ACUTE INHIBITION OF THE ENDOGENOUS XANTHINE OXIDASE IMPROVES RENAL HEMODYNAMICS IN HYPERCHOLESTEROLEMIC PIGS

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Running Head: Xanthine oxidase in kidney of hypercholesterolemic pigs.

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ABSTRACT

OBJECTIVE: Hypercholesterolemia (HC), a major risk factor for onset and progression of renal disease, is associated with increased oxidative stress, potentially causing endothelial dysfunction. One of the sources of superoxide anion is xanthine oxidase (XO), but its contribution to renal endothelial function in HC remains unclear. We tested the hypothesis that XO modulates renal hemodynamics and endothelial function in HC pigs.

METHODS: Four groups (n=23) of female domestic pigs were studied 12 weeks after either normal (n=11) or HC diet (n=12). Oxidative stress was assessed by plasma isoprostanes and oxidized LDL, and the XO system by plasma uric acid, urinary xanthine, and renal XO expression (by immunoblotting and immunohistochemistry). Renal hemodynamics and function were studied with electron beam computed tomography before and after endothelium-dependent (acetylcholine) and –independent (sodium-nitroprusside) challenge, during a concurrent intra-renal infusion of either oxypurinol or saline (n=5-6 in each group).

RESULTS: HC showed elevated oxidative stress, higher plasma uric acid (23.8±3.8 vs. 6.2±0.8 μM/mM creatinine, p=0.001), lower urinary xanthine, and greater renal XO expression compared to normal. Inhibition of XO in HC significantly improved the blunted responses to acetylcholine of cortical perfusion (13.5±12.1 and 37.2±10.6 %, p=0.01 and p= n.s. vs. baseline, respectively), renal blood flow, and GFR, restored medullary perfusion, and improved the blunted cortical perfusion response to sodium-nitroprusside.

CONCLUSIONS: This study demonstrates that the endogenous XO system is activated in swine HC. Furthermore, it suggests an important role for XO in regulation of renal hemodynamics, function, and endothelial function in experimental HC.

KEYWORDS: oxidative stress, endothelium, oxypurinol, uric acid
INTRODUCTION

Hypercholesterolemia (HC) is a major risk factor for development and progression of atherosclerosis (45), and is associated with an increase in the incidence of coronary artery disease and cardiac events (1). Even at an early stage, HC can alter vasomotor regulation in both large vessels and the microcirculation (26, 46), and is responsible for the impairment of both the function and the structure of various vascular beds.

Moreover, HC has been demonstrated to be an independent risk factor for onset (10) and progression (22) of renal disease, and can both induce and worsen renal glomerular, interstitial, and vascular damage (30, 35). We have previously shown (11, 20, 42) that even a short exposure to diet-induced HC is associated with increased formation of oxidized low density lipoprotein (ox-LDL) and reactive oxygen species (ROS). Increased oxidative stress impairs endothelial function in both humans and animal models (13, 14, 37), partly by reducing bioavailability of nitric oxide (NO) via its reaction with ROS. Moreover, ROS can induce renal injury both by direct cellular toxicity (3) and by promoting production of ox-LDL, which in turn further inactivates NO (12, 33), and directly contributes to tubulointerstitial disease (2) and glomerulosclerosis (16).

Superoxide anions and other ROS may be generated by several different enzymatic and nonenzymatic mechanisms. In the vascular endothelium the main source for superoxide is NAD(P)H-oxidase, but additional enzymes can induce ROS production, e.g. cyclooxygenase, uncoupled eNOS and xanthine oxidase (XO) (28). XO can lead to superoxide production during the purine degradation process, which involves metabolism of hypoxanthine and xanthine to uric acid (4). XO activity has been demonstrated to be elevated in the plasma of hypercholesterolemic subjects and to contribute to endothelial dysfunction in HC animals (52) and humans (6). In the kidney, XO is also involved in ischemic injury (23). However, the contribution of XO-derived ROS to endothelial dysfunction in the
kidney in early atherosclerosis has not been determined.

The purpose of the present study was to assess the role of XO in the hemodynamics and endothelial function in the kidney of pigs with diet-induced HC. For this purpose we used electron beam computed tomography (EBCT), which provides accurate and noninvasive measurement of single-kidney regional hemodynamics and function in vivo, and allows detection of subtle alterations in renal hemodynamics and function (8, 10, 11, 20, 31, 32, 42).

MATERIALS AND METHODS

This study was approved by the Institutional Animal Care and Use Committee. Four groups of female domestic crossbred pigs (n=23, mean body weight 49.2 ± 10.9 Kg) were studied with EBCT after 12 weeks of either a normal (N, n=11) laboratory chow diet (Land O Lakes Purina Feed, Shoreview, MN), or high cholesterol diet (HC, n=12), which contained 2% cholesterol and 15% lard by weight (TD 93296, Harlan Teklad, Madison, WI, Table 1).

After completion of 12 weeks of diet, blood and urine samples were collected from all pigs for measurement of serum lipid profile (Roche, Nutley, NJ) and creatinine (spectrophotometry, Creatinine Analyze 2, Beckman Coulter, Fullerton, CA) (21). In addition, plasma levels of PGF2 alpha-isoprostanes (EIA, Cayman Chemical) (32, 53) and ox-LDL (ELISA, Mercodia) (8) served to assess systemic oxidative stress. Plasma levels of uric acid and urinary levels of xanthine (spectrophotometry) served as measures of the activity of the XO system.

The pigs were then randomized to obtain constant intra-renal infusion of either oxypurinol (oxy) or vehicle during performance of the subsequent EBCT studies. In each acute EBCT study, cortical, medullary, and papillary perfusion were measured before and after infusion of acetylcholine or sodium nitroprusside, representing endothelium-dependent and –independent challenges, respectively.
Following completion of studies, the pigs were euthanized with i.v. (100 mg/kg) Sleepaway (sodium pentobarbital, Fort Dodge Laboratories, Inc., Fort Dodge, IA). The kidneys were immediately dissected, and sections shock-frozen in liquid nitrogen (and maintained at -80°C) or preserved in formalin. Renal XO expression was then assessed using western blotting and immunohistochemistry.

**Spectrophotometric measurements of urinary xanthine levels.**

For measurement of xanthine level, diluted, filtered urine was mixed with an internal standard (8-13 Adenine) and analyzed by liquid chromatography tandem mass spectrometry (PE Sciex API 3000 LC/MS/MS, Applied Biosystem, Foster City, CA). LC/MS/MS was performed using a mobile phase composed of 50 mM ammonium formate, pH=5, and 1:1 mixture of 50 mM ammonium formate, pH=5: methanol, and ran using a gradient. An Xterra MS C18 column (2.1x150 mm) was used to separate xanthine and hypoxanthine from the bulk of the specimen matrix. The MS/MS was operated in the selected reaction monitoring (SRM) scanning mode. The ratios of the extracted peak areas of xanthine and hypoxanthine to an internal standard was used to calculate the concentration of xanthine in the sample (27).

**EBCT studies**

EBCT studies were performed as was previously described (31, 42). On the day of the studies, each animal was anesthetized with intra-muscular ketamine (20 mg/kg) and xylazine (2 mg/kg), intubated, and mechanically ventilated with room air. Anesthesia was maintained with a mixture of ketamine (15.7 mg/kg/h) and xylazine (2.3 mg/kg/h) in saline, administered via an ear-vein cannula (0.05 ml/kg/min).

Under sterile conditions and fluoroscopic guidance, a 7F arterial guide was advanced from the left carotid artery to the abdominal aorta; a tracker catheter was advanced within the guide into one renal
artery to serve for intra-renal infusions, as we have previously shown (10, 31). The arterial guide was
maintained at a level above the renal arteries and served for vasodilator infusion and for monitoring
mean arterial pressure (MAP) throughout the experiment. A pigtail catheter advanced through a
vascular sheath in the left jugular vein was positioned in the right atrium for contrast media injections.
ECG leads served for monitoring heart rate.

Animals were then transferred to the EBCT (Imatron C-150, Imatron Inc. South San Francisco, CA)
scanning gantry. In one normal group of pigs (Noxy, n=5) and one HC (HCoxy, n=6), after a 15 min
recovery period, a constant infusion of oxypurinol was initiated into the renal artery catheter. In each
group, baseline renal perfusion and function were measured after a 30-minute intra-renal infusion of
oxypurinol (300 mg/min/kg) (6). This dose has been shown to achieve more than 90% inhibition of XO
activity (15). The other normal (n=6) and HC (n=6) groups were infused with saline (0.1 ml/kg/min).
After a 30-minute stabilization, hemodynamic measurements were recorded and the EBCT studies
were performed to determine baseline renal hemodynamics and volume in both kidneys. Forty
consecutive scans (over 3 min) were obtained at variable time intervals after a bolus injection (0.5
cc/kg over 1 s) of the non-ionic, low-osmolar contrast medium iopamidol (Isovue®370, Squibb
Diagnostics, Princeton, NJ) into the right atrial catheter. After 15 min, a 10-min infusion of
acetylcholine (4 μg/kg/min) or sodium nitroprusside (6 nmol/kg/min) in random order was performed
and the EBCT scans repeated.

**Western Blotting**

To measure renal XO expression, frozen renal tissue of 5 HC and 5 normal pigs (including both cortex
and medulla) was pulverized and homogenized at 4°C in chilled protein extraction buffer. The
homogenate was incubated in buffer for 1 hour at 4°C and the homogenized lysates were then
centrifugated for 15 minutes at 14000 rpm. The supernatant was removed and the protein concentration
determined by spectrophotometry with a protein assay (Coomassie Plus, Pierce). The lysate was then diluted 1:4 in 1 x polyacrylamide gel electrophoresis sample buffer, sonicated and heated at 95°C to denature the proteins. The lysate were then loaded into a gel and run for standard western blotting protocols with the rabbit anti-xanthine oxidase polyclonal antibody (1:10000, Chemicon International) as primary antibodies, and anti-rabbit IgG Horseradish Peroxidase linked whole antibodies from donkey (1:500, Amersham Biosciences) as secondary antibodies. The membrane was exposed for 5 minutes to a chemiluminescence developing system (SuperSignal West Pico Chemiluminescent Substrate, Pierce) and then finally exposed to x-ray film (Kodak), which was subsequently developed and intensities of the protein bands were determined by densitometry. The specificity of the immunoblotting was confirmed with negative and positive controls obtained by parallel experiments performed in the absence of the the primary antibody or with a known concentration of XO (enzyme purified from buttermilk, 0.05µg, Sygma, S.Louis, MO), respectively.

**Immunostaining**

Immunohistochemistry for XO was performed on deparaffinized renal tissue of 5 HC and 5 normal pigs, using pre-diluted monoclonal primary antibodies (LabVision Corporation, CA). The secondary antibody, IgG Envision Plus (Dako), was followed by staining with the Vector NovaRED substrate kit (Vector-Laboratories, Burlingame, CA), and slides were counterstained with hematoxylin. Kidney sections (2 sections/each pig) were examined and the staining quantified (as fraction of surface area) using a computer-aided image-analysis program (MetaMorph, Meta Imaging Series 4.6).

**EBCT data analysis**

The methodology used for EBCT data analysis has been previously described in detail (8, 10, 11, 20, 31, 32, 42). Briefly, regions of interest were selected from the images by tracing the aorta and the
bilateral renal cortex, medulla, and papilla, and their densities sampled. Time-density curves were generated for each region, and described the change in tissue density consequent to transit of contrast in that region. The curves were then fitted using a modified gamma-variate fit (31). From each segment of the curve, the area enclosed under the curve and its mean transit time were calculated from the curve-fitting parameters. Renal regional perfusion (ml/min/cc tissue), normalized single-kidney glomerular filtration rate (GFR, ml/min/cc tissue), cortical, medullary volumes, and renal blood flow (RBF) were subsequently calculated as previously described (31).

**Statistical analysis**

Results are expressed as mean±SEM. Comparisons between experimental periods within groups were performed using paired Student's t-test, and among groups using analysis of variance (ANOVA), with the Bonferroni correction for multiple comparisons, and unpaired Student's t-test if applicable. Statistical significance was accepted for p<0.05.
RESULTS

Group characteristics

Systemic characteristic of the study groups are shown in Table 2. At the end of the diet period, the pigs fed with HC and normal diets had similar body weights, MAP, heart rate and serum creatinine. Total and LDL cholesterol levels were significantly elevated in the HC group compared with normal (p<0.0001, Table 2), as were plasma isoprostanes and oxidized LDL (p<0.05).

XO system

HC animals showed a significant increase of plasma levels of uric acid compared to normal (p=0.001, Table 2) while urinary levels of xanthine were significantly lower in HC (p<0.01). Renal XO expression was significantly (p<0.05) increased in HC compared to normal pigs (Figure 1A) and immunostaining showed that it was expressed throughout the kidney, but particularly prominent in the cortical proximal and distal tubules (p<0.05 vs normal, Figure 1B). No differences in XO expression or activity were observed between HC or normal pigs that were randomized to vehicle compared to oxypurinol infusion (data not shown).

Renal hemodynamics and function

Under basal conditions, cortical and medullary perfusions, volumes, and blood flows, as well as single kidney blood flow and GFR, were similar among all the groups (p= n.s., Table 3). Intrarenal infusion of oxypurinol did not modify heart rate or blood pressure. In addition, it did not alter basal kidney hemodynamics in either normal and HC groups compared to vehicle infused (Table 3) or contralateral kidneys (data not shown).
In the normal groups acetylcholine induced a significant increase in the EBCT-derived perfusion of the renal cortex, medulla, and papilla, as well as in RBF and GFR (p<0.01 vs. baseline for all). In HC the increase in cortical, medullary, and papillary perfusion in response to acetylcholine was not significant compared to baseline and reached values significantly smaller than in the normal group (p<0.05 for all regions), as was the increase in RBF (p<0.01 vs normal). GFR showed a modest but significant (p<0.05) increase, which was significantly attenuated compared to normal group (p<0.05). On the other hand, in HCoxy the perfusion of renal cortex significantly increased in response to acetylcholine (p<0.001 from baseline), although it did not reach normal values (p<0.05), and RBF showed a similar pattern (p<0.05 vs. HC, and p=n.s vs. normal). Furthermore, medullary and papillary perfusion response both normalized, increased significantly (p<0.005) in response to acetylcholine, and were not different from normal (Figure 2A and 3, p=n.s.). However, oxypurinol did not modify GFR response to acetylcholine, which remained significantly lower compared to normal (Fig. 3).

In normal animals EBCT-derived cortical perfusion and RBF increased significantly in response to sodium nitroprusside (p<0.01 and p<0.05, respectively), while medullary and papillary perfusions remained unchanged (Figure 2B). GFR was also not modified by sodium nitroprusside. In HC both cortical perfusion and RBF failed to increase (p=n.s.) in response to the drug, with a significant attenuation compared with the normal group (p<0.01 and p<0.05, respectively). Similar to the normal group, neither medullary and papillary perfusion nor GFR changed in response to sodium nitroprusside. Oxypurinol improved cortical perfusion response to sodium nitroprusside in HC pigs (Figure 2B).
DISCUSSION

This study suggests that the endogenous xanthine oxidase system plays an important role in regulation of renal hemodynamics in HC. We observed that our hypercholesterolemic porcine model was characterized by increased activity of the XO system, as indicated by the elevated levels of uric acid, decreased urinary levels of xanthine, and increased renal protein expression of XO. Furthermore, acute blockade of this system in HC significantly improved cortical and restored medullary perfusion responses to both endothelium-dependent and –independent challenges.

HC is an established cardiovascular risk factor in humans (1), and is associated with impaired coronary artery epicardial and microvascular endothelium-dependent vasodilation (44) and myocardial perfusion and permeability (43). Similarly, we have shown that this condition can induce functional vascular changes in the kidney, both in vivo and in vitro (50), which precede the onset of overt atherosclerotic lesions (10, 20, 42). Subsequently, HC subjects may develop glomerulosclerosis (24), ox-LDL deposition (35, 36) and chronic tubulointerstitial damage (25). Increased oxidative stress appears to be one of the main pathogenic mechanisms mediating lipid-induced nephropathy, and involves increased generation of ROS, decreased NO bioavailability, and consequently endothelial dysfunction, a condition characterized by a blunted endothelium-dependent vasodilator response.

As we have observed before (8, 32), in the present study HC pigs showed augmented oxidative stress, as demonstrated by the increase of plasma isoprostanes and ox-LDL compared to normal animals. In the presence of increased abundance of ROS, LDL particles can become oxidized to form a particularly atherogenic lipoprotein species (48). The main source of superoxide production in the arterial wall in HC has been identified in the endothelium, and implicated various ROS-generating enzymatic pathways such as NAD(P)H oxidase, uncoupled eNOS, and XO (28). Circulating XO has been suggested to be specifically involved in the mechanism of peripheral endothelial dysfunction in
HC (6, 52). XO can play a crucial role in generation of ROS in the kidney in pathological conditions, such as renal ischemia-reperfusion injury (23) and hypertension induced renal hypertrophy (34), and in the present study we assessed its involvement in HC-induced changes in renal hemodynamics. Indeed, we observed increased expression of XO throughout the kidney of HC pigs, especially in the cortical proximal and distal tubules. The significant increase in plasma levels of uric acid and decrease in xanthine levels in the urine also suggested increased activity of this enzyme.

Our previous studies in a porcine models of HC showed blunted kidney perfusion responses to both acetylcholine and sodium nitroprusside (20). The present study extends our previous observation and shows that acute XO inhibition with oxypurinol improves both acetylcholine and sodium nitroprusside-induced renal vasodilation. Interestingly, XO was mainly expressed in renal tubules, yet its inhibition improved renal perfusion responses, suggesting cross-talk between renal tubules and blood vessels (40), or possibly involvement of tubuloglomerular feedback in the hemodynamic effect of XO. The improvement in endothelial function might have resulted from decreased generation of ROS, and thereby increased availability of NO. In addition, the blunted response to sodium nitroprusside in HC may result from increased vasoconstrictor activity, and the improved response to sodium nitroprusside in HCoxy may implicate XO in regulation of renal vasoconstrictors.

The HC model also exhibited increased plasma levels of uric acid, which may constitute an independent cardiovascular risk factor (29, 38, 47). Interestingly, uric acid has anti-oxidant properties, because it can scavenge superoxide, hydroxyl radical, and peroxynitrite (29), so that an increased level of uric acid in HC could represent a compensatory effect to the increased oxidative stress. However, contrarily, uric acid is also a pro-oxidant, because urate and urate metabolites can amplify lipid peroxidation and inflammation (29). Furthermore, in addition to the ROS produced during the reaction catalyzed by XO, evidence suggests that uric acid generated during the same reaction might also directly and independently contribute to endothelial dysfunction (17, 39).
Notably, oxypurinol improved but not completely restored cortical perfusion in HC pigs. Although the relative response to Ach was increased in all the regions of the kidney in HCoxy (Figures 2A and 2B), the absolute values that cortical perfusion reached after Ach infusion (Table 3) were improved, but remained significantly lower than those reached by normal or Noxy pigs. In contrast, the response of medullary and papillary perfusion was completely normalized. Since the expression of XO was similarly increased in all renal regions, these results imply a greater sensitivity of the medulla and papilla to XO-derived radicals. The incomplete improvement in the cortical perfusion and GFR responses could reflect activation of other ROS-generating enzymes, such as NAD(P)H oxidase or uncoupled eNOS, that could account for the persistence of impaired renal hemodynamic response. Indeed, we have previously demonstrated increased expression of NAD(P)H oxidase in the kidney of HC pigs (7). Alternatively, other mechanisms could be involved in the lack of complete restoration of endothelial function by oxypurinol, including elevated levels of asymmetric dimethylarginine (ADMA), an endogenous competitive inhibitor of eNOS (5), decreased availability the eNOS substrate L-arginine (18), or reduced availability of the eNOS cofactor tetrahydrobiopterin (49).

Previous studies have shown that a single dose of allopurinol or oxypurinol increased urinary xanthine clearance and decreased plasma uric acid (51), and that the dose that we used resulted in more than 90% inhibition of XO (15). However, it is possible that acute blockade of ROS formation by either XO or other mechanisms may not suffice to improve renal hemodynamics, because of chronic renal tissue injury that resulted from the HC-diet (7, 8, 10, 42). This is supported by our recent study (9) demonstrating that in the stenotic kidney of hypertensive pigs, chronic administration of antioxidants can achieve greater improvement in renal hemodynamics than acute infusion of antioxidants.

Indeed, benefits of chronic inhibition of XO in cardiovascular disease continue to emerge (19). For example, a recent study (34) showed a protective blood pressure-independent effect of the blockade of XO in a rat model of hypertension-induced renal hypertrophy, suggesting a role for locally
synthesized XO in the development of hypertension-associated end-organ damage. On the other hand, a 4-week allopurinol administration did not affect endothelial function in forearm microcirculation in hypercholesterolemic patients (41), suggesting that the effects of chronic XO inhibition on cardiovascular and renal function may be variable and may depend on the model and on the treatment regimen.

In summary, we observed that a 12-week experimental HC was associated with increased plasma level of uric acid and renal expression of XO. Short-term inhibition of XO using intra-renal oxypurinol infusion significantly improved renal perfusion response to an endothelium-dependent challenge, suggesting that oxypurinol may be renoprotective under conditions of increased oxidative stress. Therefore, this study suggests an important role for XO in regulation of renal endothelial function in HC, although chronic tissue injury and other sources of ROS likely contribute to renal vascular dysfunction as well. Further studies will be needed to assess the relative contribution of these different systems to regulation of renal hemodynamics, function, and structure in HC.

ACKNOWLEDGMENTS

This study was partly supported by NIH grants number HL-63282, HL-77131, the AHA, and by the University of Study of Pisa, Italy.
REFERENCES


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44. **Rodriguez-Porcel M, Lerman LO, Holmes DR, Jr., Richardson D, Napoli C, and Lerman**


FIGURE LEGEND

Figure 1. A). Representative immunoblots (top) and densitometric measurement (bottom, arbitrary densitometric units) showing increased renal expression of xanthine-oxidase in hypercholesterolemic (HC) pigs. * p<0.05 vs normal pigs.

B). Representative immunostaining (top) and densitometric measurement (bottom, arbitrary densitometric units) showing renal expression of xanthine-oxidase in normal (top) and HC (bottom) pig renal cortex (left) and medulla (right). * p<0.05 vs normal pigs

Figure 2. Percent change (mean±SEM) from baseline in cortical, medullary and papillary perfusion in normal and hypercholesterolemic (HC) pigs either untreated or infused with oxypurinol (Noxy and HCoxy, respectively) in response to acetylcholine (Ach, A) and sodium nitroprusside (SNP, B).

* p<0.05 vs normal; † p<0.01 vs. HCoxy; ‡ p<0.01 vs normal.

Figure 3. Response (mean±SEM) to acetylcholine of RBF (left panel) and GFR (right panel) in normal and hypercholesterolemic (HC) pigs either untreated or infused with oxypurinol (Noxy and HCoxy, respectively). * p<0.05 vs normal
Table 1. Composition of the normal and HC diet:

<table>
<thead>
<tr>
<th>Composition</th>
<th>Normal Diet</th>
<th>High-cholesterol diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein</td>
<td>17%</td>
<td>17%</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>65%</td>
<td>50%</td>
</tr>
<tr>
<td>Fat</td>
<td>2% (crude)</td>
<td>17% (15% lard, 2% Cholesterol)</td>
</tr>
<tr>
<td>Salt</td>
<td>0.1-0.6%</td>
<td>0.4%</td>
</tr>
<tr>
<td>Others</td>
<td>15.1-15.9%</td>
<td>15.6%</td>
</tr>
</tbody>
</table>

The vitamin content was similar in both diets.
Table 2. Systemic characteristics (mean±SEM) in normal and hypercholesterolemic (HC) pigs.

<table>
<thead>
<tr>
<th></th>
<th>Normal (n=11)</th>
<th>HC (n=12)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (Kg)</td>
<td>48.2±2.8</td>
<td>52.1±3.5</td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td>83.5±12.8</td>
<td>98.2±9.9</td>
</tr>
<tr>
<td>Heart rate (bpm)</td>
<td>79.7±4.4</td>
<td>70.4±5.1</td>
</tr>
<tr>
<td>Serum creatinine (mg/dl)</td>
<td>1.66±0.27</td>
<td>1.75±0.09</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>70.4±4.2</td>
<td>371.7±38.3*</td>
</tr>
<tr>
<td>HDL cholesterol (mg/dl)</td>
<td>26.7±2.7</td>
<td>114±10.1*</td>
</tr>
<tr>
<td>LDL cholesterol (mg/dl)</td>
<td>39.0±3.5</td>
<td>231.1±37.9*</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>23.4±2.8</td>
<td>28.3±3.3</td>
</tr>
<tr>
<td>Isoprostanes (pg/mL)</td>
<td>125.6±33.7</td>
<td>270±51†</td>
</tr>
<tr>
<td>Ox-LDL (U/mL)</td>
<td>9.29±0.83</td>
<td>14.53±2.4†</td>
</tr>
<tr>
<td>Plasma uric acid (µM/mM Creatinine)</td>
<td>6.2±0.8</td>
<td>23.8±3.8†</td>
</tr>
<tr>
<td>Urine xanthine (µM/mM Creatinine)</td>
<td>12.0±0.85</td>
<td>7.8±1.1*</td>
</tr>
</tbody>
</table>

p<0.0001 vs. normal; † p<0.05 vs. normal.

MAP: mean arterial pressure, HDL: high density lipoprotein, LDL: low density lipoprotein, ox-LDL: oxidized low density lipoprotein.
Table 3. Renal hemodynamic and function in normal and hypercholesterolemic (HC) pigs, either untreated or infused with oxypurinol (Noxy and HCoxy, respectively).

<table>
<thead>
<tr>
<th></th>
<th>Normal</th>
<th>HC</th>
<th>Noxy</th>
<th>HCoxy</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PERFUSION (ml/min/ cc)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Cortex</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>4.17±0.4</td>
<td>4.41±0.44</td>
<td>4.27±0.3</td>
<td>3.72±0.17</td>
</tr>
<tr>
<td>Acetylcholine</td>
<td>5.93±0.65*</td>
<td>4.68±0.39†</td>
<td>5.69±0.35*</td>
<td>4.96±0.33*†</td>
</tr>
<tr>
<td>Sodium nitroprusside</td>
<td>5.10±0.39*</td>
<td>4.3±0.32†</td>
<td>5.2±0.49*</td>
<td>3.88±0.36†</td>
</tr>
<tr>
<td><strong>Medulla</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>2.63±0.33</td>
<td>3.14±0.56</td>
<td>3.36±0.34</td>
<td>2.8±0.28</td>
</tr>
<tr>
<td>Acetylcholine</td>
<td>4.37±0.52*</td>
<td>3.39±0.71†</td>
<td>4.39±0.32*</td>
<td>4.07±0.44*‡</td>
</tr>
<tr>
<td>Sodium nitroprusside</td>
<td>3.61±0.45</td>
<td>2.78±0.38</td>
<td>3.71±0.44</td>
<td>3.22±0.29</td>
</tr>
<tr>
<td><strong>Papilla</strong></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Baseline</td>
<td>2.51±0.43</td>
<td>2.81±0.54</td>
<td>3.8±0.54</td>
<td>2.78±0.57</td>
</tr>
<tr>
<td>Acetylcholine</td>
<td>4.59±0.58*</td>
<td>2.66±0.41†</td>
<td>5.7±1.2*</td>
<td>4.41±0.73*‡</td>
</tr>
<tr>
<td>Sodium nitroprusside</td>
<td>3.51±0.64</td>
<td>3.76±1.18</td>
<td>3.72±0.53</td>
<td>3.59±0.61</td>
</tr>
<tr>
<td><strong>RBF (ml/min)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>511.5±50.2</td>
<td>514.5±54.43</td>
<td>487.29±55.8</td>
<td>408.96±22.52</td>
</tr>
<tr>
<td>Acetylcholine</td>
<td>746.5±67.7*</td>
<td>548.1±52.9†</td>
<td>630.4±47.1*</td>
<td>543.45±36.47*†</td>
</tr>
<tr>
<td>Sodium nitroprusside</td>
<td>760.1±40.5*</td>
<td>487.9±40.2†</td>
<td>624.8±79.3*</td>
<td>456.7±28.5†</td>
</tr>
<tr>
<td><strong>GFR (ml/min/cc)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>64.54±3.85</td>
<td>56.13±5.08</td>
<td>64.65±7.98</td>
<td>60.15±3.09</td>
</tr>
<tr>
<td>Acetylcholine</td>
<td>98.02±11.8*</td>
<td>70.88±5.4*†</td>
<td>88.78±11.14*</td>
<td>73.12±3.36*†</td>
</tr>
<tr>
<td>Sodium nitroprusside</td>
<td>73.08±6.04</td>
<td>62.95±6.19</td>
<td>66.64±8.33</td>
<td>57.32±4.55†</td>
</tr>
</tbody>
</table>

* p≤0.05 vs. baseline. † p≤0.05 vs. normal and Noxy; ‡ p≤0.05 vs. HC.

RBF: renal blood flow, GFR: glomerular filtration rate.
Figure 1A

A

+ control  Normal  HC

Xanthine oxidase

β-actin

275 kDa  42 kDa

0  50  100  150  200

Densitometric units (AU)

Normal  HC

*
Figure 1B

B

<table>
<thead>
<tr>
<th>Normal</th>
<th>Cortex</th>
<th>Medulla</th>
</tr>
</thead>
<tbody>
<tr>
<td>HC</td>
<td>Cortex</td>
<td>Medulla</td>
</tr>
</tbody>
</table>

Fraction of renal surface area (%)

- Normal
- HC

- cortex
- medulla

Normal

HC

*
Figure 2A

Increase in perfusion in response to Ach (%)
Figure 2B

Increase in perfusion in response to SNP (%)

- Normal
- Noxy
- HC
- HCoxy

B
Figure 3