In Vivo Renal Vascular and Tubular Function in Experimental Hypercholesterolemia

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Abstract—Hypercholesterolemia (HC) is often associated with impaired peripheral and coronary vascular responses to endothelium-dependent vasodilators, which are probably due to low bioavailability of nitric oxide. To examine the effect of HC on renal vascular and tubular function, 22 domestic pigs were studied after being fed a 12-week normal (n=11) or HC (n=11) diet. Renal regional perfusion and intratubular contrast media concentration in each nephron segment (representing fluid reabsorption) were quantified in vivo with electron-beam computed tomography before and after a suprarenal infusion of either acetylcholine (6 pigs of each diet) or sodium nitroprusside (SNP; 5 pigs of each diet). An increase in cortical perfusion, observed in normal pigs with acetylcholine (+35±6%, P=0.002) and SNP (+12±4%, P=0.005), was blunted in the HC group (+8.8±4.0, P=0.01, and −4.6±4.0%, P=0.1, respectively, P=0.003 and P=0.005 compared with normal) as was an increase in medullary perfusion (+58±21 in normal versus +24±11% in HC, P=0.04). A decrease in the intratubular contrast media concentration in the distal tubule and collecting duct of normal pigs was observed in all tubular segments (and was significantly enhanced in the proximal tubule and Henle’s loop) in the HC group, which was associated with increased sodium excretion. The tubular and renal excretory responses to SNP were similar between the groups. In conclusion, early experimental HC in the pig attenuates renal perfusion response to both endothelium-dependent and -independent vasodilators possibly because of decreased bioavailability or decreased vascular responsiveness to nitric oxide. This vascular impairment may play a role in maladjusted renovascular responses and contribute to renal damage in later stages of atherosclerosis. (Hypertension. 1999;34[part 2]:859-864.)

Key Words: kidney ■ hypercholesterolemia ■ atherosclerosis ■ blood flow ■ renal function

Altered regulation of vasomotion by the endothelium is an early development in atherosclerosis and involves vasoconstriction and/or altered reactivity to vasoactive agents in most vascular beds in large vessels and microcirculation. Endothelial dysfunction can be demonstrated in humans and pigs with hypercholesterolemia (HC) and is characterized by blunted vasodilation in response to endothelium-dependent vasodilators, whereas the response to the smooth muscle relaxants is often maintained. Increased levels of oxidized LDL have been implicated in these vascular abnormalities, partly by decreasing bioavailability of nitric oxide (NO).

Most of the vascular effects of HC have been thus far described in the peripheral and coronary vascular beds and may modify regulation of tissue perfusion. For example, in coronary circulation, HC-induced endothelial dysfunction of anatomically normal coronary arteries may be associated with clinical symptoms and signs during increased cardiac demand. In the kidney, similar alterations may conceivably have renal functional and eventually structural consequences. However, the functional effect of diet-induced HC on the kidney has not been fully elucidated. In particular, the effect of diet-induced HC on the intrarenal microcirculation and tubules in vivo has been difficult to assess because of the lack of reliable, noninvasive techniques.

Electron-beam computed tomography (EBCT) is an ultrafast scanner that provides accurate, reproducible, and noninvasive measurements of single-kidney regional volume, perfusion, and segmental tubular function which are difficult to obtain with comparable spatial and temporal resolutions by other technologies. Therefore, the present study was designed to examine the functional intrarenal microcirculatory and excretory alterations associated with diet-induced HC in the in vivo, intact pig kidneys.

Methods

Animals
This study was performed according to institutional animal care and use guidelines (Mayo Clinic). The pig model was selected because of its humanlike anatomy and physiology. Twenty-two domestic female pigs (55 to 65 kg) were studied with EBCT after 12 weeks of being fed either a normal laboratory chow diet (n=11), which contained 0.2% to 0.7% sodium, or an HC diet (n=11), which contained 2% cholesterol, including 15% lard (Harlan Teklad), and...
EBCT Studies
In each acute EBCT study, cortical, medullary, and papillary perfusion and segmental tubular dynamics were measured before and after suprarenal infusion of acetylcholine (Ach; 6 normal and 6 HC pigs) or sodium nitroprusside (SNP; 5 normal and 5 HC pigs). On the day of the EBCT study, each animal was anesthetized with 0.5 g of intramuscular ketamine and xylazine, intubated, and mechanically ventilated with room air. Anesthesia was maintained with a mixture of ketamine (0.2 mg · kg⁻¹ · min⁻¹) and xylazine (0.03 mg · kg⁻¹ · min⁻¹) in normal saline administered via an ear vein cannula at a rate of 0.05 mL · kg⁻¹ · min⁻¹. Under sterile conditions and fluoroscopic guidance, a 7F arterial guide was inserted in the left carotid artery and advanced to the abdominal aorta; a tracker catheter was advanced within the guide into the aorta above the level of both renal arteries to serve for infusion of saline or vasodilators. The arterial guide was maintained at a level above the scanning plane and served for monitoring mean arterial pressure (MAP) throughout the experiment. A pigtail catheter was positioned in the right atrium for contrast media injections and another was positioned in the urinary bladder for urine collection. ECG leads served for monitoring heart rate.

After the animals were prepared for surgery, they were positioned in the EBCT (Imatron C-150, Imatron Inc) scanning gantry. After a 1-hour recovery period during which saline infusion (3 to 4 mL/min) was initiated into the tracker, an EBCT study was performed to determine baseline hemodynamics and tubular dynamics in both kidneys. After a 15-minute recovery period, infusion of either Ach (4.5 μg · kg⁻¹ · min⁻¹) or SNP (5.5 [nmol/L] · kg⁻¹ · min⁻¹) into the aortic tracker catheter was initiated. The EBCT study was then repeated 15 minutes into vasodilator infusion. Urinary volume losses were repleted by an equivalent volume of saline.

Urine was collected for 10 minutes before each EBCT study for determination of urinary flow rate (with a graduated cylinder), sodium and potassium (with a flame photometer), and creatinine (by spectrophotometry) excretion. A blood sample was collected in the middle of each collection period for measurement of sodium, potassium, and creatinine. The sample obtained before any drug infusion was also used to measure lipid profile and plasma renin activity.

EBCT Scanning Sequence
Each EBCT study was performed during respiratory suspension at end expiration. With localization scans, all tomographic levels that contained both kidneys (from cranial to caudal pole) were identified, and 2 mid-hilar tomographic levels were selected. For the study of renal perfusion and tubular dynamics, the kidneys were scanned in the standard resolution (50 ms/image) multi-slice flow mode, which resulted in 2 contiguous 8-mm thick mid-hilar sections. Forty consecutive scans (over 3 minutes) were initiated and performed at variable time intervals 4 seconds after a bolus injection (0.5 cm³/kg over 1 to 2 seconds) of the nonionic, low-osmolar contrast medium iopamidol ( Isovue-370, Squibb Diagnostics) into the right atrial catheter, as previously described. These were followed by a renal volume study that was performed during iopamidol infusion (5 mL/s), as previously described. All of the studies were completed, the pigs were euthanized with a lethal infusion of pentobarbital sodium (Sleepaway, Fort Dodge Laboratories, Inc).

Data Analysis
All images were reconstructed with a filtered back-projection algorithm on the EBCT workstation and then transferred and displayed on a Sun workstation for density measurements. Regions of interest were selected by manually tracing the aorta, right and left 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 (Figure 1a). From each segment of the curve, the area enclosed under this segment, its peak height, and its mean transit time (MTT) were calculated.

MTT (seconds) through each vascular or tubular segment was calculated as: \( \frac{f(t)dt}{f(t)} \), where \( f(t) \) is contrast concentration (tissue density) at time \( t \), \( t \) is time from appearance of the curve, and \( f(t) \) is the area under the segmental time-density curve. Perfusion (blood flow normalized per unit tissue, mL · min⁻¹ · cm⁻³ tissue⁻¹) was calculated in each region from the first peak of its time-density curve, which represents passage of contrast through the regional vascular compartment. This flow calculation is based on the translocation of the tissue vascular volume per unit time: Area under tissue curve/Area under aortic curve/MTT > 60.

Intramural fluid concentration (ITC) was assessed by the degree of concentration or dilution of intratubular contrast media. Each peak observed after the vascular phase in each regional time-density curve was analyzed separately. ITC relative to cortical vascular blood was calculated for each nephron segment as the ratio of its peak height to that under the cortical vascular curve. Renal blood flow was calculated as the sum of the products of cortical and medullary perfusions and their corresponding volumes.

Statistical Analysis
Results are expressed as mean±SEM and were compiled for both kidneys. Statistical comparisons between experimental periods within groups were performed with paired Student’s t test and among groups with an unpaired Student’s t test and ANOVA. Statistical significance was accepted for \( P<0.05 \).
Results

Comparison of Hypercholesterolemic and Normal Pigs Under Resting Conditions

Levels of total, HDL, and LDL cholesterol were significantly elevated in the HC group compared with the normal group (Table). Plasma and urinary electrolytes and creatinine clearance were similar, but serum creatinine was mildly increased in the HC pigs (Table).

No significant difference existed between the normal and HC groups in either heart rate (85±4 versus 90±5 bpm, respectively, P=0.21) or MAP (94±5 versus 101±4 mm Hg, respectively, P=0.13). Cortical and medullary perfusions, volumes, and blood flows, as well as single kidney blood flow, were similar between the groups under basal conditions, as were ITC in the various nephron segments (Table, P>0.2 for all parameters).

Effect of Acetylcholine

Ach infusion into the suprarenal aorta was followed by a transient decrease in MAP in both groups, which returned to baseline levels within 2 minutes. These stabilized levels of MAP were not significantly different from baseline in either the normal (from 99±6 to 90±4, P=0.1) or the HC (from 86±7 to 88±8, P=0.4) groups. Heart rate was similarly unchanged.

In the normal group, creatinine clearance did not change (P=0.3) during Ach infusion, but there was a trend toward an increase in both urinary flow rate (+995±786%, P=0.07) and sodium excretion (+944±514%, P=0.08). Furthermore, Ach induced a significant increase in the EBCT-derived perfusion of the renal cortex, medulla (Figure 1), and papilla (to 5.0±0.3 mL·min⁻¹·cm⁻¹) (P=0.0001 each region), as well as the single-kidney blood flow (+36±6%, P=0.0002). This was associated with a significant decrease in ITC (Figure 1b) in the distal tubule (P=0.047) and collecting duct (P=0.039) segments, which suggested intratubular dilution and decreased fluid reabsorption. In addition, intratubular transit times decreased significantly in the proximal tubule, distal tubule, and collecting duct (−6±2%, −3±4%, and −8±5, respectively, P<0.05).

In the HC group, creatinine clearance was also unchanged (P=0.3) during Ach infusion. However, in this group, there was a significant increase in urinary flow rate (+742±301%, P=0.036) and a 22-fold increase in sodium excretion (P=0.019). The increase in cortical and medullary perfusion (Figure 1b, P=0.01 and P=0.019, respectively) was significantly attenuated compared with the normal group (P=0.006 and P=0.046, respectively). Papillary perfusion did not change (P=0.3), which was also significantly attenuated (P=0.03) compared with the normal group. Renal blood flow increased by only 15±4% (P=0.02), which was significantly less than in the normal group (P=0.019). Nevertheless, in the HC pigs, Ach induced a marked EBCT-measured decrease of ITC in all nephron segments (Figure 1b). The Ach-induced decrease in ITC in the proximal tubule and in the loop of Henle was significantly greater in the HC group versus the normal group (ANOVA, P=0.034 and P=0.0006, respectively). This dilution of intratubular content was accompanied by a shortening of intratubular transit times in all tubular segments: the proximal tubule, loop of Henle, distal tubule, and collecting duct (−7±3%, −8±4%, −8±4, and −14±6, respectively, P<0.05).

effect of nitroprusside

SNP did not elicit a change in MAP or heart rate in either the normal (P=0.2 and P=0.3, respectively) or the HC (P=0.6 and P=0.4, respectively) pigs. In the normal group, SNP did not affect creatinine clearance (P=0.4) but induced a mild increase in urinary flow rate (+54±24%, P=0.012) and sodium excretion (+56±24%, P=0.036). EBCT-derived cortical perfusion increased significantly (P=0.005, Figure 1c), and renal blood flow showed a strong tendency to increase (+15±8%, P=0.06) although medullary and papillary perfusions did not change (P=0.1 and P=0.4, respectively). This was accompanied by a significant decrease in ITC in the proximal (P=0.049) and distal (P=0.02) tubules and collecting duct (P=0.005) but not in the loop of Henle (P=0.17). A decrease in intratubular transit times did not reach statistical significance levels.
In the HC group, creatinine clearance and urinary flow rate did not change during SNP infusion ($P=0.3$ and $P=0.4$, respectively), although urinary sodium excretion tended to increase (+96 ± 5%, $P=0.054$). However, both cortical perfusion (Figure 1c) and renal blood flow failed to increase ($P=0.1$ and $P=0.3$, respectively) in response to the drug, which was a significant attenuation compared with the normal group ($P=0.005$ and $P=0.045$, respectively). Similar to the normal group, neither medullary nor papillary perfusions changed ($P=0.1$ and $P=0.3$, respectively). Nevertheless, in the HC pigs, SNP decreased ITC in the proximal tubule ($P=0.029$), distal tubule ($P=0.03$), and collecting duct ($P=0.001$), which was similar to the normal group. Again, the loop of Henle showed no change in fluid reabsorption ($P=0.48$). There were no significant differences in tubular responses to SNP between the normal and HC groups (ANOVA, $P>0.2$). There was also no significant change in intratubular transit times.

**Discussion**

The present study demonstrates that in the intact kidney of pigs with diet-induced HC, EBCT-derived regional renal perfusion and tubular function are normal under basal conditions, but the increase in renal perfusion in response to both endothelium-dependent (Ach) and -independent (SNP) vasodilators is significantly attenuated. Conversely, renal tubular response to SNP is relatively preserved and tubular response to Ach is enhanced.

Ach is a vasodilator and diuretic agent, which exerts its action through increased synthesis of NO and release of vasodilator prostaglandins (PG), and is commonly used to test endothelium-dependent vasodilatory capacity and bioavailability of NO. Renal synthesis of NO is governed by the NO synthase (NOS) family of enzymes, of which the inducible (i-NOS) form is expressed constitutively in epithelial cells along the nephron, whereas the endothelial (e-NOS) isoenzyme is expressed constitutively in both cortical and medullary vessels. The present study shows that in normal pigs the increase in renal blood flow elicited by Ach indeed results from a marked increase in both cortical and medullary perfusions. Moreover, Ach decreased fluid reabsorption in the distal tubule and collecting duct and tended to increase urinary flow rate and sodium excretion. These changes were probably due to Ach rather than the effect of the contrast medium per se, because we have previously observed only minimal contrast-induced alterations in renal tubular dynamics. Previous in vitro studies also demonstrated that a major tubular site of action of both NO and PG was the collecting duct. NO also inhibits both basal and angiotensin II–stimulated fluid reabsorption in the proximal tubule.

In contrast to normal pigs, we found that in HC pigs, both cortical and medullary vascular responses to Ach were blunted. The mechanisms of an attenuated vascular response to Ach have been proposed to include impairments in endothelial cell signal transduction, substrate (L-arginine) deficiency, alterations in the NOS enzymes or one of its cofactors, excess destruction of NO by an increased superoxide anion, or an imbalance between vasodilators and vasoconstrictors. The latter may include factors such as endothelin, angiotensin II, or PG. Although plasma renin activity was not elevated in our HC experimental group, this does not exclude local activation of the renin-angiotensin system. In cholesterol-fed rabbits, angiotensin-converting enzyme activity in aortic tissue, but not in plasma, was significantly elevated after a short feeding period when no atherosclerotic lesion was observed in the aortic tissue. Indeed, the beneficial effects of angiotensin-converting enzyme inhibitors on endothelial dysfunction have been demonstrated. Alternatively, Ach infusion during the increased supply of arachidonic acid, and in the presence of oxygen radical species, may shift the PG synthesis pathway toward greater production of vasoconstrictor PG. Indeed, the blunted response to Ach in HC rats was normalized by a thromboxane receptor antagonist, which also partly blocks the potent renal vasoconstrictor 8-epi-PGF2 alpha isoprostane. Any of these mechanisms may have participated in the blunted perfusion response observed in our study.

Our results are supported by previous studies in HC rats in which renal blood flow response to suprarenal Ach infusion was blunted. However, in rabbits, an 8- to 10-week HC diet did not impair the increase in renal blood flow in response to intrarenally infused Ach, although the response of the rabbit renal artery in vitro was slightly impaired. These differences may relate to the animal model, the duration of diet, or the route of Ach administration (systemic versus intrarenal infusion) in each study. Notably, EBCT measurements of perfusion represent an integrated response of the renal regional vasculature. These are, therefore, difficult to directly compare with in vitro studies of isolated renal arterial segments because some of the reactivity changes that we observed may, in fact, reside in the intrarenal microvasculature. EBCT thereby allows examination of the functional significance of pathophysiological hemodynamic alterations in each intrarenal region.

In contrast to the attenuated vascular response, our unique EBCT technology enabled us to demonstrate that in the intact kidney of HC pigs, tubular response to Ach was in fact enhanced. We observed marked dilution (decreased ITC) and faster transit (shortening of transit times) of intratubular fluid in all nephron segments, in association with a significant increase in urinary flow rate and sodium excretion. In particular, as opposed to the normal group, in the HC group the proximal tubule exhibited decreased ITC, which was still evident in the loop of Henle and was marked in the distal nephron. The mechanism for this augmentation may be speculated. Although in the coronary vascular wall e-NOS immunostaining is decreased in HC, the expression of the tubular i-NOS may not necessarily be reduced. Furthermore, although in the kidney i-NOS is expressed constitutively, its maximal expression typically requires stimuli such as cytokines, which are activated in early atherosclerosis. Interestingly, increased renal expression of i-NOS may even locally inhibit e-NOS, which may in turn contribute to the attenuated vascular response. A scenario of decreased vascular e-NOS expression, associated with sustained or augmented tubular i-NOS expression, could hypothetically account for the dissociation between the vascular and tubular response to Ach in this model. In line with this speculation was our...
observation that renal tubular response to exogenous NO (donated by SNP) was relatively preserved rather than enhanced. Because the natriuretic effects of Ach are mediated by NO, but not vasodilator PG,\textsuperscript{21} the tubular response cannot be fully explained as a compensatory PG effect for low NO availability. Moreover, this would not explain why the response was augmented rather than sustained. Alternatively, high cholesterol levels may possibly decrease sodium reabsorption by altering the cholesterol-tubular membrane fluidity-ionic transport relationships.\textsuperscript{36}

Nonetheless, low bioavailability of endogenous vascular NO does not appear to be the sole mechanism responsible for impaired vascular reactivity in our model, because we observed that the increase in cortical perfusion in response to SNP was also blunted. Previous studies reported attenuated vasodilatory response to exogenous, in addition to endogenous, NO donors in humans with HC\textsuperscript{37,38} or diabetes\textsuperscript{39} and suggested that the abnormality was due to increased inactivation of NO or decreased reactivity of the vascular smooth muscle to NO. A substantial resistance to NO was also observed in vascular smooth muscle cells obtained from rabbits after a 10-week HC diet, and NO failed to blunt the rise in intracellular calcium induced by angiotensin II,\textsuperscript{40} which may indicate enhanced sensitivity to vasoconstrictors. Indeed, both increased intravascular inactivation of NO and increased levels of vasoconstrictors may have played a role in the attenuated renal vascular response to SNP (and potentially Ach as well). The reduced responsiveness of the renal vasculature did not appear to fully extend to the tubular compartment, in which ITC decreased during SNP infusion. Nevertheless, because the urinary flow rate failed to increase in this group, it is possible that the tubular response to exogenous NO is mildly impaired in the HC pigs.

Alterations in vasomotor regulation may be responsible for development of hypertension, altered tissue perfusion, and an enhanced propensity for vasoconstriction.\textsuperscript{4} Our findings do not support impairment in sodium excretion as a prohypertensive mechanism in early HC. However, a longer duration of HC and the vascular effects of HC per se may lead to more severe renal functional and structural changes, which may eventually have a detrimental effect on renal excretory function. Although ischemia of the kidney occurs only during extreme reductions in renal blood flow, it is not unlikely that impaired renal vascular reactivity could lead to maladjusted renal blood flow responses to physiological challenges and translate into repetitive renal insults. Hence, even at its early stage, HC may be a risk factor for the kidney.

In summary, we found that in diet-induced HC, EBCT-derived renal vascular response to both endothelium-dependent (Ach) and -independent (SNP) vasodilators was attenuated, in association with relatively preserved tubular response to exogenous NO and enhanced response to endogenous NO. Thus, these results suggest dissociation of renal vascular from tubular dynamics in HC. The mechanism for these renal changes may be related to intravascular inactivation or low sensitivity to NO and increased levels of vasoconstrictors.

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