Myogenic Responses of Mouse Isolated Perfused Renal Afferent Arterioles
Effects of Salt Intake and Reduced Renal Mass

En Yin Lai, Maristela L. Onozato, Glenn Solis, Shakil Aslam, William J. Welch, Christopher S. Wilcox

Abstract—Because defects in renal autoregulation may contribute to renal barotrauma in chronic kidney disease, we tested the hypothesis that the myogenic response is diminished by reduced renal mass. Kidneys from 5/6 nephrectomized mice had only a minor increase in the glomerular sclerosis index. The telemetric mean arterial pressure (108±10 mm Hg) was unaffected after 3 months of high-salt intake (6% salt in chow) or reduced renal mass. Afferent arterioles from 5/6 nephrectomized mice and sham-operated controls were perfused ex vivo during step changes in pressure from 40 to 134 mm Hg. Afferent arterioles developed a constriction and a linear increase in active wall tension above a perfusion pressure of 36±6 mm Hg, without a plateau. The slope of active wall tension versus perfusion pressure defined the myogenic response, which was similar in sham mice fed normal or high-salt diets for 3 months (2.90±0.22 versus 3.22±0.40 dynes·cm⁻¹/mm Hg; *P* value not significant). The myogenic response was unaffected after 3 days of reduced renal mass on either salt diet (3.39±0.61 versus 4.04±0.47 dynes·cm⁻¹/mm Hg) but was reduced (*P*<0.05) in afferent arterioles from reduced renal mass groups fed normal and high salt at 3 months (2.10±0.28 and 1.35±0.21 dynes·cm⁻¹/mm Hg). In conclusion, mouse renal afferent arterioles develop a linear increase in myogenic tone around the range of ambient perfusion pressures. This myogenic response is impaired substantially in the mouse model of prolonged reduced renal mass, especially during high salt intake. *(Hypertension. 2010;55:983-989.)*

Key Words: kidney ■ renal autoregulation ■ chronic kidney disease ■ hypertension ■ salt sensitivity

Renal autoregulation implies a proportionate increase in renal vascular resistance with increasing perfusion pressure. Autoregulation is mediated predominantly by myogenic and tubuloglomerular feedback (TGF) components. Louzenhis and colleagues demonstrated a myogenic response in the renal afferent arteriole of hydronephrotic kidneys from rats that contributed 31% to the autoregulation of renal blood flow.⁹ Takenaka et al reported a one-third decrease in afferent arteriolar diameter and a maintained blood flow velocity in juxtamedullary nephrons of the rat during increases in renal perfusion pressure. Impaired autoregulation and systemic hypertension in chronic kidney disease (CKD) have been proposed to cause the elevated glomerular capillary pressure that has been linked to progressive glomerular injury.⁵ Dietary salt restriction reduced glomerulosclerosis and renal damage in rats with reduced renal mass (RRM)⁶-⁷ and reduced proteinuria in patients with CKD.⁸ A preliminary study reported that a high salt intake impaired autoregulation of the juxtamedullary afferent arterioles in the rat.⁹ However, the myogenic response of isolated afferent arterioles has not been studied in models of CKD or during changes in salt intake. Renal afferent arterioles are the main renal resistance vessels and, unlike the arcuate or interlobular arteries,⁶ can have strong myogenic contractions.¹⁰ We tested the hypothesis that the myogenic responses of renal cortical afferent arterioles were impaired by prolonged RRM and by dietary salt loading. The aim was to study the myogenic responses in isolated perfused afferent arterioles where the perfusion pressure could be controlled and changes in the luminal diameter measured directly without confounding effects of the TGF or circulating factors. We evaluated myogenic responses at 3 days, 3 weeks, and 3 months after RRM or sham operations during normal or high levels of dietary salt intake.

Methods and Protocols

Male C57BL6 mice weighing 22 to 30 g (Jackson Laboratory, Bar Harbor, ME) were fed a 0.4% NaCl control-test diet (normal salt [NS]; Harlan Teklad) or an equivalent 6% NaCl diet (high salt [HS]; TD92055, Harlan Teklad) and allowed free access to tap water. All of the procedures conformed to the Guide for Care and Use of Laboratory Animals prepared by the Institute for Laboratory Animal Research. Studies were approved by the Georgetown University Animal Care and Use Committee.
Animal Preparation and Surgery
A 2-step surgical 5/6 nephrectomy procedure was used to create RRM under inhalational anesthesia with 2% isoflurane and oxygen mixed with room air in a vaporizer. Two thirds of the mass of the left kidneys was ablated by stitching off each pole using an absorbable hemostat (Ethicon, Inc.). At a second surgery after 1 week, the right renal vessels were dissected, clipped, and cut and the kidney removed. Sham-operated control mice (sham) were subject to a similar 2-stage surgery without the removal of kidneys. After a 1-week recovery period, mice were randomized to a normal or high salt intake for 3 days, 3 weeks, or 3 months. Parallel groups of RRM and sham mice fed normal or high salt intakes were equipped with telemetric blood pressure recorders (Data Sciences International) connected to a cannula in the carotid artery.13 The telemeters were implanted during the second surgery and were switched on 1 week later. The data for the first and last 3 weeks corresponding with the times of assessment of myogenic responses (3 weeks and 3 months) were averaged.

Dissection and Microperfusion of Afferent Arterioles
Renal afferent arterioles were dissected, mounted, and perfused as described in detail.16 Briefly, the kidneys were sliced along the corticomedullary axis immediately after euthanization, placed in 4°C dissection solution, and an afferent arteriole with glomerulus attached was microdissected under a stereomicroscope using sharpened forceps, transferred to a thermostated chamber on the stage of an inverted microscope (Olympus IX70, Olympus America, Inc.), and perfused using a micromanipulator system (Vestavia Scientific) with concentric holding and perfusion pipettes. The arteriole was aspirated into the holding pipette, which had an OD of 2.13 mm, an ID of 1.63 mm, and a tip aperture of 20 μm. The inner perfusion pipette had an OD of 1.19 mm, an ID of 1.02 mm, and a tip diameter of 6 μm. It was advanced into the arteriolar lumen. The pressure at its tip was calibrated using a closed chamber connected to a model DPM-1B pneumatic transducer calibrator (Bio-Tek Instruments, Inc.). Microdissection and cannulation were completed within 120 minutes, after which the bath was gradually warmed to 37°C and the arteriole stabilized for 20 minutes. The cannulated afferent arteriole was perfused with DMEM/Nutrient Mixture F-12 Ham (Sigma) at 40 mm Hg. For calibrating the pressure in the lumen of the perfused arteriole stabilized for 20 minutes. The cannulated afferent arteriole was perfused with DMEM/Nutrient Mixture F-12 Ham (Sigma) at 40 mm Hg. For calibrating the pressure in the lumen of the perfused arteriole, a special holding pipette was applied at the end of the afferent arteriole, and perfusion with these 2 solutions. Because active wall tension was calculated as the difference between the tensions measured during perfusion with these 2 solutions. Because active wall tension increased linearly with perfusion pressure above ~40 mm Hg (see Figure 2), the myogenic response of each arteriole was calculated as the slope of the regression of active wall tension on perfusion pressure. The calculated intercept on the x axis defined the threshold pressure that initiated an active wall tension response.

Chemical Methods: Plasma Creatinine and Urinary Albumin
Plasma and urinary creatinine were measured with high performance capillary electrophoresis (Beckman Coulter, P/ACE MDQ system) equipped with UV detection. Briefly, 5 mL of plasma ultraltrate were injected under vacuum at the short end of an uncoated fused silica capillary (Polymicro) with an effective length of 10.2 cm, total length of 60.2 cm, and ID of 50 μm. The background buffer consisted of 40 mmol/L sodium phosphate at pH 2.35. The peaks were detected at 200 nm wavelength were confirmed by spiking with a known amount of creatinine. The urine samples were diluted 10-fold. All of the samples were run in duplicate. The values for plasma creatinine for normal mice were in good agreement with a previous report using tandem mass spectrometry.17 Urinary albumin was measured by a murine microalbuminuria ELISA using a microplate reader equipped to determine absorbance at 450 nm (Albuwell M kit, Exocell).

Statistics
Data are expressed as mean±SEM. GraphPad Software Prism 3.0 was used for statistical analysis (GraphPad Prism, GraphPad Software). A 2×2 ANOVA was used to compare effects of RRM and salt intake and any interaction (ie, effects of salt intake on the response to RRM). When appropriate, these calculations were followed by Bonferroni post hoc Student’s t tests. Changes were analyzed using parametric statistics. P<0.05 was considered statistically significant.

Results
A high salt intake increased body and kidney weights, whereas RRM reduced total kidney weight (Table 1). There was an increase in albuminuria in mice with RRM. Plasma creatinine increased more in mice with RRM fed a high salt intake. There was no effect of RRM or salt on the basal diameter of the afferent arteriole perfused at 40 mm Hg without activating the myogenic response. There was a small but significant increase in the glomerular sclerosis index of mice with RRM. Morphological changes were correspondingly mild in all of the groups (data not shown), as described previously.18

The mean arterial pressure (MAP) was measured telemetrically starting 1 week after the second surgery, corresponding with the allocation to normal or high salt intakes (Figure 1). There were no differences in the MAP of sham mice on NS or HS diets. However, the MAPs of mice with RRM during HS (n=9) and NS diets (n=7) were significantly (P<0.0001) higher than the MAPs of sham HS (n=8) and NS (n=6) mice during the first 3 weeks of recording. Thereafter, the pressure differences between the RRM and sham groups became less prominent or disappeared. When assessed over the last 3 weeks of the RRM protocol, corresponding with the period of the 3-month myogenic response measurements, there were no significant differences among the 4 groups. The

T=Pi×R, where Pi was the intravascular perfusion pressure and R was the internal radius.10 A set of pressure steps from 40 to 134 mm Hg was undertaken in each arteriolo in physiological solution and in a perfusate without Ca2+ and containing 5×10−3 M EGTA (Sigma) to abolish active tone. The active wall tension was calculated as the difference between the tensions measured during perfusion with these 2 solutions. Because active wall tension increased linearly with perfusion pressure above ~40 mm Hg (see Figure 2), the myogenic response of each arteriole was calculated as the slope of the regression of active wall tension on perfusion pressure. The calculated intercept on the x axis defined the threshold pressure that initiated an active wall tension response.

Renal Glomerulosclerosis Score
Kidney sections were stained with hematoxylin-eosin and periodic acid-Schiff. Glomerulosclerosis was scored in all of the glomeruli of each section, as described,16 with 0 for a normal glomerulus, 1 for mild sclerosis (<25%), 2 for moderate segmental sclerosis (25% to 50%); 3 for severe segmental sclerosis (50% to 75%), and 4 for global sclerosis. The score was calculated in 6 mice in each group as (0×number of S0+1×number of S1+2×number of S2+3×number of S3+4×number of S4)/(number of S0+number of S2+number of S3+number of S4), where S represents the glomerulosclerosis index.

Measurement of Myogenic Tone
Data were analyzed by proprietary Windows-based advanced software. The experiments were recorded by a video system of Panasonic VHS linked to an analog analysis system, digitized, and monitored in real time. Wall tension (T) was calculated as follows:

T=Pi×R, where Pi was the intravascular perfusion pressure and R was the internal radius.10 A set of pressure steps from 40 to 134 mm Hg was undertaken in each arteriolo in physiological solution and in a perfusate without Ca2+ and containing 5×10−3 M EGTA (Sigma) to abolish active tone. The active wall tension was calculated as the difference between the tensions measured during perfusion with these 2 solutions. Because active wall tension increased linearly with perfusion pressure above ~40 mm Hg (see Figure 2), the myogenic response of each arteriole was calculated as the slope of the regression of active wall tension on perfusion pressure. The calculated intercept on the x axis defined the threshold pressure that initiated an active wall tension response.
heart rates and the activity records were not different among the 4 groups (data not shown).

Figure 2 depicts data in isolated perfused afferent arterioles from 9 normal mice fed an NS diet. When perfused in a bath with a calcium-free solution and EGTA to abolish active tone, there was an increase in luminal diameter with perfusion pressure, but when perfused in a physiological calcium-containing solution (vehicle), the diameter of the afferent arterioles decreased significantly \( (P<0.05) \) by 11% from 10.22±0.55 to 9.06±0.51 \( \mu m \) during an increase renal perfusion pressure from 40 to 80 mm Hg (Figure 2A). The corresponding wall tensions are shown in Figure 2B, and the active wall tension (the difference between the 2 sets of data in Figure 2B) is shown in Figure 2C. The slope of the line in Figure 2C gives the myogenic response that averaged 2.98±0.37 dynes \( \cdot \) cm\(^{-1}\) \( \cdot \) mm Hg.

### Table 1. Body and Total Kidney Weights, Kidney Function, Afferent Arteriolar Diameter, and Glomerulosclerosis Index

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Normal Salt (3 mo)</th>
<th>High Salt (3 mo)</th>
<th>Effect of Salt</th>
<th>Effect of RRM</th>
<th>Effect of Interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight, g</td>
<td>25.86±0.51</td>
<td>25.30±0.97</td>
<td>27.10±0.9</td>
<td>27.00±0.38</td>
<td>( P&lt;0.05 ) NS</td>
</tr>
<tr>
<td>Kidney weight, g</td>
<td>0.32±0.01</td>
<td>0.18±0.02</td>
<td>0.35±0.01</td>
<td>0.20±0.01</td>
<td>( P&lt;0.0001 ) NS NS</td>
</tr>
<tr>
<td>Pcr, mg/dL</td>
<td>0.082±0.003</td>
<td>0.102±0.005</td>
<td>0.077±0.007</td>
<td>0.175±0.021</td>
<td>( P&lt;0.0001 ) P&lt;0.001</td>
</tr>
<tr>
<td>Albuminuria, ( \mu g/d ) per g of body weight</td>
<td>6.01±0.52</td>
<td>8.53±1.68</td>
<td>9.72±1.48</td>
<td>12.35±0.98</td>
<td>( P&lt;0.01 ) P&lt;0.05 NS</td>
</tr>
<tr>
<td>Afferent diameter, ( \mu m ) at 40 mm Hg</td>
<td>9.41±0.53</td>
<td>10.48±0.97</td>
<td>10.38±0.64</td>
<td>10.74±0.99</td>
<td>NS NS NS</td>
</tr>
<tr>
<td>Sclerosis index, arbitrary unit</td>
<td>0.02±0.002</td>
<td>0.073±0.024</td>
<td>0.044±0.008</td>
<td>0.081±0.012</td>
<td>NS P&lt;0.01 NS</td>
</tr>
</tbody>
</table>

Data show the mean±SEM values for mice with a sham operation (sham) or with RRM fed an HS or NS diet for 3 months (n=6 to 7 per group). NS in last 3 columns indicates \( P \) value not significant. Pcr indicates plasma creatinine concentration.

---

**Discussion**

These results demonstrate a consistent and significant myogenic response of renal afferent arterioles isolated from the mouse and extend studies in the rat.\(^{1,2}\) The main new findings were that there was a linear increase in active wall tension of the mouse afferent arteriole with perfusion pressure above a threshold of \( \approx 40 \) mm Hg with no evidence of a plateau up to 134 mm Hg. A 15-fold increase in dietary salt intake over 3 months did not modify myogenic responses from arterioles of...
sham-operated mice, but these responses were reduced over 3 weeks to 3 months after a surgical reduction in renal mass.

Unlike the rat model of RRM and many human subjects with CKD, the mouse model of RRM, even when studied with prolonged telemetric recording, did not display salt sensitivity. However, mice with RRM had a higher MAP than sham-operated controls for the first 3 weeks of recording (4 weeks total after RRM), with no apparent effects of salt intake. This might relate to activation of renal afferent reflexes initiated by the renal injury and the healing process from the 2/3 nephrectomy, but this was not studied further. The mouse model of RRM had an adaptive increase in the size of the remaining kidney tissue but only modest albuminuria. There were corresponding modest renal morphological changes, confirming a previous report, and only a minor

Figure 2. Mean±SEM values from studies in normal mice (n=9) fed an NS diet of the afferent arteriolar diameter (A), the wall tensions (B), and the active wall tension (C) during pressure steps from 40 to 134 mm Hg. Studies were undertaken in a physiological solution and in a perfusate with a calcium-free solution containing EGTA to abolish active tone. Compared with data at 40 mm Hg; *P<0.05; ***P<0.005.

Figure 3. Mean±SEM values for the afferent arteriolar diameters and active wall tensions from sham mice fed an NS diet for 3 days (n=5), 3 weeks (n=5), or 3 months (n=5; C and D).
increase in the glomerular sclerosis index. Because salt intake did not affect the blood pressure, the defective myogenic responses of renal afferent arterioles in this strain of mice with RRM, and the enhancing effects of salt, should be related primarily to the RRM and not to hypertension.

A preliminary study reported that an HS diet reduced the autoregulatory responses of rat juxtaglomerular arterioles and impaired responsiveness to purinergic receptor stimulation.9 The failure of a high salt intake to modify myogenic responses of afferent arterioles from sham-operated mice in the present study suggests that the diminished autoregulation in the rat study may relate to additional factors, such as a diminished TGF response,23 which contributes to autoregulation in intact kidneys, but this requires study.

Human CKD is usually accompanied by a normal or low level of plasma renin activity unless it is because of segmental renal ischemia.24 This has been modeled in the rat by surgical reduction of renal mass in 1 kidney and contralateral nephrectomy, which leads to a low-renin, low-angiotensin, salt-sensitive form of hypertension accompanied by an adaptive increase in single nephron glomerular filtration rate followed by a slowly progressive loss of kidney function.25,26 The mouse model described here develops an initial modest hypertension of uncertain cause that is independent of salt intake and lasts ≈3 or 4 weeks. Thereafter, the BP returns to normal even during rather severe dietary salt loading. These mice developed only modest albuminuria and glomerular injury, confirming previous studies in this mouse strain.18 Mice with RRM had a 3.4-fold increase in weight of the partially nephrectomized kidney. This demonstrates considerable adaptive responses to the reduction in renal mass and dissociates glomerular injury in this model from the adaptive growth of remaining nephrons. Studies in rat models led to the conclusion that glomerular injury results from the combination of systemic hypertension and impaired autoregulation8,27 that permits transmission to the elevated pressure into the glomerulus.28 Thus, the absence of hypertension might explain the absence of significant glomerular injury in this study.

Autoregulation preserves a constant renal blood flow and glomerular filtration rate over a physiological range of renal perfusion pressures. It is mediated primarily by an afferent arteriolar myogenic and TGF response.29 The myogenic response entails a proportionate contraction of the vascular smooth muscle cells with increasing stretch. The TGF response translates flow-dependent changes in the composition of the tubular fluid at the macula densa into proportionate changes in the resistance of afferent arterioles. Recent studies have identified additional mechanisms that contribute to autoregulation of renal blood flow.29–31 The mechanism of the afferent arteriolar myogenic response is incompletely understood and might be clarified by future studies in gene-deleted mice. Inscho et al9,32 demonstrated that ATP-sensitive purinoceptors linked to Rho kinase regulated myogenic responsiveness of pregglomerular microvascular elements. Pregglomerular resistance is regulated by vascular smooth muscle cell calcium influx- and calcium mobilization-dependent mechanisms.33

The present study is the first to quantify the myogenic component in isolation in the afferent arteriole of the mouse. A doubling of perfusion pressure in the afferent arterioles from normal mice within a physiologically relevant range of 40 to 80 mm Hg elicited an 11% reduction in diameter from normal mice within a physiologically relevant range of 80 to 160 mm Hg,1 whereas the TGF response was blocked (as in the isolated arteriole), the response was reduced to 0%4 or 8% to 10%.24 Studies with the hydronephrotic kidney of normal rats (which lacks a TGF response) report a 7% to 23% response during a doubling of perfusion pressure from 80 to 160 mm Hg,1 whereas studies in isolated afferent arterioles from normal rats or rabbits report no significant contraction with a doubling of perfusion pressure.35,36 However, there was a 34% reduction in the diameter of afferent arterioles isolated from spontaneously hypertensive rats.12 Measurement in the rat juxtamedullary arterioles with a doubling of perfusion pressure but, if the resistance is regulated by vascular smooth muscle cell calcium influx- and calcium mobilization-dependent mechanisms.33
ullary nephron preparation show that a 20% reduction in luminal diameter during a 40-mm Hg pressure increase maintained a stable arteriolar blood flow (perfect autoregulation). Clearly, there is much variability in response that may relate to technical differences between preparations. Because resistance to flow is proportional to the fourth power of the radius, there would be more than a doubling in the calculated resistance from the reduction in the afferent arteriolar radius recorded in the present study in normal mice across the pressure range of 40 to 80 mm Hg. The impaired myogenic response in vessels from mice with RRM may contribute to the impaired ability to autoregulate glomerular capillary pressure and whole kidney hemodynamics that have been described in rats with RRM.

The glomerular capillary pressure, which approximates the pressure at the end of the afferent arteriole, can be calculated indirectly from the sum of the plasma protein oncotic pressure and the proximal tubule stop flow pressure. The oncotic pressure of mouse plasma averaged $20.9 \pm 1.8$ mm Hg. The proximal tubule stop flow pressure in anesthetized mice was 32 to 42 mm Hg (average: 36 mm Hg; unpublished data from our laboratory). Thus, the calculated glomerular capillary pressure in the mouse is $\approx 57$ mm Hg. Because this is above the threshold pressure to elicit a myogenic response, which we found to average 36 mm Hg, the afferent arteriolar myogenic response could contribute to the maintenance of renal blood flow at perfusion pressures above and below the normal range. Indeed, whole kidney blood flow in response to 20-mm Hg pressure changes was well autoregulated in the mouse. The myogenic component contributed more to overall autoregulation during reductions than increases in renal perfusion pressure.

In summary, renal afferent arterioles from mice develop a contraction and a linear increase in myogenic tone above ambient perfusion pressure. This myogenic response is impaired substantially in the mouse model of prolonged RRM, especially during high salt intake.

**Perspectives**

Rats with RRM develop hypertension, proteinuria, and glomerulosclerosis that are exacerbated by a high salt intake. In contrast, the mouse model of RRM developed hypertension over 3 weeks but thereafter maintained a normal blood pressure and developed only modest albuminuria and glomerular injury even at 3 months of high salt intake. The myogenic response has been considered to protect the kidney from barotrauma during hypertension. A reset TGF response and an impaired autoregulation of glomerular capillary pressure or renal blood flow have been related to renal damage in rat models. However, renal damage in the rat RRM model, or in patients with CKD, is generally slight, as in the present and previous study, in this strain of mice, unless they develop hypertension. Thus, one might speculate that the delayed development of an impaired afferent arteriolar myogenic response in mice after 3 weeks might even be protective if it permitted better transmission of the arterial pressure into the kidneys and thereby allowed correction of the hypertension that developed during the first month. Development of a

![Figure 4](https://example.com)
transient salt-resistant hypertension early after induction of RRM was not apparent in studies in the rat.7

Acknowledgments
We thank Emily Wing Kam Chan for preparing and editing the article.

Sources of Funding
The work described in this study was supported by research grants to C.S.W. from the National Institute of Diabetes and Digestive and Kidney Diseases (DK-049870 and DK-036079) and from the National Heart, Lung, and Blood Institute (HL-68686) and by funds from the George E. Schreiner Chair of Nephrology.

Disclosures
None.

References
19. Yllatalo P, Hepp R, Mohring J, Gross P. Effects of varying sodium intake on blood pressure and renin-angiotensin system in subtotaly nephrec-
29. Just A, Arendshorst WJ. A novel mechanism of renal blood flow auto-
33. Yuan BH, Robinette JB, Conger JD. Effect of angiotensin II and norepi-
</text>
Myogenic Responses of Mouse Isolated Perfused Renal Afferent Arterioles: Effects of Salt Intake and Reduced Renal Mass
En Yin Lai, Maristela L. Onozato, Glenn Solis, Shakil Aslam, William J. Welch and Christopher S. Wilcox

Hypertension. 2010;55:983-989; originally published online March 1, 2010;
doi: 10.1161/HYPERTENSIONAHA.109.149120
Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2010 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/55/4/983

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Hypertension can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Hypertension is online at:
http://hyper.ahajournals.org//subscriptions/