS + HT (Figure 2a-DHE and d, $P = 0.006$ vs. sham and $P = 0.03$ vs. HT).

Compared with sham, renal capillary density decreased in HT (Figure 2, $P = 0.02$), yet in CAS + HT it decreased further compared with all groups ($P < 0.04$ each). Microvascular wall thickening (media-to-lumen ratio) increased in CAS ($P = 0.0003$ vs. sham), but further in HT and CAS + HT

**Table 1 | Systemic characteristics and cardiac function in the four groups (mean ± s.e.m., $n = 7$ each)**

| | | | | | *P*-value for two-way ANOVA | | |
|---|---|---|---|---|---|---|---|
| | Sham | HT | CAS | CAS + HT | HT | CAS | HT×CAS |
| Body weight, kg | 48.0 ± 2.8 | 48.3 ± 5.2 | 51.6 ± 4.6 | 46.1 ± 5.0 | | | |
| MAP, mm Hg | 96.7 ± 2.8 | 116.5 ± 7.8[a] | 101.3 ± 5.6 | 112.5 ± 7.0[a] | 0.028 | 0.961 | 0.519 |
| PRA, pg/ml/min | 0.17 ± 0.10 | 0.25 ± 0.16 | 0.22 ± 0.07 | 0.24 ± 0.15 | 0.505 | 0.786 | 0.648 |
| Creatinine, mg/dl | 1.17 ± 0.31 | 1.58 ± 0.17[a] | 1.62 ± 0.32[a] | 1.80 ± 0.16[a] | 0.016 | 0.008 | 0.326 |
| Urine protein, μg/ml | 16.9 ± 2.5 | 22.1 ± 5.9 | 18.4 ± 6.1 | 33.4 ± 4.7[a,b] | 0.047 | 0.199 | 0.319 |
| Norepinephrine, ng/ml | 0.08 ± 0.02 | 0.10 ± 0.02 | 0.04 ± 0.01[c] | 0.05 ± 0.01 | 0.290 | 0.01 | 0.583 |
| Degree of CAS, % | 0 | 0 | 77.3 ± 8.9[a,c] | 74.0 ± 10.3[a,c] | | | |
| Stroke volume, ml | 48 ± 2 | 50 ± 3 | 48 ± 5 | 37 ± 3[a,c] | 0.262 | 0.045 | 0.078 |
| Ejection fraction, % | 50 ± 3 | 57 ± 4 | 45 ± 5 | 45 ± 3 | 0.421 | 0.046 | 0.426 |
| *E/A* | 1.07 ± 0.06 | 1.02 ± 0.15 | 1.20 ± 0.15 | 1.14 ± 0.12 | 0.652 | 0.313 | 0.946 |

Abbreviations: ANOVA, analysis of variance; CAS, coronary artery stenosis; *E/A*, early-to-late left ventricular filling velocities; HT, hypertension; MAP, mean arterial pressure; PRA, plasma renin activity.
[a]$P < 0.05$ vs. sham.
[b]$P < 0.05$ vs. CAS.
[c]$P < 0.05$ vs. HT.